



# **20th Congress of the International Society for Animal Hygiene**

**5. – 7. October 2022**



**DEPARTMENT OF VETERINARY MEDICINE**

**FREIE UNIVERSITÄT BERLIN**

Lecture hall of the equine clinic

Oertzenweg 19B | 14163 Berlin, Germany

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## Opening remarks Prof. Dr. Uwe Rösler

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Dear colleagues, dear members and friends of ISAH,

It is my great pleasure to welcome you to the 20<sup>th</sup> Congress of the International Society of Animal Hygiene, ISAH 2022 in Berlin.



The ISAH 2022 Congress takes place at a time of great uncertainty and changes. We are now more than two and a half years into the Covid 19 pandemic. This crisis showed us how a zoonotic infectious agent found its way into the human population, adapted to this new host species within a very short time and then spread rapidly worldwide, even finding its way back to new animal hosts. Destruction of natural areas, over-consumption of natural resources and intensive global travel have all contributed to this dramatic development. In the meantime, millions of people have been affected and sadly, many victims have been reported. As dramatic and drastic as this development was, it also showed that correct, consequently taken hygiene measures and new, revolutionary vaccines brought the pandemic under control.

However, the Covid-19 pandemic also had dramatic consequences for science itself.

Many institutes and universities around the world closed down and only conducted their teaching online. Laboratories were closed and many research projects were suspended. This also applied to ISAH 2021, which was to take place in Pattaya, Thailand, in 2021 and which many of our community had already prepared for and looked forward to. Due to the travel restrictions and the associated planning uncertainties, it was decided to postpone the 20th ISAH Congress by one year and to organise it in Berlin in 2022.

ISAH 2022 is the first ISAH Congress as a hybrid event. This will enable many ISAH members and interested persons to participate, even if the situation and the available travel budget would not otherwise allow it. Moreover, supporting participants from low and middle income countries has always been one of ISAH's main objectives! Online participation is, therefore, free of charge for participants from these countries. This also applies to online participants from Ukraine. The people of Ukraine are going through a major crisis and are heroically resisting the aggression. We hope to be able to help the colleagues and friends of ISAH in Ukraine a little and are very happy to be able to meet them in person again in the future - healthy and in freedom.

I wish you and all of us together a successful ISAH 2022 and good meetings and conversations.

Yours,

Uwe Rösler

## Opening remarks Prof. Dr. Dr. h.c. mult. Jörg Hartung

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On behalf of the Organizing Committee, the Scientific Committee and the Executive Board of ISAH, I cordially welcome you all in Berlin, Germany to the 20th Congress of the International Society for Animal Hygiene (ISAH).

First of all, I want to thank Prof Dr Uwe Rösler, Professor for Animal and Environmental Hygiene of the Free University of Berlin, and his team, for taking initiative, responsibility and courage in these times troubled by Covid-pandemia and war to host the congress in his Institute and adjacent facilities.



Animal Hygiene is the third pillar of veterinary medicine besides curing medicine and care taking of animals. Hygiene is science and application of preventing diseases in a holistic sense. This is mirrored in the broad range of oral and poster presentations in these proceedings. The topics cover infectious diseases, antimicrobial resistance problems (AMR) in the One Health context and specific issues relate to pig production, such as ASF and disease preventing measures, recent developments in poultry production incl. novel technologies.

Animal welfare themes, farming systems with outdoor access and biosecurity concepts encompassing recent knowledge of all relevant aspects of the abiotic and biotic environment of animals in a housing and keeping environment and the understanding of the principles of the interactions of animals with their technical, physical (thermal), chemical and biological surrounding. The main goal is always to improve animal health, welfare / wellbeing and performance without the unnecessary use of drugs being the precondition for the production of safe and healthy food. Furthermore, it requires a profound knowledge of management, handling and behaviour of animals on individual and herd level, and the understanding of the effects of animal husbandry systems on the surrounding environment by airborne emissions, manure disposal and spread of diseases (environmental hygiene) in order to define prevention strategies incl. bio-security measures and disinfection. Animal Hygiene integrates the relevant aspects of animal health and welfare in livestock production, ethics, ecology, and consumer demands on a sound economic basis. The potential of precision livestock farming (PLF) is addressed, maintaining animal health and welfare and recognizing early spread of diseases and pathogens incl. zoonosis.

These Proceedings present abstracts of 36 oral presentations and 42 Posters in nine sessions.

The organisation of such a congress requires the help and input of many people, and I hereby would like to express my deep thanks to all who contributed to realise the 20th ISAH2022 Congress in Berlin.

Last but not least, it is my privilege to thank all participants, contributors, chairpersons, organisational and technical assistants for their considerable efforts and inputs. Now, we wish you all an interesting and pleasant ISAH Congress, an enjoyable time in Berlin and much value reading this Proceedings booklet.

Jörg Hartung

In the name of the Executive Board of ISAH  
President of ISAH

## Programme

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### Day 1: 5. October 2022

1:00 pm – Opening  
2:00 pm

ISAH President Prof. Dr. Dr. h.c. mult. Jörg Hartung  
Congress president Prof. Dr. Uwe Rösler  
Vice Dean Research Prof. Dr. Marcus Fulde

2:00 pm coffee break

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2:30 pm – Session 1  
4:10 pm *Chairs: U. Rösler & S. Opalinski*

#### Pig production: infectious diseases and disease preventing measures (I)

2:30 pm	Prof. Dr. Franz Conraths (Keynote Lecture):	African Swine Fever: Epidemiology of a pandemic disease in pigs.
3:10 pm	Ceruti A.:	Successful deployment of a mobile suitcase lab for rapid detection of African Swine Fever Virus in Uganda
3:30 pm	Tanneberger F.:	Tenacity and disinfection of Modified vaccinia virus Ankara and African swine fever virus in forest soil
3:50 pm	Kemper N.:	Evaluation of cleaning and disinfection practices in pig barns using boot swab sampling
4:10 pm	coffee break	

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4:30 pm – Session 2  
5:30 pm *Chairs: H. Schobesberger & J. Paeshuyse*

#### Pig production: infectious diseases and disease preventing measures (II)

4:30 pm	Fablet C.:	Salmonella excretion level in pig farms and impact of quaternary ammonium compounds based disinfectants on Escherichia coli antibiotic resistance
4:50 pm	Schulz J.:	Disinfection of contaminated anesthetic masks for piglets
5:10 pm	von und zur Mühlen F.:	Vaccination of pigs against <i>Lawsonia intracellularis</i> can help to improve efficiency and with this have a positive effect on sustainability
5:45 pm	EEB Meeting	

## Day 2: 6. October 2022

8:30 am - **Session 3**  
 10:00 am *Chairs: J. Hartung & C. Fablet*

### Infectious diseases in the One Health context (I)

8:30 am	Prof. Dr. Dr. hc. Lothar H. Wieler (Keynote Lecture):	The silent pandemic, which needs to be contained by global concerted action NOW
9:10 am	Desvars-L. A.:	SARS-CoV-2 and One Health approach: filling the data gap
9:30 am	Jarynowski A.:	Perception of infectious diseases with animal and humans hosts on the Polish internet
9:50 am	Adukkadukkam S.:	Antimicrobial resistance profiling of <i>Acinetobacter baumannii</i> isolated from the environment least exposed to antibiotics.
10:10 am	coffee break (30 min, including Poster Session)	

10:40 am - **Session 4**  
 12:40 pm *Chairs: U. Rösler & S. Schwarz*

### Infectious diseases and AMR in the One Health context (II)

10:40 am	Hanford T.:	Determining the fitness of fluoroquinolone resistant <i>Campylobacter</i> using in vivo competition models in chickens
11: 00 am	Ahmed M.:	Broiler insects' role in antimicrobial resistant <i>Campylobacter</i> , <i>Salmonella</i> and <i>Staphylococcus aureus</i> carry over between broiler flocks
11:20 am	Feßler A.:	Antimicrobial and biocide resistance of zoonotic pathogens from dogs and cats
11:40 am	Prasek J.:	On farm culture system as a tool to reduce the antimicrobial consumption at selective dry cow therapy in Czech farms.
12:00 pm	Tenhagen B.-A.:	The Devil and the Beelzebub - Controlling MRSA in cattle in the dry period
12:20 pm	Lunch (60 min, including Poster Session)	

1:20 pm – **Session 5**  
 2:40 pm *Chairs: A. Friese & B.A. Tenhagen*

### Infectious diseases and AMR in the One Health context (III)

1:20 pm	Robé C.:	Genetic analysis of specific antibiotic resistances in <i>E. coli</i> of veal calves up to eight months
1:40 pm	Schwenker J.:	Teat disinfection - (Co-)selection of udder pathogens and antibiotic-resistant bacteria? A longitudinal experiment.
2:00 pm	Kivali V.:	Microbial contamination at slaughter and retail points of the pork value chain in Uganda.
2:20 pm	Pöppe J.:	Experimental exposure of <i>Salmonella enterica</i> from farm animals to a glyphosate-containing herbicide: effects on tolerance response, cross- tolerance, cross-resistance and fitness.
2:40 pm	coffee break (40 min, including Poster Session)	

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3:20 pm – **Session 6**  
 4:40 pm *Chairs: A. Aland & C. Fablet*

### Poultry production: infectious and non-infectious issues, novel technologies (I)

3:20 pm	Olejnik K.:	Hemp oil and cannabidiol in broilers diet – impact on productivity and behaviour
3:40 pm	Abd El-Wahab A.:	A preliminary study on the impacts of early nutrition of hatched chicks on well-being and growth performance
4:00 pm	Reichelt B.:	<i>Campylobacter</i> spp. transmissions in the environment of commercial poultry farms in Germany
4:20 pm	coffee break (10 min)	
4:30 pm – 6:30 pm	ISAH General Assambly (with Elections)	
8:00 pm – 12:00 am	ISAH Dinner; SEMINARIS Campus-Hotel Berlin	

## Day 3: 7. October 2022

8:30 am-  
10:10 am **Session 7**  
*Chairs: J. Hartung & N. Kemper*

### Animal Welfare (I)

8:30 am	Prof. Dr. Donald M. Broom (Keynote Lecture):	How consumer demand for sustainability may change animal and plant production.
9:10 am	Schüpbach-R. G.:	Smart Animal Health – The development of an animal-based and data-driven method for assessing health and welfare in farm animals
9:30 am	Spieß F.:	Innovation in broiler housing: Environmental Enrichment and a Farmer Assistance System
9:50 am	van Eerdenburg F.:	Quality of drinking water for cattle.
10:10 am	coffee break	

10:40 am –  
12:40 pm **Session 8**  
*Chairs: J. Paeshuyse & A. Aland*

### Animal Welfare (II)

10:40 am	Opalinski S.:	Expectations and concerns about the use of information and communication technology tools at poultry and pig farm - results of a survey of Polish producers
11: 00 am	Kovandžić M.:	Assessment of dairy donkey welfare from five farms in Northern Serbia
11:20 am	Sentamu D.:	Prevalence of gross lesions and handling practices in pigs and their association with pork quality, Kiambu, Kenya
11:40 am	Kongsted H.:	Danish welfare-label pig production systems: Usage of antibiotics and occurrence of disease
12:00 pm	Gumbert S.:	Anesthetic incidents, labor time and occupational safety of automated isoflurane anesthesia during piglet castration.
12:20 pm	Kemper N.:	Hygienic samples in a mobile house for laying hens
1:00 pm	Lunch	



2:00 pm - **Session 9**  
 3:40 pm *Chairs: U. Rösler & H. Schobesberger*

### Disinfection, Probiotics, ...

2:00 pm	Eglite S.:	Use of probiotics containing Lactobacteria to improve the microclimate and foot health of broilers
2:20 pm	Jagielski T.:	Canine protothecosis – a genotyping and meta-analytical study
2:40 pm	Mattauch M.:	Efficacy of disinfection of various structural timbers in livestock farming
3:00 pm	Rausch F.:	Development of a rapid molecular assay for validating the efficacy of air purifier in virus inactivation
3:20 pm	Abd El Wahed A.:	Raising awareness and hygiene practices for paratuberculosis in Africa

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### Closing

3:40 pm Closing ceremony (Prof. Dr. Jörg Hartung & Prof. Dr. Uwe Rösler)  
 3:55 pm coffee and farewell

### Sponsors:



## Abstracts Session 1

### Pig production: infectious diseases and disease preventing measures (I)

#### Successful deployment of a mobile suitcase lab for rapid detection of African Swine Fever Virus in Uganda

Arianna Ceruti<sup>1\*</sup>, Rea Maja Kobialka<sup>1</sup>, Judah Ssekitoleko<sup>2,3</sup>, Julius Boniface Okuni<sup>2</sup>, Sandra Blome<sup>4</sup>, Ahmed Abd El Wahed<sup>1</sup>, Uwe Truyen<sup>1</sup>

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#### Introduction

African swine fever (ASF) is a severe viral disease which affects swine populations worldwide. It is endemic in sub-Saharan Africa and outbreaks are regularly reported. No vaccine is currently available. Thus, surveillance and rapid identification of cases and subsequent control measures are the best strategies to avoid outbreaks in domestic pigs. Collected samples from suspected cases are usually sent to centralized laboratories for testing using real-time polymerase chain reaction (PCR). The results can be delivered within 24 hours to several days depending on the capacity of the accredited laboratory and the transfer times. An equally sensitive, but faster, simpler, and more user-friendly test approach offers significant advantages, especially in low resource settings. This study demonstrates the successful deployment of the mobile suitcase laboratory (suitcaselab) to detect suspected ASF cases in domestic pigs in Uganda.

#### Material & Methods

The suitcaselab operates an isothermal amplification assay based on the recombinase polymerase amplification (RPA), for the highly conserved B646L gene of ASFV. Two different extraction approaches were applied: a commercial silica-based extraction method and a rapid extraction protocol, consisting of three steps: dilution, lysis buffer and heating; Cross-reactivity was investigated using various genotypes of ASFV as well as other relevant swine pathogens' nucleic acid. The suitcaselab was deployed on-site of the suspected outbreak farm in the Kibaale district of Uganda.

#### Results

The RPA assay has an analytical sensitivity of 3.5 DNA copies per µl and detected all tested ASFV genotypes, but no amplification was observed for other viruses. The combination of rapid extraction and RPA showed 97% sensitivity and 100% specificity compared to real-time PCR by screening (n=73) under lab conditions. With silica-based extracted DNA, all assays sensitivity and specificity were 100%. In the Ugandan farm, both RPA approaches detected the samples identically: eleven afebrile animals

were assigned as negative, while six febrile, two afebrile and one pig without temperature reading as positive.

### **Discussion**

In summary, the suitcaselab was successfully implemented in a farm with a suspected ASF-outbreak in Uganda, detecting not only febrile pigs but also pre-symptomatic cases. This indicates a high sensitivity and underlines the potential of the assay as an accurate screening method. Moreover, the combination with the simple extraction protocol allows the use of the suitcaselab at point of need. The circulating ASF strain can be rapidly detected and thus control measures can be improved.

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## **Tenacity and disinfection of Modified vaccinia virus Ankara and African swine fever virus in forest soil**

Franziska Tanneberger <sup>1</sup>, Ahmed Abd El Wahed <sup>1</sup>, Melina Fischer <sup>2</sup>, Paul Deutschmann <sup>2</sup>, Hanna Roszyk <sup>2</sup>, Tessa Carrau <sup>2</sup>, Sandra Blome <sup>2</sup> and Uwe Truyen <sup>1</sup>

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### **Introduction**

African swine fever (ASF) is a highly contagious animal disease that exclusively affects pigs. In September 2020, the first ever case of African swine fever (ASF) was reported in Germany. The main reservoir for the virus in the European region is wild boar, therefore, an effective interruption of this infection chain is essential, in particular, the removal and safe disposal of infected carcasses and the direct disinfection of contaminated soil. For the disinfection, highly potent as well as environmentally compatible disinfectants must be used. Thus, in this study, various disinfectant solutions were selected for efficacy testing against the test virus recommended by the German Veterinary Society (DVG), Modified Vaccinia Ankara virus (MVAV), as well as ASF virus (ASFV).

### **Material & Methods**

The humus and upper mineral soil layers of soil types from six different sites in Saxony were collected and used to test the tenacity of ASFV and MVAV at different time points (0.5 up to 72 hours) using a germ carrier model. Investigations on the ability of different concentrations of citric and peracetic acid (approx. 0.1 to 2%) as well as slaked lime, quicklime and milk of lime (1 to 10%) to inactivate both viruses in the various soil types with increased protein load were carried out in BSL-2 for MVAV and in high containment biosafety for ASFV.

### **Results**

For all soil types, a reduction in infectivity of ASFV and MVAV of about 2-3 log<sub>10</sub> (TCID<sub>50</sub>) was observed after a contact time of two hours. For MVAV, a loss of 4 log<sub>10</sub> was further recorded after 72 hours. Overall, 0.1% peracetic acid (5 L/m<sup>2</sup>) was sufficient to inactivate both viruses. Application of 1% citric acid resulted in a 4 log<sub>10</sub> decrease in infectivity of MVAV, whereas only a 2 log<sub>10</sub> decrease was recorded for ASFV. In contrast, the inactivation of ASFV and MVAV by the three lime products slaked lime, quicklime and milk of lime was equally effective at concentration of 10%.

## **Discussion**

Both viruses showed high tenacity in different soil types. The application of MVAV as a substitute of ASVF for the efficacy screening of disinfection solutions showed many similarities. Peracetic acid reduced the infectivity of both viruses independent of the soil type. Slaked lime, quicklime and milk of lime achieved equally satisfactory results under the same experimental conditions.

## **Acknowledgement & Funding**

We acknowledge the financial support by the Saxon State Ministry for Social Affairs and Cohesion and the help with the characterization and definition of the soil types by the state—owned enterprise Sachsenforst. We thank Nadja Leinecker, Dana Rüster, Mario Reinhardt and Robert Küchler for the expert technical support. The authors thank Saxon State Ministry for Social Affairs and Cohesion for collaboration and support.

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## Evaluation of cleaning and disinfection practices in pig barns using boot swab sampling

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### Introduction

Cleaning and disinfection (C&D) of animal houses within and between production cycles are important hygienic measures to control the transmission of pathogens between flocks. To adequately assess the level of success of C&D procedures in the corresponding barns via bacteriological examination, sampling methods should have a high level of sensitivity and repeatability. In this matter, the use of so-called swab methods may represent an appropriate alternative to other established methods (e.g. agar contact plates) for evaluating the effectiveness of C&D. The aim of the study was to evaluate a boot swab sample method to assess the success of C & D in pig barns.

### Material/Methods

The study was performed in pig barns (farrowing, rearing and fattening) with slatted floors on two farms located in Northern Germany. Animal houses were cleaned and disinfected by a specialised contractor according to standard protocols. Success of C & D procedures was assessed using a boot swab method. Microbiologic examinations of swabs included the quantitative determination of total bacteria counts and the count of *Enterobacteriaceae*. Differences on bacterial loads were statistically analyzed between sample times, and production types.

### Results

It was possible to accurately determine the bacterial counts before water cleaning (BC), after water cleaning (AC), and after disinfection (AD). At BC, total bacteria counts and counts of *Enterobacteriaceae* differed moderately between production types. However, results at AC showed different outcomes of cleaning practices. Results varied between clear reductions of both indicators (around 2 log<sub>10</sub> cfu 250 mL<sup>-1</sup>) to a slight increase after a C&D bout in one farrowing barn. Similar tendencies were observed at AD in comparison to bacterial loads before disinfection (AC). Differences on outcomes at AC and AD seemed to be related to variations on C&D procedures between production cycles, and not to the production types.

## Conclusion/Discussion

The use of this boot swab sampling method allowed the assessment of the effectiveness of C&D procedures at different time points in barns of different pig production types. Through this method, it was possible to detect unexpected outcomes, which may indicate incorrect performance of C & D protocols. Such results may be advantageous, when the efficiency of C&D practices are evaluated by animal owner or specialized contractors. However, the suitability of boot swab sampling for the assessment of the hygiene measures on structured floors should be considered with caution. In most cases, only the microbial contamination on the upper surface may be recorded, and the bacterial loads in the slots in the floor may be disregarded.

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## Abstracts Session 2

### Pig production: infectious diseases and disease preventing measures (II)

#### **Salmonella excretion level in pig farms and impact of quaternary ammonium compounds based disinfectants on Escherichia coli antibiotic resistance**

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#### **Introduction**

The control of contamination of food by *Salmonella* and the reduction of antibiotic resistance are two major public health issues. *Salmonella* is the second foodborne pathogen responsible for gastroenteritis in the European Union (EFSA and ECDC, 2021). Ten to 20% of human salmonellosis in the EU are attributed to the consumption of pork. Meat pork ranks second among the meat associated with human salmonellosis outbreaks (Bonardi, 2017). There is little data on the level of *Salmonella* in the different steps of the pork production from farm to retail whereas these data are important to assess the risk along the food chain. Therefore, in order to estimate what can potentially happen in the food chain, it is important to acquire data on the level of *Salmonella* shedding in pigs at the farm level (Anses opinion 2016-SA-037). Moreover, *Salmonella* resistance to antimicrobials in food-producing animals can spread to humans and consequently infections by multi-antimicrobial resistant *Salmonella* may result in treatment failures. On the other hand, *Escherichia coli* (a well-distributed microorganism on farms) can also be a reservoir of resistance genes, which may be transferred between bacterial species, including organisms able of causing human and animal diseases.

One way to control *Salmonella* and other bacteria all along the food chain is to clean and disinfect the equipment and surfaces of empty premises. However, bacteria are able to adapt to repeated biocides exposure and may sometimes develop cross-resistance to biocide and antimicrobials (Soumet et al., 2012; FAO, 2018). A large amount of in vitro assays supports the role of some disinfectants in the selection or induction of antibiotic resistance in bacteria (Branen and Davidson 2004, Karatzas et al. 2008, Curiao et al. 2016) but field data are very limited (Anses opinion 2016-SA-238). Since disinfectants are regularly used on farms to break down transmission of infectious pathogens, it is relevant to examine these disinfectant and antimicrobial resistances at this first step in the food chain. This study therefore aimed at acquiring data on the level of *Salmonella* excretion by pigs on infected farms and assessing the impact of disinfectants used in these farms on the evolution of antibiotic resistance in *Escherichia coli* strains.



## Material & Methods

Four French farrow-to-finish pig farms deemed to be *Salmonella* positive were visited 3 times from May to November 2019. At each visit, individual faeces from 10 lactating sows located in the same farrowing room and individual faeces from 20 finishing pigs from the same batch were collected. The detection of *Salmonella* was carried out according to the standardized NF-U47-102 method and enumeration using a method based on the most probable number (MPN) (ISO/TS 6579-2-2012). Isolates were serotyped by agglutination according to the Kauffmann-White scheme and ISO/TR 6579-3:2014 method.

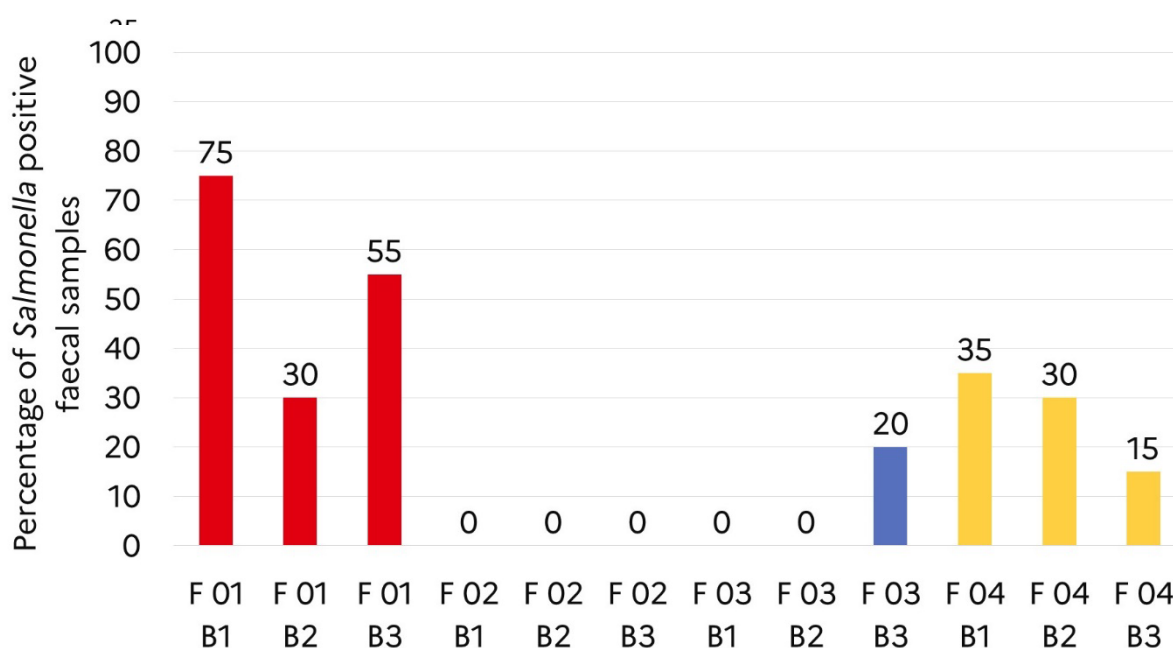
In addition, on three farms, the pen partitions and floor surfaces of 4 pens of the farrowing room (where faeces were collected) were swabbed with sterile gauze swabs (one swab = 1 m<sup>2</sup>) in presence of the pigs and then after cleaning and disinfection (C&D) procedures of the rooms (3 rooms/farm before CD procedures and the same 3 rooms/farm after CD procedures; n=36 samples before CD procedures and n=36 samples after CD procedures). All these farms used quaternary ammonium compounds (QAC) based disinfectants. Total *E. coli* and total *E. coli* resistant to antibiotic were respectively enumerated on Petrifilm™ Select *E. coli* from swab samples without and with a concentration of one of the four antibiotics considered: 16 µg/ml for Ampicillin (AMP), Tetracycline (TET) and for Florfenicol (FFN), and 0.125 µg/ml for Ciprofloxacin (CIP). Incubation of petrifilms was performed at 44°C ±1°C for 20h ± 2h. Total bacteria were also enumerated on non-selective media (Plate Count Agar) at 37°C for 20h ± 2h.

Moreover, Minimum Inhibitory Concentration (MIC) for antimicrobials and for biocides were obtained for pools of *E. coli*. The pools of *E. coli* were recovered from Petrifilms™ and were tested for their sensibility to 14 antimicrobials according the Sensititre™ method. They were also tested using a home-made protocol for their sensibility to 4 active substances of biocides among those most commonly used in swine farms in France (BTC50, CDDA, H<sub>2</sub>O<sub>2</sub> and NaClO).

## Results

No sow tested positive for *Salmonella*. For finishing pigs, 52 of the 240 samples of faeces (21.6%) were positive and 44 were enumerable, of which 63.5% were below 10 MPN/g. A low *Salmonella* excretion level was measured on most of the positive samples (figure 1). However, the excretion level was variable between farms and between pigs within a farm (figure 2). *Salmonella* strains were mainly the monophasic variant of *S. Typhimurium* (mVST) (83/97 strains). The second most frequent serotype was *S. Derby* (14/97 strains). Before C&D procedures, the total bacteria and total *E. coli* were detected in all samples. The mean counts of total bacteria and total *E. coli* were 10<sup>9.4</sup> UFC/m<sup>2</sup> and 10<sup>6.6</sup> UFC/m<sup>2</sup>, respectively. The mean reductions between before C&D procedures and after C&D procedures were around 10<sup>2.8</sup> and 10<sup>3.9</sup> UFC/m<sup>2</sup> for total bacteria and total *E. coli*, respectively. After C&D procedures, 10 of the 36 swab samples were found positive for *E. coli*. Levels of antibiotic resistance in *E. coli* strains from these 10 samples were globally lower than those before C&D procedures. The mean resistance in *E. coli* strains was around 50 % for Tetracycline and Ampicillin and about 8% for Ciprofloxacin and Florfenicol.

The results of MIC on pools of *E. coli* showed that the MIC for antimicrobials and for biocides do not change following repeated exposure to biocides.



**Figure 2 : Percentage of *Salmonella* positive faecal samples from finishing pigs, for each farm (F) and batch (B)**

## Conclusion & discussion

This study enabled to collect, for the first time in France, quantitative data on *Salmonella* excretion by pigs under field conditions. These data are required to build accurate risk assessment models and ultimately allow better control of the risk associated with *Salmonella* contamination of food. In our study, *Salmonella* was excreted by finishing pigs in all farms but was not detected in any of the sow faeces samples. Although *Salmonella* excretion by lactating sows was not measured in our study, a previous study showed that sows could shed *Salmonella* (Denis et al., 2013).

The level of *Salmonella* excretion by naturally infected pigs estimated in this study was low for a majority of the samples in contrast to the excretion levels observed in experimental infections (Cevallos-Almeida et al., 2019).

The main serotypes identified in this study were mVST and S. Derby, which are currently the most reported serotypes in Europe in the pig industry (Bonardi, 2017). These serotypes are among the top 10 serotypes found in human cases in France.

In our study, the disinfection protocols (with QAC based disinfectants) used on these farms in farrowing rooms were effective to reduce by 1,000 to 10,000-fold the level of total *E. coli* and antibiotic resistant *E. coli*. The antibiotic resistance profiles for *E. coli* strains were similar to those reported by the French National Reference Laboratory for Antimicrobial resistance and, EFSA and ECDC (2019). Even though experimental trials carried out under in vitro conditions (Soumet et al., 2016) indicate that repeated exposure to biocides may select to bacteria with antibiotic resistance, such a selection was not observed in this first field study involving a limited number of samples. Further field studies are needed to better assess these preliminary findings.

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## Disinfection of contaminated anesthetic masks for piglets

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### Abstract

Suckling piglets are castrated predominately under automated isoflurane anesthesia in Germany. The used anesthetic masks can represent potential vectors for pathogens between groups of piglets. A standard operation procedure (SOP) was used to clean and disinfect the masks after usage on 12 different breeding farms to analyze the effect of this hygiene measure. On each farm, samples were taken at three different days. Pooled swab samples were taken from the inner surface of masks after disinfection and before usage (A, n=36), after usage (B, n=36), after usage, cleaning and disinfection (C, n=36), and after storing the cleaned and disinfected masks (D, n=24). The total bacteria count in colony forming units (cfu) per pooled sample and the detection of *Escherichia coli* (*E. coli*) as fecal indicator bacteria were chosen to evaluate the effect of cleaning and disinfection. A statistical model was used to test significant differences between the total bacteria counts from different samplings. Furthermore, *E. coli* were enriched from samples and identified by MALDI-TOF to compare the frequencies of these indicators in treated and untreated masks. Medians of total counts were  $2.7 \times 10^2$  (A),  $5.9 \times 10^6$  (B),  $3.2 \times 10^2$  (C) and  $4.1 \times 10^3$  (D). The statistical model revealed significant differences ( $p < 0.0001$ ) of total bacteria counts between all combinations of samplings except between (A) and (C). Additionally, 89% of the masks carried *E. coli* after anesthesia (B). The *E. coli* occurrence in cleaned and disinfected masks varied from 6% (A, C) to 8% (D). Using a SOP to disinfect used anesthetic masks on pig breeding farms showed significant reductions of total bacteria counts ( $> 4$  log-reduction), and obviously reduced the occurrence of fecal indicator bacteria. The application of such a hygiene measure to prevent the transmission of harmful microorganisms within or between farms is highly recommended.

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## Vaccination of pigs against *Lawsonia intracellularis* can help to improve efficiency and with this have a positive effect on sustainability

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### Introduction

One of the most important enteric diseases in swine is caused by the bacterium *Lawsonia intracellularis* (*Li*). After entering the gut, the pathogen colonizes the ileal enterocytes. Infected animals react depending on their age at the time of infection as well as influenced by other factors, differently. So that symptoms can vary between subclinical ileitis with reduced growth performance and decreased feed conversion without markedly clinical illness up to diarrhoea and bloody diarrhoea that can peak in sudden death. Regardless of symptoms even subclinical *Li* infections result in economic losses. Furthermore, the reduction in animals' performance has an impact on feed efficiency with resulting ecological consequences. The question appears whether a prophylactic approach of intramuscular vaccination can not only reduce clinical occurrence of the disease but also improve performance and efficiency of pig meat production.

### Material & Methods

Groups of animals were vaccinated with an intramuscular *Li* vaccine on six farms under field conditions. Farms varied in size, clinical signs and strategy of treating *Li* infection and were all detected positive for *Li* before vaccine introduction. In the vaccinated (vac+) and comparison group (vac-) performance parameters (daily gain, total losses, feed conversion ratio, antibiotic use, economic result) were recorded and compared on farm level – detailed results are shown in a poster abstract.

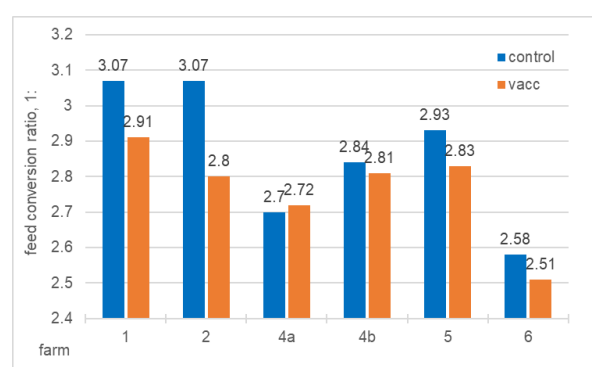
**Table 1: Overview of the farms: structure, clinic, previous measures, details of *Lawsonia intracellularis* intramuscular vaccination (individual / combined; age)**

intracutaneous intramuscular vaccination (individual / combined, age)						
farm	1	2	4a	4b	5	6
fattening places, appr.	3500	1400	11500	5000	1920	1980
clinical expression	acute-chronic	acute	chronic		sub acute	acute
L.i. measure, so far	AB	oral L.i. vaccination			AB	AB
vaccination L.i. im single or combined	mixed in PCV & M. hyo combined vacc.		as single vaccine			
age / weight at vaccination	4. week				appr. 33 kg	appr. 26 kg
stalling / evaluation	time-shifted				at same time	time-shifted

To evaluate the impact on efficiency and sustainability a model calculation on the base of an average standard fattening feed mixture with 13.0 MJ ME, 16.4 % crude protein and 3.4 g phosphorus and an increase of bodyweight during fattening of 94 kg was used. Due to principles of DLG leaflet 418 the N and P excretion was determined on farm base. The CO<sub>2</sub> balance was calculated using the license-free program FeedPrint NL, version 2020.00-18 June 2020 from the University of Wageningen.

## Results

Clinical *Li* situation could be improved in all farms after using the i.m. vaccine. This was seen in the documentation of *Li* related symptoms and in the use of antibiotics particularly oral group medications. The performance data in all farms increased, especially feed conversion ratio was improved. Having a level of feed conversion from 1:2.58 up to 1:3.07 before i.m. vaccination the decrease reached values up to 0.27.



**Figure 1: Feed conversion ratio on the analysed farms before (control) and after (vacc) introducing the intramuscular *Lawsonia* vaccine**

The positive effect of an efficient use of feed (mean improvement of 0.1) resulted in a calculated reduction of nutrient excretions (-230 g N and - 35.1 g P per pig) and an improved CO<sub>2</sub> balance by 5,321 g per pig.

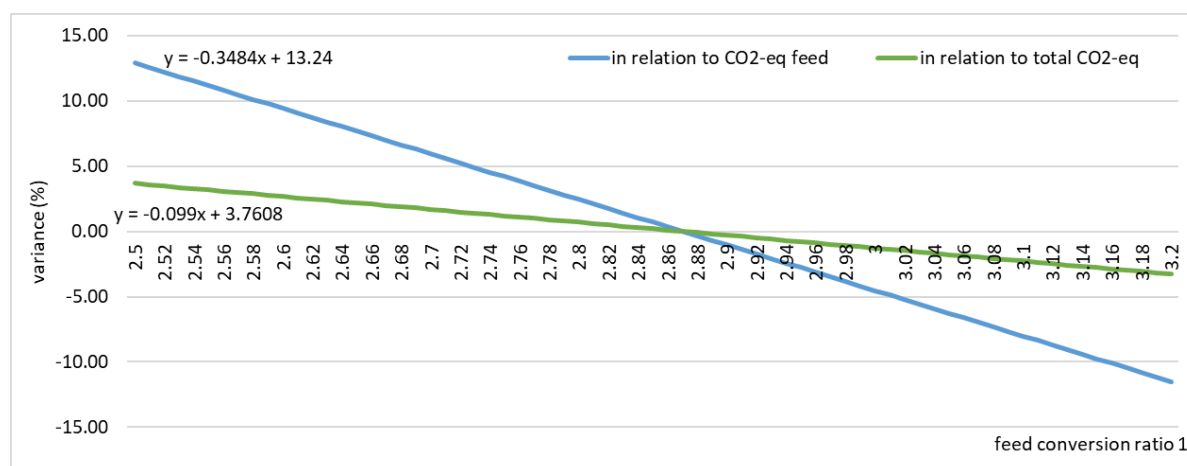
**Table 2: Influence of feed conversion on the evaluated farms and the resulting effects on N / P excretion and hectare (ha) requirement**

farm	number of animals	deviation FCR (%)	deviation N (%)	deviation P (%)	deviation ha-requirement N*	deviation ha-requirement P <sub>2</sub> O <sub>5</sub>
Surveyed from field observation			Calculated values based on standard fattening mixture			
1	2034	-5.21	-7.91	-9.40	-3.52	-3.08
2	419	-8.79	-13.34	-15.86	-1.22	-1.07
4a	11183	+0.74	+1.21	+1.50	+2.42	+2.11
4b	4142	-1.06	-1.67	-2.04	-1.34	-1.17
5	962	-3.41	-5.31	-6.40	-1.04	-0.91
6	1533	-2.71	-4.56	-5.77	-1.16	-1.01

\* incl. 20% stable and storage losses; at 170 kg N / ha and 85 kg P<sub>2</sub>O<sub>5</sub> / ha

## Discussion and Conclusion

The use of the intramuscular Lawsonia vaccine resulted not only in a major improvement of pig health in German fattening farms leading to a significant reduction in the use of antibiotics.



**Figure 2: Influence of feed conversion on the CO<sub>2</sub>-eq (g / kg live weight) compared to the CO<sub>2</sub>-eq feed and the total CO<sub>2</sub>-eq (g / kg live weight) with a feed conversion of 1: 2.87**

The clear positive effects on key production parameters furthermore resulted in a countable economic benefit for each farm. One highlighting parameter in this course is feed conversion. Due to climate changes and reduced availability of nutrients the efficiency of meat production is crucial. Even governmental restrictions are made to reach ecological goals during the next years and decades. The results shown are not a new approach but even more than a positive side effect: fattening of healthier pigs due to efficacious vaccination contributes to a more efficient and sustainable pork production.

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## Abstracts Session 3

### Infectious diseases in the One Health context (I)

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#### SARS-CoV-2 and One Health approach: filling the data gap

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#### Abstract

The zoonotic origin of SARS-CoV-2, the etiological agent of COVID-19, is not yet fully resolved. Emergence in humans most likely involved at least two independent zoonotic spillover (i.e. animal-to-human transmission) events from wild animals kept at the Huanan Seafood Market in Wuhan, China. Although natural infections in animals are reported in a wide range of species and the number of reported SARS-CoV-2 events in animals has been steadily increasing, large knowledge and data gaps remain regarding SARS-CoV-2 animal hosts.

To date, secondary animal-to-human transmissions have been observed from farmed mink, pet hamster, cat, and possibly free-ranging white-tailed deer. These secondary spillovers resulted in the mass culling of mink and occasionally led to subsequent human-to-human transmission. In this context, the World Health Organization (WHO), the World Organisation for Animal Health (WOAH), and the Centers for Disease Control and Prevention (CDC) are now advocating for a One Health approach that includes multi-sectoral, coordinated, and transparent monitoring of SARS-CoV-2 in animals. However, human and economic resources available to support veterinary surveillance are restricted in many countries. Therefore, prioritizing surveillance efforts appears as a cost-effective solution. One limiting factor to develop such strategies is the availability of comprehensive, machine-readable data on SARS-CoV-2 in animals.

We used two major health databases to extract unstructured data and generated a comprehensive, structured global dataset of SARS-CoV-2 events in animals. The dataset integrates relevant epidemiological and clinical data on each event and is readily usable for analytical purposes. We also created a user-friendly dashboard that intends to facilitate access to the data, favour animal health information sharing, and foster global understanding of the data among the scientific community, stakeholders, and the public.

Data on SARS-CoV-2 occurrence in animals is critical to adapting One Health monitoring strategy, preventing the formation of animal reservoirs, and tailoring future human and animal vaccination programs. The FAIRness and analytical flexibility of the data will support One Health research efforts on SARS-CoV-2 at the human-animal-environment interface.



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## Perception of infectious diseases with animal and humans hosts on the Polish internet

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### Introduction

Timeliness and precision for infectious human/animal disease prevalence and outbreaks detection (infovelliance) and its impact on the society (Infodemiology) from data published on the web is crucial for prevention against their spread (Eysenbach, 2009; Jarynowski, 2022). Infodemiology may be very useful in understanding social perception of infectious diseases by quantifying dynamics of interest (demand and supply of content) and discourse patterns (behavioral and affective). It plays a complementary role to standard tools such as surveys and allows for the analysis in real time. There is a complex interaction landscape between animal breeders and society as well as their interaction with nature (Jarynowski et al., 2019) and will be more and more challenging (van der Ploeg, 2020; Dobbins et al., 2021). Dutch farmers took to the roads in tractors in November 2020 to protest against culling minks due to detection of a mink-associated SARS-CoV-2 variant, the so-called Cluster 5 (Dyer, 2020). The COVID-19 pandemic has dominated the socio-economic picture of the last 2 years (Jarynowski, Wójta-Kempa, 2020) almost everywhere around the world and is also speeding up research in infodemiology. Disinformation and misinformation around infectious diseases (Eysenbach, 2008) spread up propagation during the COVID-19 crisis, the World Health Organization (WHO) has stated that the world is not “just fighting an epidemic; we’re fighting an infodemic” (Eysenbach, 2020). The advent of the Internet and social networks has changed the way the world communicates, digital communities have given rise to new socio-cultural scenarios compared to the past, thus some new tools are needed that mixes the theories of the communication sciences and psychological ones with IT techniques to collect and process data. However, non-English European languages are highly underrepresented and other zoonotic diseases are hardly covered by this research. At the same time, the African Swine Fever (ASF) and Avian Influenza (HPAI) are continuing to spread, covering more and more regions, affecting the economics of animal production.

Animal epizootics show a widely destructive impact on livestock production, but also cause tensions between various groups of interest. Hence, lack of effective mitigating actions to control ASF for instance (Iacolina et al., 2021) has been deeply triggering in European regions. There were attempts to map the landscape by surveys, interviews or media perception analysis with a special focus on conflicts and protests (Gorlach, 2000) in agriculture. Pre-existing social conflicting matters of animal production. Such a structural configuration facilitates mobilization of own groups of interest and ends up with conflict between animal breeders, far-right, agricultural parties, Christian organizations as well as opposing, government, pro-European opposition and ecological organizations (Jarynowski et al., 2019; Jarynowski, Płatek, 2022). COVID-19 crisis and pressures on farmers related to economic and

climate challenges cause more and more farmers to escape from animal production (Baker et al., 2022). Moreover, special operation in Ukraine during 2022 threatens supplies for fertilizers and some crops, causing increases in animal feed prices.

The model disease which affects animal hosts only is ASF, which is not only the biggest current threat to veterinary public health, but also could be important trigger of social protest of both animal breeders and wide life conservation associations from SE Asia, through Eastern Europe till Dominican Rep (Jarynowski et al., 2022). The disease has evolved to give rise to an even more intricate situation, reflecting a complex interaction between sanitary, economic, environmental and sociological factors in the European region (Cwynar et al., 2019) and other parts of the world. Although the virus does not cause disease in humans, the impact it has on the global economy (Stoffel et al., 2020), especially through trade and farming disruption especially in European countries (EFSA, 2020), is substantial, causing more than one billion EUR yearly losses (ter Beek, 2018; Jarynowski et al., 2020) in Europe. Moreover, ASF has societal implications as well (Chenais, 2020). It raises important ethical and animal rights issues and questions the justification of particular agricultural business models or mitigation strategies as wild boar depopulation (Bush et al., 2021).

Besides, ASF perception and attitudes could serve as a proxy indicator in the context of other societal phenomena that have the potential to polarize. In particular, the recent COVID-19 pandemic has a big impact on ASF perception and containment strategies (Standaert, 2020; Stoffel et al., 2020).

## Material & Methods

We have attempted to quantify differences in the perception of infectious diseases on the Polish Internet (demand measured by Google search queries/topics occurrence in digital traditional media and supply measured by tweeting behavior from a user perspective) dependent on host type:

- 1) mainly human host diseases such as COVID-19;
- 2) zoonotic diseases: such as COVID-19 in animal reservoirs (mainly livestock such as Minks) and Lyme disease;
- 3) animals only host diseases such as ASF and HPAI.

The internet is a digital footprint of social activities – secondary data source. Thus, data from Twitter, internet news media, Google Trends were collected and processed. To understand how people, perceive infectious diseases and what role the traditional and social Internet media plays in it, the acquisition of this information is necessary for effective disease control policies. In particular, we analyzed Polish Google Trends, Digital Traditional Media and Twitter from 01.01.2019 to 31.07.2022, using the internet as a digital footprint of social activities – secondary data source. Google trends measures interest of a particular search query (topic) in relative search volume (RSV) - normalized from 0 to 100. Frazeeo.pl service is using a broad range of sources (e.g. online news, articles, blogs) and is a large monitor of web-based Polish corpus. This tool was used to extract time series of interest in given topics in digital traditional media. Frazeeo.pl corpus is used for analysis of the most frequent Polish complementary words and the associations they trigger found in the Polish digital press (Satoła-Staśkowiak, 2021).

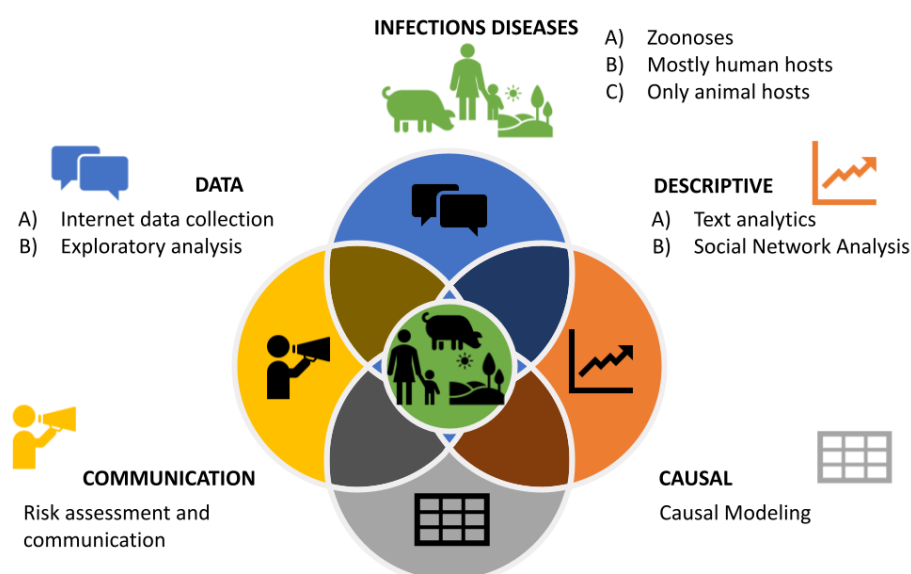


Fig. 1) Diagram of project implementation (with data and descriptive analysis already included in this preliminary research, while causal modeling and risk communication guidelines are future works)

For a model disease (ASF) we have performed additional text analysis in 2 case studies of protest triggered by the disease. We primarily applied Social Network Analysis (SNA) of the Internet media users connected via their tweets sharing activities. Nodes are Twitter accounts (after filtering), link is a retweet. Louvain algorithm for community detection was used and node color denotes the community it belongs to. Node size corresponds to frequency of occurrence in the data-set. Simple NLP (natural language processing) techniques such as sentiment analysis and keyword analysis were also applied.

A) Case Study farmers (9739 tweets between 1-31.10.2020 in Polish language with hashtag #StrajktRolników (farmers protest)). Farmers call for significant reduction of the wild boar population. In September 2020, the governing party (Law and Justice) proposed the so-called "Five for Animals" which attempted to extend animal rights, but at the same time it impacted a number of sectors of Polish agriculture (Jarynowski, Patek, 2022). Moreover, the feeling of abandonment (by the state) and powerlessness (for instance due to ongoing outbreaks of ASF and HPAI) led to protests distributed over 1000 locations across the country in October 2020.

B) Case study ecologists (5285 retweets with #ASF language also in Polish from 19.12.2018 to 18.01.2019 (animal right defenders protest)). Series of national-wide protests started January 2019 with protests against wild boar depopulation (Jarynowski et al., 2019). In turn, in several Polish cities animal right defenders organized demonstrations against hunters and government plans of wild boar depopulation as a way to slow down the spread of ASF.

## Results

Interest of internet users in selected infectious diseases usually peaks up during (re-) emergence of diseases in a new region and can be also driven by social-induced events such as street protests (e.g.

against ASF control strategies or due to lack of government support in infectious disease managements).

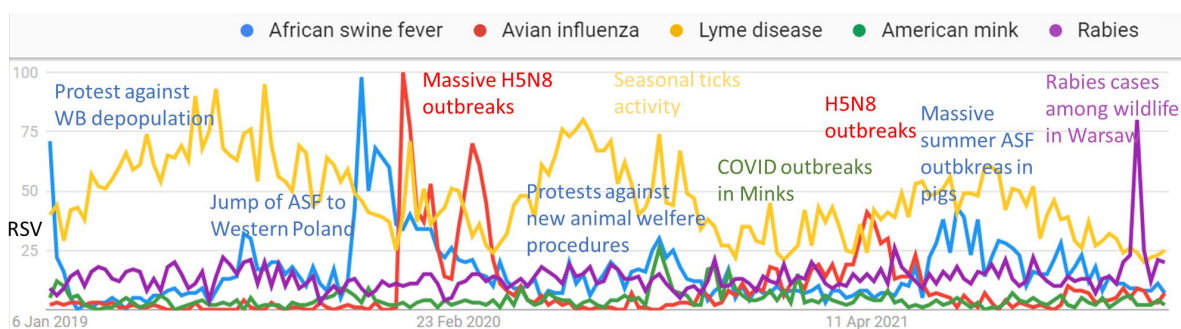


Fig. 2) Trajectories of relative search queries (weekly) of given keywords with related events (collected using <https://trends.google.com/>)

Seasonal patterns can be clearly detected. E.g. ASF gains the highest interest during summers, Lyme disease during springs, and HPAI during winters [Fig. 2, 4, 5].

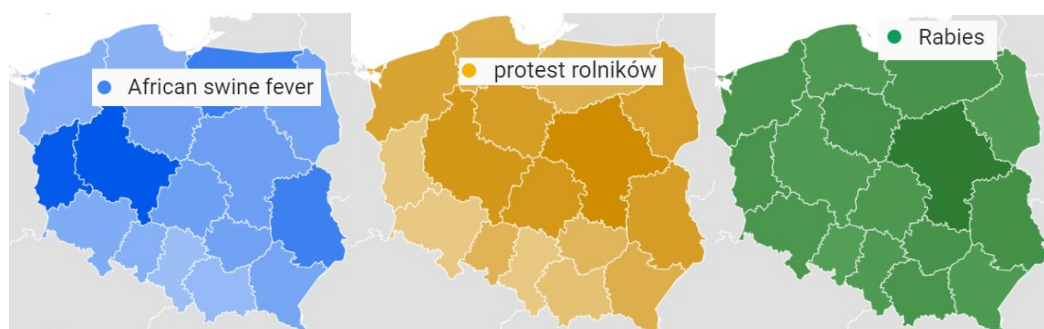


Fig. 3) Interest by sub-region on Google Trend (darker - more interest): ASF (left), Farmer Protests (center) and Rabies (right) (collected using <https://trends.google.com/>)

Although infoveillance does not represent the next frontier of infectious disease prevalence estimation as it was expected (Eysenbach, 2009; Jarynowski, Wójta-Kempa, 2020; Jarynowski, 2022), in the case of Rabies the interest overlaps with surveillance [Fig. 3] geographically (Masovia is the most affected region). Moreover, COVID-19 pandemic demonstrated that biological paradigms where the interactions are described in purely biological terms, have some limits (Jarynowski, Wójta-Kempa, Belik, 2020; Jarynowski, 2022). In particular, actions of human actors, participating in the disease transmission process, such as farmers or pet owners, are driven by social dynamics (Broz, et al., 2020).

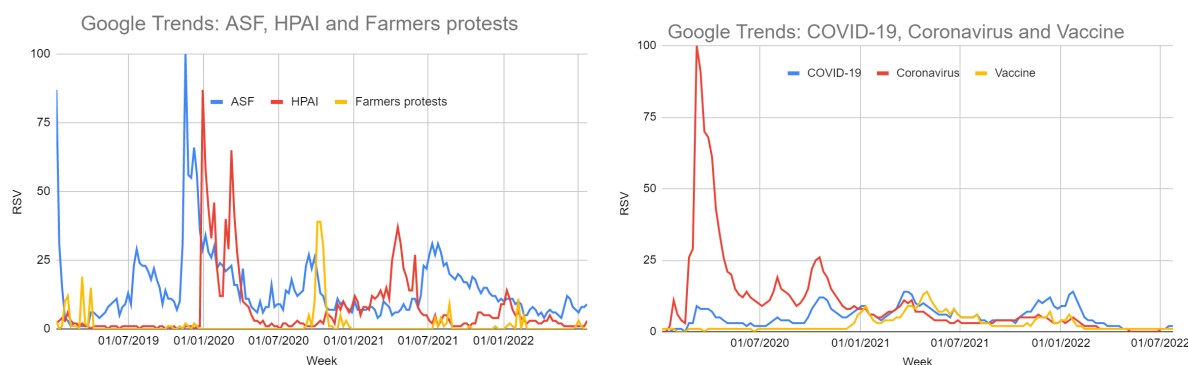


Fig. 4) Trajectories of relative search queries: RSV (weekly) of given keywords (collected using <https://trends.google.com/>) Please note that 100 is maximum separately for both left and right graphs thus maximum of interest in Coronavirus (right) is different (over 3 magnitude higher in fact) than for ASF (left)

Digital traditional media has been also already used in infection control (Arsevska, et al., 2016) for the early detection, assessment, and monitoring of current infectious disease threats to some extent.

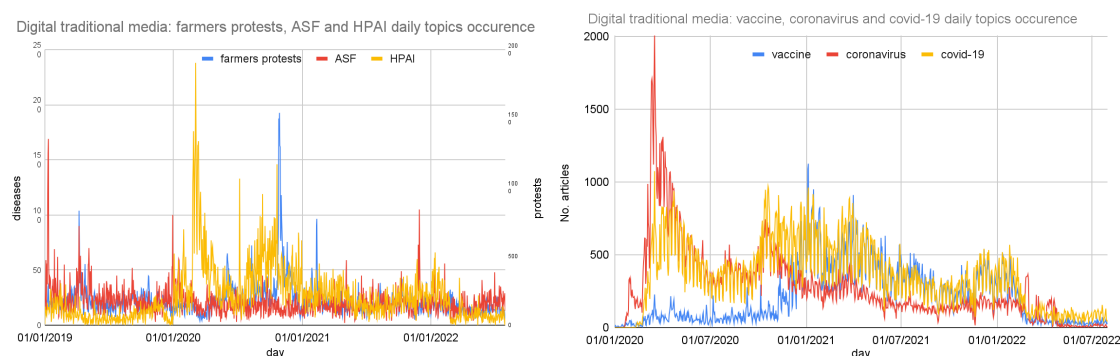


Fig. 5) Trajectories of number of news/articles (daily) with given keywords in Polish traditional digital news media (collected using <http://frazeeo.pl>)

It worth to mention that discrepancy between new media (Google Trends [Fig. 4]) and digital traditional media [Fig. 5] can be driven by multiple factors. For instance, the narration on traditional media does not need to correspond to people information needs. Thus, we can see multiple and constant information campaigns on ASF and HPAI in digital traditional media [Fig. 5], however it does not resonate on new media as human diseases.

Tab. 1) Summary interest across selected terms (disease) and medium (areas under curves Fig. 4, 5)

term(topic)/summary interest	weekly RSV Google Search (01.2020-07.2022)	daily No. article (01.2020-07.2022)
COVID-19	713	330390
Coronavirus	1368	255620
HPAI	2.2	29857
ASF	3.3	17893

There is over 621-fold higher interest during pandemic years in Coronavirus than HPAI topics in Google trends and only 8 folds higher among news articles [Tab. 1].

Summarizing our preliminary results of interest time series:

1) The highest interest is observed in mostly human host diseases among the general population with over million tweets and hundred million search queries monthly (for instance COVID-19 in peak time (Jarynowski, 2020)). The peak of interest was in the march 2020 [Fig. 5], when restrictions were announced (Jarynowski, Wójta-Kempa, Belik, 2020).



2) Interest in zoonotic diseases is usually concentrated in selected areas [Fig. 3]. E.g. an average interest with some peaks during local events as SARS-CoV-2 outbreaks among Minks or cases of Rabies in companion animals with around million search queries monthly.

3) On the other hand, the interest in non-zoonotic diseases (animal only host) is observed only in engaged groups, such as farmers, gamekeepers, ecologists, hunters, veterinarians, public administration with few thousand tweets (Jarynowski et al., 2019; Jarynowski, Płatek, 2022) and less than million search queries monthly. Interest is concentrated in selected areas (i.e. in Greater Poland where the hub of pigs' production is, there is 5 fold higher interest in ASF than in Lesser Poland on Google Trends [Fig. 3]). However, high presence of ASF and HPAI in digital traditional media [Fig. 5, Tab. 1] suggest that awareness campaigns have been performed, even its effectiveness can be questionable due to low reach in a general population.

In the second part of our exploratory study, we also process tweets to illustrate public attitudes towards ASF during protests.

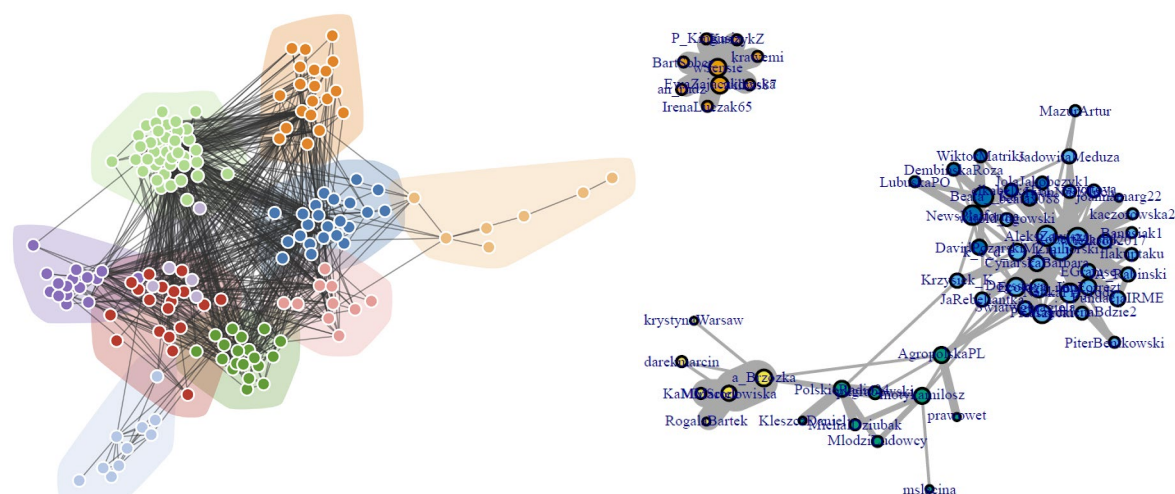


Fig. 6) Networks of Twitter users (vertices) and edges representing retweets with tagged language Polish in ASF context (collected using <https://developer.twitter.com/en/docs/twitter-api>) [Left] Retweeting network (only user with more than 10 retweets are shown) for hashtag farmers protest (case A) in October 2020 ([http://belik.userpage.fu-berlin.de/asf/asf\\_vis.html](http://belik.userpage.fu-berlin.de/asf/asf_vis.html)). Colorcode: pink – opponents of protests from the opposition; blue – opponents of protest associated with PiS – a governing party; orange – a mix of supporters and opponents of protest; light blue/gray – Protestant protesters; red – protesting farmers and agricultural organizations; light green – supporters of protests within right-wing (conservative) organizations; violet – supporters of protests within right-wing (liberal) organizations; dark green – supporters of protests associated with agricultural party PSL. [Right] Retweeting network for animal right defenders protest (case B) from 19.12.2018 to 18.01.2019. Colorcode: orange – right wing politicians, blue – mass media, yellow – animal rights activists, green – farmers representatives.

Let us compare two types of discussion triggered by ASF [Fig. 6], among A) farmers (Jarynowski, Płatek, 2022) and B) animal right defenders (Jarynowski et al., 2019).

A) Animal breeders' protest communication has a highly modular and hierarchical structure with farmers as a general component, but with clear boundaries between internet communities and opinion leaders. There was only a small presence of the general public (i.e. mainstream media) in the discourse. Level of optimism (text sentiment) among farmers protesters than animal right defender protesters was significantly higher than - probably due to a feeling of unity.

B) Ecological protests have been massively discussed by mainstream media and the general population constitute a general component linked closely to ecological activists' clusters. Level of verbal aggression was greater among animal right defenders' than farmers, which suggests that language repertoires differ.

## Discussion

There is totally different perception in human than animal diseases: almost 100-1000-fold [Tab. 1] higher interest (i.e. No. articles/No. search daily/ No. tweets). There are no technical reasons (according to distinguished veterinarians) why animal diseases (such as ASF, even causing massive financial implications), should catch the interest of the public and become newsworthy, because the main reason is making people sick or killing, so only if animal disease has a zoonotic potential it may gain popularity in a general population (Bush et al., 2021; Trotta et al., 2022). However, not only COVID-19, but animal infectious diseases (mainly ASF and HPAI) are currently the major problems in animal breeding immediately affecting the food production market. We observed social and economic impacts of animal infection, and these diseases amplify tensions contributing to the vulnerability of some groups as farmers. E.g. the average price of hog's body mass (0.75EUR/kg) in Poland (Jarynowski et al., 2021) in the middle of Autumn 2020 was the lowest in the history of exchange markets, far below production cost (see protest in October 2020 [Fig. 3, 4, 5]). The European Green Deal is projected to affect livestock production by decline between 5% and 15%, with the animal breeder's income being the hardest hit among all farmers in the perspective of the next 10 years (Barreiro Hurle et al., 2021). Thus, social tensions (ecologists and animal breeders protests) propagate from East to West of Europe with ASF. We see in June/July 2022 that animal breeders and supply chain cooperators took to the streets all over the Netherlands (due to planned 25% cattle and milking farms reduction). This brings us closer to answering a question: how attitudes towards infectious disease in One Health paradigm are influenced by psychosocial, sociolinguistic, cultural profiles and social interaction patterns? Thus, real time monitoring of internet media as suggested in this study should be a core element of preventing spread of human, wildlife and livestock infectious diseases.

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## Antimicrobial resistance profiling of *Acinetobacter baumannii* isolated from the environment least exposed to antibiotics

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### Introduction

Multidrug-resistant *Acinetobacter baumannii* (*A. baumannii*), an opportunistic nosocomial bacterium is of great global health concern due to the fast emergence of antimicrobial resistance and limited treatment options. *A. baumannii* ranked among the top six dangerous priority microorganisms and listed in the “Critical” category of the World Health Organization’s (WHO) first ever Pathogen Priority List (PPL) for urgent research prioritization towards identification of new antibiotics. Despite all these developments, the resistance development and molecular epidemiology are not completely elucidated.

### Material & Methods

A total of seven *A. baumannii* strains were isolated from agricultural lands of Neerukonda village, Andhra Pradesh, India, which is away from the cities, no cultivation activities were carried out for past five years and least exposed to the antibiotic’s residues. The bacteria were isolated using selective growth media Hichrome *Acinetobacter* agar base and species identification was carried out using biochemical methods. A total of 16 antibiotics belonging to eight antibiotic classes, Aminoglycosides (streptomycin, gentamycin), cephalosporins (ceftriaxone, cefepime), Tetracyclines (tetracyclines, doxycycline), penicillin (ampicillin, amoxicillin), fluoroquinolones (ciprofloxacin, levofloxacin), carbapenem (ertapenem, meropenem), lincosamides: (clindamycin, lincomycin) and glycopeptides (vancomycin, teicoplanin) were chosen to perform antibiotic disc diffusion experiment on the effectiveness against isolated *A. baumannii* infections and the results were compared with the CLSI standard.

### Results

The antimicrobial activity of *A. baumannii* displayed variations from the CLSI standard. All the isolates were 100% susceptibility to ciprofloxacin, levofloxacin and meropenem. And 100% resistance towards clindamycin, lincomycin, vancomycin, and teicoplanin. Intermediate resistances were observed towards aminoglycosides, cephalosporins, tetracyclines, penicillin and ertapenem of carbapenem. Lincosamides and glycopeptides have no effect on the control of environmental *A. baumannii* isolates.

### Discussion

*A. baumannii* isolated from environment display a unique pattern of antibiotics resistance. Further research is needed to confirm in terms of increased number of isolates and molecular especially the resistant gene expression level.

## Acknowledgement & Funding

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## Abstracts Session 4

### Infectious diseases and AMR in the One Health context (II)

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#### Determining the fitness of fluoroquinolone resistant *Campylobacter* using *in vivo* competition models in chickens

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#### Introduction

*Campylobacter* is a major cause of bacterial foodborne illness worldwide. Fluoroquinolones (FQs) are broad-spectrum antimicrobials used to treat unidentified gastrointestinal bacterial infections. Due to increasing levels of resistance in *Campylobacter* the WHO have listed fluoroquinolone resistant *Campylobacter* as a high priority pathogen in urgent need of new treatment. Our data suggest that in samples derived from broilers throughout the UK, fluoroquinolone (FQ) resistance (FQR) has increased from 7% in 1995 to 65% in 2020. Significant reduction in the use of FQ in broiler meat production in the past decade has not led to reduced levels of resistance which has led scientists to hypothesise that FQR might confer a fitness advantage in *Campylobacter*. This project aims to determine the fitness cost/benefit of FQR in *C. jejuni* derived from broilers. Four FQ susceptible *C. jejuni* strains, isolated from broiler caeca, were selected from the APHA archives, with two isolates from clonal complexes with a high association with FQR and FQ susceptibility (FQS). The strains were used in colonisation studies using specific pathogen-free White Leghorn chickens (SPFCs) firstly to generate isogenic FQ resistant mutants, then to evaluate the relative fitness of the resistant and susceptible isogenic pairs in *in vivo* competition trials.

#### Material & Methods

FQR *C. jejuni* mutants were generated by inoculating SPFCs with one of four FQS isolates and then treating them, via the drinking water, with enrofloxacin (a veterinary FQ) with final caeca. Faecal samples were taken pre-treatment (for FQS isolates) and final caeca content samples taken post-mortem (for FQR isolates). Competition studies were then done using FQR/FQS isogenic pairs of MLST types CC354 and CC443. For each MLST type, three groups of SPFCs (n=15), housed in separate isolators, were dosed by oral gavage with approx. 10<sup>9</sup> CFU of either the chicken-adapted FQS variant, the FQR variant or a combined dose. Faecal samples, collected throughout the study, and caecal contents obtained post-mortem on day 35 were plated onto mCCDA plates, and resultant colonies replica plated onto MH agar supplemented with 2mg/l ciprofloxacin (a human medicinal FQ) to determine the proportion of FQR isolates.

## Results

FQ resistant mutants were successfully generated from each strain following enrofloxacin treatment. The first competition trial using CC354 FQR/FQS variants, showed significant dominance of the FQR mutant in birds dosed with co-inoculum. In contrast, for the second competition trial, with CC443, results showed no dominance by the FQR mutant over FQS variant, both FQR and FQS isolates persisted throughout the trial in mono-inoculated SPFCs.

## Discussion

The dominance of a FQR mutant over its FQS parental strain as seen for CC354 may explain why FQR is spreading despite a reduction in selective pressure. Further work is underway to understand if FQR *Campylobacter* confers a fitness advantage in other MLST profiles.

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## Broiler insects' role in antimicrobial resistant *Campylobacter*, *Salmonella* and *Staphylococcus aureus* carry over between broiler flocks

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### Introduction

Intensive poultry production raises the risk of emergence and contamination with potential antimicrobial resistant foodborne pathogens that represent a direct hazard in global public health. Therefore, there is need to understand the way of persistence and dissemination of antimicrobial resistant pathogens from broiler flocks. The current study performed to determine the role of housefly (*Musca domestica*) and darkling beetles (*Alphitobius diaperinus*) in the transmission of antimicrobial resistant *Campylobacter*, *Salmonella* and *Staphylococcus* (*S.*) *aureus* between production cycle and efficiency of cleaning and disinfection to mitigate the spread of those pathogen between animals, humans and the environment.

### Material and Methods

Boot swabs, houseflies and darkling beetles were collected from ten broiler flocks at Dakahlia province, Egypt for three times: start of empty period, after cleaning and disinfection, and at the end of empty period. *Salmonella*, *Campylobacter*, and *S. aureus* were isolated from each sample according to ISO 6579-1:2017, ISO 10272-1:2017 and ISO 6888-1:2018, respectively. The recovered isolates were identified with matrix-assisted laser desorption ionization time-of-flight mass spectrometry. (MALDI-TOF MS) and the antimicrobial resistance phenotypes were determined using broth microdilution test.

### Results

*Salmonella* and *S. aureus* are widely distributed in the flies and both are detectable to a lesser degree in beetles. Occurrence is associated with positive flocks. However, *Campylobacter* was isolated from flies and beetles in affected broiler flocks only. With regards to the effects of cleaning and disinfection, insects could transmit *Salmonella*, and *S. aureus* even after cleaning and disinfection, meanwhile, this was not the case for *Campylobacter*. By looking to antibiotic resistance, 66.7%, 40.0% and 35.0% of *Campylobacter*, *Salmonella*, and *S. aureus* strains showed resistance to at least three different classes of antimicrobials.

### Conclusion

This results confirm that broiler farm insects constitute a potential reservoir of antibiotic resistance *Campylobacter*, *Salmonella*, and *S. aureus* strains and support carry-over from one flock to the next especially at low biosecurity level, at least, in the breaching of biosecurity around *Campylobacter*-negative flocks.

## Antimicrobial and biocide resistance of zoonotic pathogens from dogs and cats

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### Introduction

Antimicrobial resistance (AMR) of bacteria is a major public health issue. In addition to AMR, resistance to biocides is emerging. The aim of the present study was to evaluate Gram-positive and Gram-negative pathogens from infections of cats and dogs for their susceptibility, not only to antimicrobial agents commonly used for the treatment of infections caused by the respective bacteria, but also to four different widely used biocides.

### Material & Methods

A total of 329 isolates from infections of dogs and cats, including 62 *Staphylococcus aureus*, 52 *Staphylococcus pseudintermedius*, 49 *Enterococcus faecalis*, 37 *Enterococcus faecium*, 59 *Escherichia coli*, 56 *Pseudomonas aeruginosa*, and 14 *Acinetobacter baumannii*, were investigated for their susceptibility to 27 (Gram-positive bacteria) or 20 (Gram-negative bacteria) antimicrobial agents/combinations by broth microdilution according to the recommendations of the Clinical and Laboratory Standards Institute [1]. Moreover, all isolates were analysed for their susceptibility to the biocides benzalkonium chloride, chlorhexidine, polyhexanide, and octenidine by a recently published broth microdilution biocide susceptibility testing method [2,3].

### Results

Among *S. aureus* and *S. pseudintermedius*, penicillin resistance (83.3%) was the dominant resistance property. About one quarter of the isolates (25.4%) proved to be methicillin-resistant. Macrolide resistance was the second most prevalent resistance property (27.2%) and fluoroquinolone resistance was detected in 21.1% of all isolates tested, whereas tetracycline resistance occurred in 19.3% of the isolates. Resistance to last resort antimicrobial agents in human medicine was seen only in single isolates, if at all. While the *E. faecalis* isolates did not show expanded resistances, considerable numbers of the *E. faecium* isolates were resistant to penicillins, macrolides, tetracyclines, and fluoroquinolones. Even a single vancomycin-resistant isolate, that carried the *vanA* gene cluster, was detected. Expanded multiresistance phenotypes were also detected among the *E. coli* isolates, including a single carbapenem-resistant isolate which carried the gene *bla*<sub>OXA-48</sub>. In addition, multiresistant *A. baumannii* isolates were identified during this study [4,5].



The minimal inhibitory concentrations of the biocides showed unimodal distributions, but differed with respect to the biocide and the bacterial species investigated. Although there were no indications of a development of biocide resistance, due to solubility limits some *P. aeruginosa* isolates exhibited benzalkonium chloride MICs higher than the highest test concentration [4,5].

## Discussion

As large numbers of (multi)resistant isolates were seen in most bacterial species investigated, antimicrobial susceptibility testing is highly recommended before the start of an antimicrobial chemotherapy. Moreover, no hints towards the development of biocide resistance were detected among the bacteria investigated.

## Acknowledgement & Funding

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## On farm culture system as a tool to reduce the antimicrobial consumption at selective dry cow therapy in Czech farms

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### Introduction

Most dairy farms in Czech Republic utilized blanket dry cow therapy as a part of the dry off management to treat existing quarter infections and to prevent the new infections during the dry period. Since the new regulation of EU (Regulation EU 2019/6) prohibited the preventive use of antimicrobials in food producing animals in January 2022, selective dry cow therapy (SDCT) became mandatory.

### Material & Methods

The aim of the study was to evaluate the impact of SDCT on the antimicrobial consumption and on the herd SCC, % of high SCC cows, % of high SCC fresh cows and % of chronic infections in consequence with the compliance of the proposed methodology during the 12 month trial.

Two selected farms with different type of breeding environment were included into the trial. Both of them have implemented mastitis management based on DHI data analysis and culture diagnostics using on farm culture system (OFC) MicroMast™ at the level of clinical and subclinical mastitis before the start of the study. Blanket dry cow therapy was used.

Farm A breed 480 Red Holstein cows with milk yield 11.500 l/lactation in straw bedding barns. Farm B breed 110 Czech fleckfieh cows with milk production 7.100 l/lactation in deep bedding barn. Cows were milked in the milking parlor on both farms twice a day. Standard milking protocol was used.

Cow selection strategy was based both on the algorithms and culture based decision (Vaquez et al., 2018). Algorithm method determines a low-risk cows (cows dried-off using internal sealants without antibiotics) based on these parametres:

- Monthly SCC less than 100,000 cells/ml for three last test of the current lactation.
- No clinical mastitis in the 30 days prior to dry-off.

All other cows were cultured using MicroMast™ triplate set (Prasek et al. 2010) to identified high-risk cows. The growth of the major mastitis pathogens, the intensity of the infection and the history of previous infections were included into the antibiotic therapy decision.

### Results

On Farm A were dried-off in total 296 cows during the trial. As a low-risk cows were identified 153 of them (51.7% reduction of antibiotics use). Antibiotic dry cow therapy administrated into all quarters

were used in 95 (32.1%) of cows and in 48 (16.2%) cows were used antibiotics in selected quarters. On the other hand herd SCC elevated from 189,000 cells/ml at the beginning of the trial to 244,000 cells/ml at the end of the study. Percentage of high SCC cows increase during 12 months from 11% to 18%. Percentage of high SCC fresh cows elevated as well from 8% to 17% and the percentage of chronic cows elevated from 7% to 9%.

On Farm B were dried-off 56 cows. As a low- risk cows were identified 55 (98.2% reduction of antibiotic use). Antibiotic dry cow therapy was used in 1 cow (1.8%) during one year study. Herd SCC elevated from 101,000 cells/ml to 129,000 cells/ml at the end of the study. Percentage of high SCC cows increase from 12% to 15%. On the other hand percentage of high SCC fresh cows even decreased from 13% to 10% and the percentage of chronic cows elevated from 8% to 10%.

## Conclusion/Discussion

Our results show the reduction of antibiotics use and we can confirm the importance of the method of SDCT because even cows with elevated SCC but without the evidence of the infection were not treated by antibiotics. The combination of methods of cow selection can increase the reduction of the antibiotics consumption. The elevation of SCC parameters were not significant and our results are consistent with Rowe et al. (2021) and other authors. Moreover we prove even decrease of high SCC fresh cows at farm B.

We can conclude that the selective dry cow therapy has a potential to reduce the antimicrobial consumption on farms. Our results showed reduction for 51.7% and 98.2% respectively. The successful implementation of the SDCT program is based on proper following of the protocol and proper employee training especially hygiene and technique of antimicrobials and sealants intramammary infusion.

## Acknowledgement & Funding

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## The Devil and the Beelzebub - Controlling MRSA in cattle in the dry period

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### Introduction

It has been repeatedly shown that Methicillin-resistant *S. aureus* can be found in bulk tank milk of dairy herds and can be associated with mastitis [1]. Mastitis caused by MRSA in dairy herds may lead to substantial financial losses and is associated with occupational health risks for farm staff and potential risks to food safety. In the mammary gland, *S. aureus* leads to long lasting infections and spontaneous self cure is rare. In the control of *S. aureus*, treatment during the dry period traditionally plays an important role as cure rates tend to be higher than during lactation. Besides curing the infection, successful dry cow treatment may also curb transmission of MRSA from dams to their offspring through contaminated colostrum [2]. Unfortunately, most dry cow products predominantly rely on beta-lactam antimicrobials that are not efficient against MRSA. It was therefore the objective of this study to control MRSA around drying off using non-beta-lactam drugs.

### Material & Methods

MRSA positive cows from a dairy farm were identified by analysis of quarter milk samples 14 days prior to dry off. Cows were assigned to two groups. Both groups received dry cow treatment with a licensed dry cow product at dry off containing a penicillin and an aminoglycoside. The test group was additionally treated intramammarily over 8 days with pirlimycin, a licensed lincosamide, prior to the dry off treatment. Success of treatment was defined as an increase in the proportion of previously positive quarters being negative after calving compared to the control group. Moreover, nasal and udder cleft samples were taken.

### Results

The use of pirlimycin increased the elimination of MRSA from previously MRSA positive udder quarters significantly (OR 21, 95 %CI 1.2-368). However, MRSA were still present in noses and udder clefts of cows with MRSA negative quarter milk samples. Most MRSA isolates were associated to *spa* types to11 and to34 and harbored similar antimicrobial resistance genes. Resistance to various antibiotic substances was detected in MRSA. However, resistance to pirlimycin was rare.

### Discussion

The use of pirlimycin prior to the dry-period is promising to eliminate MRSA from cows' udder provided that the prevailing MRSA strains are susceptible to pirlimycin. However, MRSA persist in other body niches. Re-infection might take place and the treatment will not eliminate the bacteria from

the herd despite successful elimination from individual udder quarters. Therefore, milking time hygiene remains the most important control measure in positive dairy herds. Antimicrobial susceptibility of MRSA needs to be closely monitored, since resistance to novel antibiotic treatments might spread across the dairy herd.

### Acknowledgement & Funding

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## Abstracts Session 5

### Infectious diseases and AMR in the One Health context (III)

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#### Genetic analysis of specific antibiotic resistances in *E. coli* of veal calves up to eight months

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#### Introduction

*Escherichia coli* (*E. coli*) is used as an indicator bacterium for surveillance to monitor the colonization of livestock animals, including veal calves, with antibiotic resistant bacteria. In Germany, half of the tested commensal *E. coli* isolates derived from the cecum of veal calves are resistant to at least one antimicrobial class<sup>1</sup>. However, the currently applied monitoring programs leave aside the variety of different production systems for veal calves in Germany and are mainly based on unselective laboratory methods to detect antibiotic resistances. We investigated fecal samples of veal calves on resistances to highest priority critically important antimicrobials using selective laboratory methods, considering different production systems in Germany. Extended-spectrum beta-lactamases (ESBL) and plasmid mediated AmpC beta-lactamases (pAmpC), resistances to colistin and fluoroquinolones in *E. coli* were phenotypically and genotypically investigated.

#### Material & Methods

Pooled feces samples of veal calves from 106 farms, representing different production systems, were screened for phenotypic resistant *E. coli* on MacConkey agar supplemented with cefotaxime (1µg/ml), enrofloxacin (2µg/ml) or colistin sulfate (2µg/ml). Distinct colony morphologies per sample and supplemented antibiotic were genotyped using pcr and sanger sequencing after species identification with MALDI-TOF/TOF. Analyses included mobile ESBL- and pAmpC- genes (*bla*<sub>CTX</sub>, *bla*<sub>SHV</sub>, *bla*<sub>TEM</sub>, *bla*<sub>CMY</sub>), mobile colistin resistances (*mcr-1* to *-5*) and chromosomal mutations in *gyrA* and *parC* (QRDR) as well as mobile fluoroquinolone resistances (PMQR: *qnr*, *qepA*, *oqxA/B*, *aac(6')-Ib-cr*).

#### Results

In total 78% of the farms were positive for ESBL-/pAmpC- producers, with the highest detection rate of *bla*<sub>CTX-M-1</sub> followed by *bla*<sub>CTX-M-15</sub> and *bla*<sub>CTX-M-14</sub>. 10% carried the *mcr-1* resistance gene and 85% (QRDR) as well as 17% (PMQR) carried resistances to fluoroquinolones with the most frequent mutations of S83L, D87N/Y/H and S80I and detection of *qnrS* and *aac(6')-Ib-cr* genes on the farms. An impact of the different production systems on the prevalence of the investigated resistance mechanisms was shown, supporting a further differentiation of 'veal calves' in surveillance programs based on the production system to improve monitoring of antibiotic resistances.

## References

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## Teat disinfection - (Co-)selection of udder pathogens and antibiotic-resistant bacteria? A longitudinal experiment

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### Introduction

Teat disinfection is a common method of hygiene management on dairy farms, which reduces bacterial colonization at the teat orifice, preventing new intramammary infections (Kumar et al. 2012). Resistance to applied concentrations of teat disinfectants has not yet been demonstrated *in vitro*. However, it is under debate whether application of teat disinfectants results in microbial selection, since bacteria differ in their natural susceptibility (Khan et al. 2017; Schwaiger et al. 2014). Such differences between bacterial species or strains could lead to changes in the bovine udder microbiota (Cassir et al. 2015). In addition, lower susceptibility to disinfectants may also be associated with lower susceptibility to antibiotics. Cross-resistance between chlorhexidine gluconate, used for teat disinfection, and several antibiotics has been reported (Köljalg et al. 2002). Considering this issue, the influence of different teat disinfectants on the susceptibility of microbiota and mastitis pathogens was investigated.

### Material & Methods

A 6-day intervention (14 days p.p.) was applied in a split-udder design, using two different postmilking teat treatments (lactic acid (LA) and chlorhexidine (CH) teat disinfectant) and an undipped control group. Quarter milk samples were collected before and after intervention and bacteria were cultured and identified by MALDI-ToF. The minimum inhibitory concentration (MIC) to the applied teat disinfectants was determined by macrodilution. MIC-values against antibiotics related to mastitis therapy were assessed using microdilution. Bacterial composition and changes in MIC-values before and after intervention were compared.

### Results

The proportion of *Corynebacterium* spp. increased significantly when LA was applied. After intervention with CH and LA, the percentage of coagulase-negative staphylococci (CoNS) decreased. The MIC-values of the bacterial community against LA were significantly higher in the LA-group after the intervention. A significant increase was also shown for CH-MIC-values upon LA-treatment. The antibiotic MIC-values of *S. aureus* and CoNS-isolates showed no significant differences before and after the intervention. However, there was a trend for CoNS to increased MIC-values towards penicillin after intervention with LA.

## Discussion

Changes in the bacterial community occurred as a result of the LA-intervention, while CH did not change the teat microbiome. This fact indicates that different bacteria are, *in vivo*, more equally reduced by CH, while LA selects corynebacteria to the disadvantage of others. MIC-values of disinfectants increased following LA-intervention. However, both applied teat disinfectants did not seem to co-select antibiotic-resistant bacteria. Further isolates (*Aerococcus viridans*, *Corynebacterium* spp., and *S. uberis*) will be tested for their antimicrobial susceptibility in order to substantiate the latter statement.

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## MICROBIAL CONTAMINATION AT SLAUGHTER AND RETAIL POINTS OF THE PORK VALUE CHAIN IN UGANDA.

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### Introduction

Unsafe food and foodborne illnesses remain a global public health concern (Havelaar et al., 2010). According to the World Health Organization (WHO), one in every ten people fall sick every year after consuming contaminated food (WHO, 2020). Specifically, bacterial foodborne pathogens are a global health issue with a huge health burden comparable to diseases like malaria, HIV / AIDs and tuberculosis (Havelaar et al., 2015). Animal source foods which include beef, pork and dairy products have been implicated in the transmission of foodborne pathogens (Grace, 2015; Havelaar et al., 2015). Pigs and pork have specifically been implicated in transmission of non-typhoidal *Salmonella* (NTS) through pork to humans (Botteldoorn et al., 2003; Hoelzer et al., 2011).

Uganda leads in per-capita consumption of pork in the region currently standing at 3.4kg per person per year (Food and Agriculture Organization, 2011). This consumption is expected to increase by up to 50% and the pig population is also expected to double, from the current 4.7 million pigs by 2050 to meet the increasing demand for pork (Food and Agriculture Organization, 2011; MAAIF, 2007). This means increased risks of zoonotic disease infections associated with increased production of pigs and consumption of pork that include bacterial food borne illnesses, specifically NTS.

This study therefore aims to establish levels of bacterial contamination at the point of slaughter and retail of the pork value chain and the factors that influence those levels. It will also establish the genetic relatedness of NTS from pigs, humans and the slaughter and retail environments establishing whether pigs in Uganda are a potential source of zoonotic NTS to humans.

### Materials & Methods

A cross-sectional study design was conducted. Pigs presented for slaughter were sampled, and the carcasses tracked to the point of retail and sampled too. Samples from slaughter and retail environments which include surfaces and water used for dressing were also collected. Laboratory microbial analyses were carried out to determine presence and levels of bacteria i.e., NTS and total coliforms. Meat handlers along the chain were sampled and investigated for presence of NTS. Demographic data and factors that influence infection with NTS in humans was collected using structured questionnaires. An observational checklist was used to collect information on factors at slaughter and retail points that influence occurrence of contamination. A total of 1100 samples (50 %

of the target sample size) have so far been cultured for isolation of NTS and evaluated for total coliforms. These includes faecal and mesenteric lymph nodes from pigs, water used for dressing at the point of slaughter, meat handlers hand swabs and stool samples from the meat handlers as shown in Table 1.

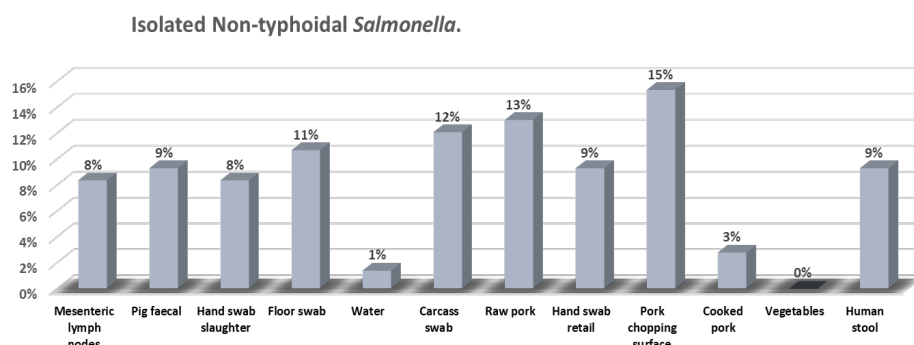
## Results

NTS has been isolated from 215 different sample types across the chain as shown in Fig 1 below. The overall *Salmonella* prevalence from all samples is 19.55%. Regarding pigs, the results show 22.22 % and 24.69 % of the pigs come in infected with *Salmonella* based on NTS isolated from mesenteric lymph nodes and faecal contents respectively. Of the carcasses swabbed before dispatch from the point of slaughter, 32.10% have *Salmonella*. The slaughter and retail environments also have presence of *Salmonella*. For instance, 33.82% of the floor swabs have *Salmonella* at the point of slaughter and 40.74% of the pork chopping surfaces at retail have *Salmonella*. Water used for dressing at slaughter also has *Salmonella* in 4.41% of the total water samples collected. The meat handlers' hands both at slaughter and retail have 22.50% and 24.69% *Salmonella* respectively. No raw vegetables samples collected has *Salmonella*. Regarding the slaughterhouse workers 6.43% of the workers were infected with NTS from their stool culture.

**Table 1\_ Non-typhoidal Salmonella isolated from each sample type.**

No	Sample type	Total collected	Non-typhoidal <i>Salmonella</i> isolates	Percentage positive
1	Mesenteric lymph nodes	81	18	22.22%
2	Faecal samples	81	20	24.69%
3	Hand swab slaughter	80	18	22.50%
4	Floor swab	68	23	33.82%
5	Water	68	3	4.41%
6	Carcass swab	81	26	32.10%
7	Raw pork	81	28	34.57%
8	Hand swab retail	81	20	24.69%
9	Pork chopping surface @retail	81	33	40.74%
10	Cooked pork	81	6	7.41%
11	Kachumbari (Mixed raw vegetables)	6	0	0.00%
12	Human stool	311	20	6.43%
	<b>TOTAL</b>	<b>1100</b>	<b>215</b>	<b>19.55%</b>

**Fig 1\_ Percentage of NTS from each sample type**



## Discussion

The preliminary findings reveal significant levels of coliforms and presence of NTS along the value chain. Isolation of NTS from up to 24% of pig lymph nodes and colon faecal content reveals on-farm NTS infection, which is the primary source of introduction of NTS into the value chain (Hoelzer et al., 2011). Significant NTS presence as you move from slaughter to retail can be attributed to poor hygiene and pork handling practices which are being assessed in this study. Pork chopping surfaces is the sample type with the most NTS infection, this could be attributed to the fact that most of them are wooden stumps which are very difficult to clean and are rarely cleaned. The ultimate findings of this research will highlight levels of contamination and presence of NTS at different points, providing useful data for control and mitigation of cross-contamination in the pork value chain in Uganda thus improving pork safety and occupational health. It will also highlight whether pigs in Uganda are a reservoir for zoonotic NTS and a potential source of NTS infection to humans.

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## Experimental exposure of *Salmonella enterica* from farm animals to a glyphosate-containing herbicide: effects on tolerance response, cross-tolerance, cross-resistance and fitness

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### Introduction

Glyphosate, the world's most used herbicide, may be ingested by farm animals via feed. Recurrent contact with the gut bacteria can potentially lead to an increased tolerance and/or resistance against the herbicide itself but also to other biocides and antibiotics. It has already been shown that glyphosate is linked to changes in susceptibility against clinically important antibiotics used in human medicine and to activation of the stress response in pathogenic bacteria. In this study we investigated the potential of the glyphosate resistance induction in pathogenic bacteria from farm animals, activation of tolerance and stress response and cross-tolerance and cross-resistance against antibiotics.

### Material & Methods

Ten clinical isolates of *Salmonella enterica* from farm animals were exposed to increasing concentrations of a glyphosate-based herbicide (GBH) in an experimental evolution approach. Subsequently, whole genome sequencing of isolates showing resistance to high concentrations of GBH was performed and genetic adaptations analyzed. Global quantitative proteomics analysis of GBH-resistant strains and their ancestors were compared, and tolerance experiments as well as susceptibility testing via minimum inhibitory concentrations against most important antibiotics were performed.

### Results

The induction of resistance against GBH in *Salmonella enterica* was hard to achieve yet possible. From 10 investigated *Salmonella enterica* isolates only three developed stable resistance against the GBH. These isolates showed changes in the *aroA* gene (responsible for the production of glyphosate's targeted enzyme) without any fitness cost. Proteome comparison revealed activation of cellular tolerance and stress response when transiently exposed to GBH in sensitive and resistant strains, but

not constitutively in resistant mutants in the absence of GBH. No cross-resistance or cross-tolerance to antibiotics was found.

## Discussion

While resistance to GBH in *Salmonella enterica* can potentially be generated, observed cellular tolerance responses were not genetically fixed in this experimental setting. Cross-tolerance and cross-resistance against clinically important antibiotic drugs were not observed.

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## Abstracts Session 6

### Poultry production: infectious and non-infectious issues, novel technologies (I)

#### Hemp oil and cannabidiol in broilers diet – impact on productivity and behaviour

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#### Introduction

Poultry meat production has changed significantly over the years. Currently, the intensive type of production is the most commonly used in large-scale production, characterized by keeping many animals in a relatively small space. One of the main problems in such conditions is a negative impact on animal welfare associated with lower physical activity [1], which is caused by a rapid weight gain. Comparing the results obtained from 1950 to 2000, taking into account differences in genetics, dietary balancing and overall management, it takes only 1/3 of the time and three times less feed intake for a "modern" broiler to reach a weight of 1815 g compared to the birds from the 1950s. [2]. Nowadays, broiler chickens get a weight of 2 - 2.5 kg within 35 - 42 days [3]. That fast growth influences leg disorders and is still one of the main health problems in these birds' production [4]. Because of the focus on its efficiency and general nature of the intensive production, deviations from the norm in terms of movement should be an essential welfare indicator [5]. The decreased activity level of birds is most often associated with the occurrence of footpad dermatitis (FPD), also called pododermatitis/footpad lesions (ulcers on the underside of the feet) and hock burn (discolouration and lesions of the hocks) [6,7]. Both diseases are genetic and related to living conditions, particularly poor-quality litter [6,8]. Foot lesions directly cause pain, reduce locomotor abilities, thus feed and water intake, and decrease weight gain [9].

Due to the proven health-promoting properties of cannabidiol (CBD), with a particular focus on the impact on the condition of the joints [10], it is a potential measure that can improve the welfare of broilers. The popularity of *Cannabis sativa*-based preparations has increased significantly in recent years in veterinary medicine. The endocannabinoid system, including CB<sub>1</sub> and CB<sub>2</sub> receptors, is present in most animals, both mammals, birds, reptiles and fish, as well as those defined as more primitive (sea snails, mussels and others) [11]. Currently, CBD is most used in companion animals. Recent studies confirm the effect of cannabidiol on reducing pain in dogs suffering from degenerative arthritis. At the same time, increased mobility of dogs was found positively influence the quality of life [12,13]. Therefore, the study aimed to evaluate the effect of a feed additive in the form of hemp oil and cannabidiol on the productivity and behavior of broiler chickens.

#### Material & Methods

714 broilers (Ross 308) were kept in 21 pens (34 birds/pen) during the experiment. The birds were randomly assigned to 3 groups (n=7): the control group (C), fed with the basal feed, the group receiving the addition of 4 ml hemp oil (HO) per kg of basal feed, and the group receiving the addition of 4 ml

hemp oil with cannabidiol (CBD) per kg of basal feed. In 50 kg of the basal feed, 200 ml of cold-pressed hemp oil or 197 ml of hemp oil were dispersed along with 3 ml of 10% CBD oil (cannabidiol content - 12 mg per kg of feed; daily CBD intake: 0.25 - 0.33 mg per bird). Bodyweight (g), daily weight gain (g), feed intake (g) and feed conversion ratio (FCR) were monitored and determined.

Factors such as eating, drinking, resting, walking, fighting, pecking other individuals/litter/equipment, and cannibalism were considered when assessing the behaviour. Video observations were carried out using recordings from cameras (GoPro Hero 4) with a wide-angle lens mounted on horizontal aluminium holders at 1.5 m above the litter. The Noldus XT Observer program was used to code the behaviour occurring during observation. It is used to evaluate behaviour in terms of the registration of behaviours occurring on the timeline and the duration and the number of occurrences of a given event.

## Results

The study aimed to evaluate the effect of adding hemp oil and hemp oil with cannabidiol on the productivity and behavior of chickens. Control birds were weighed after each week of production. The average values for each group were used for the calculations. During the first four weeks of bird life, i.e. when the tested additives were not administered, no differences in food conversion ratio (FCR) values, daily gains and body weight were observed between groups (Table 1). This suggests that there was no influence of environmental factors such as the location of pens in the building, their distance from the ventilation and ambient temperature on the overall production process. After five weeks (5 days of supplementation with hemp oil and CBD), differences in body weight were noted. Birds that received hemp oil or cannabidiol in the basal feed had a lower body weight than the control group by 7.82% and 5.66%, respectively ( $p < 0,05$ ).

Table 1. Detailed values of production parameters

Week	Production parameter	C	HO	CBD	SEM	p-value
II	Body weight (g)	478,5	463	474,7	3,22	0,17
	Daily weight gain (g)	39,4	37,8	40	0,52	0,33
	Feed intake (g)	46,8	46,5 <sup>a</sup>	47,4 <sup>b</sup>	0,22	0,04
	FCR	1,19	1,24	1,19	0,02	0,67
III	Body weight (g)	1072	1027	1045	16,67	0,19
	Daily weight gain (g)	84,8	80,7	81,5	2,29	0,57
	Feed intake (g)	98,6	98,5	98,7	0,98	0,98
	FCR	1,17	1,23	1,21	0,03	0,36
IV	Body weight (g)	1764	1684	1709	16,42	0,1
	Daily weight gain (g)	98,9	93,8	94,8	1,74	0,82
	Feed intake (g)	128	127	128	1,15	0,85
	FCR	1,31	1,36	1,35	0,03	0,6

Supplementation with hemp oil and CBD between 29 and 34 days of production						
V	Body weight (g)	2344 <sup>a</sup>	2173 <sup>b</sup>	2211 <sup>b</sup>	21,84	<0,01100
	Daily weight gain (g)	82,5 <sup>a</sup>	69,9 <sup>b</sup>	71,8 <sup>b</sup>	0,02	0,02
	Feed intake (g)	128	155	157	0,79	0,91
	FCR	1,90 <sup>a</sup>	2,23 <sup>b</sup>	2,2	0,06	0,04

<sup>a,b</sup> Mean values within the same row with no common lowercase superscript indicate significant differences ( $p < 0,05$ )

Both tested feed additives (HO and CBD) influenced the production parameters and behaviour of the birds (Tables 2 and 3). In addition, the use of CBD and HO resulted in increased mobility of birds compared to the control group. The increase in feed intake was observed in HO and CBD groups. Pecking of other individuals/litter/equipment was least frequently observed in the group fed with CBD addition. These behaviours were most common during rest. During the observation, incidents of cannibalism have not been reported.

Table 2. Behavioral observations - morning session

Assessed behaviour	Parameter	C	HO	CBD	SEM	p-value
Walking	Total duration time (s)	5671	5647	5496	201,6	0,99
	Average duration time (s)	15	329	21	3,41	0,34
Fighting	Total duration time (s)	10	9	-	1,03	0,99
	Average duration time (s)	5	3	-	0,87	0,99
Eating	Total duration time (s)	3659	3305	4439	161,1	0,61
	Average duration time (s)	325	71	86	20,7	0,77
Drinking	Total duration time (s)	1963	1170	1369	63,15	0,22
	Average duration time (s)	37	37	39	4,45	0,95
Resting	Total duration time (s)	39660	40616	39646	228,32	0,79
	Average duration time (s)	138	181	147	18,54	0,26
Running	Total duration time (s)	50	136	62	9,7	0,87
	Average duration time (s)	7	8	17	3,88	0,5
Equipment/	Total number (n)	2439	2437	3016	138,1	0,79

floor pecking	Average number (n)	32	31	37,6	8,71	0,85
Pecking of other individuals	Total number (n)	653	273	430	42,3	0,47
	Average number (n)	11,8	5,8	10,4	3,71	0,51

Table 3. Behavioral observations - afternoon session

Assessed behaviour	Parameter	C	HO	CBD	SEM	p-value
Walking	Total duration time (s)	4363	3836	4827	118,3	0,91
	Average duration time (s)	14	12	16	3,03	0,66
Fighting	Total duration time (s)	-	2	9	0,88	0,22
	Average duration time (s)	-	1	3	1	0,48
Eating	Total duration time (s)	4638	2886	5733	196	0,16
	Average duration time (s)	78	47	70	10,96	0,15
Drinking	Total duration time (s)	2471	1884	2076	106,1	0,73
	Average duration time (s)	40	37	36	4,44	0,82
Resting	Total duration time (s)	39516	42376	37692	275,6	0,09
	Average duration time (s)	151	178	140	11,87	0,1
Running	Total duration time (s)	25	31	54	3,08	0,43
	Average duration time (s)	2	3	3	0,31	0,13
Equipment/floor pecking	Total number (n)	1836	3208	1267	124,4	0,12
	Average number (n)	24,2	38,8	17	6,97	0,12
Pecking of other individuals	Total number (n)	318	129	178	9,16	0,43
	Average number (n)	7,2	3,6	5	0,86	0,54

## Discussion

Both tested feed additives had a positive impact on birds' behaviour. Broilers in groups receiving HO and CBD in the diet showed higher activity levels than those in the control group. This could be due to the anti-inflammatory properties of HO/CBD. However, the higher activity could have contributed significantly lower daily and final body weight gains with increased feed intake (higher FCR). Analysis of poultry meat quality may complement similar studies in the future. Interesting results should also be expected when the tested additives are given to animals from the first week of their life. This study was a preliminary recognition of the possibility of using CBD in poultry nutrition, and a comprehensive extension is planned. At the same time, in the available literature, we observe no reports on the use of cannabidiol in the poultry diet.

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## A preliminary study on the impacts of early nutrition of hatched chicks on well-being and growth performance

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### Introduction:

It is sometime unavoidable that one-day-old chicks can be deprived of feed for about 48 h or more before they are housed on farms. This study aimed to investigate the possibility of rearing newly hatched chicks with immediate access to feed and water in the same hatching unit one week prior to transferring them to the conventional broiler house with special regards to foot pad health and growth performance. Animals,

### Material and methods

Two trials were performed with a total of 6900/6850 (trials 1/2) broiler chickens (ROSS 308). A total of 3318/3391 chicks (trials 1/2) were transported from the hatchery (duration of about 3 h) and reared in a conventional broiler house (control group: delayed nutrition on-hatchery hatched). The control group did not receive any form of nutrition until they were taken to conventional broiler housing. Additionally, a total of 3582/3459 (trials 1/2) embryonated eggs (d 18) were obtained from the same parent flock of the same commercial hatchery and taken to the farm facility. After on-farm hatch, the chicks had immediate access to water and feed (experimental group: early nutrition on-farm hatched). After d 6/7 of life, the on-farm hatched chicks (trials 1/2) were transferred to the broiler house on the same facility.

### Results

The delayed nutrition on-hatchery hatched groups displayed a significantly lower dry matter content in the litter compared to the early nutrition on-farm hatched groups (two-factorial analysis) at d 14 of life ( $p = 0.013$ ). However, thereafter, no significant differences were noted. Based upon two-factorial analysis, the early nutrition on-farm hatched groups revealed lower foot pad lesions from d 14 of life onwards ( $p < 0.001$ ). Moreover, birds in early nutrition on-farm hatched groups showed a higher body weight throughout the rearing period compared to the delayed nutrition on-hatchery hatched groups ( $p < 0.001$ ). According to the data of the slaughterhouse, the final body weight (d 33) was higher for the birds of early nutrition on-farm hatched (experimental group) (1893 g and 1911 g for trials 1 and 2, respectively) in comparison to delayed nutrition on-hatchery hatched (control group) (1817 g and 1795 g for trials 1 and 2, respectively).

## Conclusion

Overall, early nutrition on-farm hatched chickens is of critical importance together with using new litter at d 7 to maintain healthy foot pads as well as to enhance and optimize the growth performance.



## ***Campylobacter* spp. transmissions in the environment of commercial poultry farms in Germany**

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### **Introduction**

*Campylobacter* (C.) *jejuni* is the most common cause of campylobacteriosis in humans, and broiler meat is considered one of the major sources. In general, it is believed that the poultry house environment may be a reservoir for *Campylobacter* spp. to some extent. However, to date, there are limited data on the spillover of *Campylobacter* from houses with *Campylobacter*-positive flocks and the buildup of environmental reservoirs. In addition, *Campylobacter* spp. are capable to transit into a viable but non-culturable (VBNC) state as a result of various extrinsic stress factors. In order to identify possible reservoirs of persistent and VBNC-*Campylobacter* and thus uncover relevant transmission pathways, a longitudinal study was conducted.

### **Material & Methods**

Three broiler farms and their environment close to the barn were intensively investigated at the end of two consecutive fattening cycles in summer and winter. In order to draw careful conclusions about possible transmission between consecutive fattening cycles, the selected farms were also examined after cleaning and disinfection. All samples were processed according to the semi-quantitative method for the detection and enumeration of *Campylobacter* spp. (ISO/TS 10272-3). Selected isolates were species-typed by MALDI-ToF analyses. A systematic selection of isolates from all sampling collections was examined by whole genome analyses. Moreover, environmental and selected broiler house samples were treated simultaneously with propidium monoazide (PMA) and analyzed by live/dead discrimination using real-time PCR (qPCR) in the further course of the study

### **Results**

In two out of three farms, *Campylobacter* was frequently detected in high amounts in the chicken barns, especially in summer. However, the pathogen was only occasionally detectable in the environment, particularly in water-associated matrices, especially in winter. However, *Campylobacter* could not be isolated in broiler houses after cleaning and disinfection. The emission source of

culturable *Campylobacter* was found to be primarily contaminated chicken manure. *C. jejuni* proved to be the dominant species of the isolates examined. PMA-qPCR revealed no detection of VBNC-*Campylobacter* in selected barn and environmental samples. In contrast, *Campylobacter* DNA was more frequent detected in environmental samples.

## Discussion

The present study provides insight into the significance of *Campylobacter* in the environment in relation to prevalence in the broiler farms investigated in Germany. The results established indicate sporadic environmental findings in the immediate vicinity, suggesting spread, persistence and possible reintroduction. *C. jejuni* was found in nearby water bodies, indicating that the pathogen is ubiquitous by spread and circulation. Although the findings were sporadic and no significant source of transmission has yet been identified, it should be kept in mind that even very low levels of *Campylobacter* may initiate the colonization of whole poultry flocks.

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## Abstracts Session 7

### Animal Welfare (I)

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#### Smart Animal Health – The development of an animal-based and data-driven method for assessing health and welfare in farm animals

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#### Introduction

Improving animal health and welfare in livestock production systems depends on reliable proxies for the assessment and monitoring. The aim of this project was to develop a novel method that relies on animal-based indicators and data-driven metrics for assessing the health and welfare status at farm level for the most common livestock species in Switzerland.

#### Material & Methods

Method development followed a uniform multi-stage process. Scientific literature was systematically reviewed to identify potential health and welfare indicators for cattle, sheep, goats, pigs and poultry. Suitable indicators were applied in the field and compared with outcomes of the Welfare Quality® scores of a given farm. To identify farms at risk for violations of animal welfare regulations, several agricultural and animal health databases were interconnected and various supervised machine-learning techniques were applied to model the status of farms.

#### Results

Literature reviews identified a variety of indicators for the different species, some of which are well established and widely used in the field, while others lack reliability or practicability or still need further validation. Results of these reviews were published for dairy cows (Lutz et al. 2021), goats (Minnig et al. 2021) and sheep (Zufferey et al. 2021). Data quality and availability strongly varied between animal species, with most data available for dairy cows and pigs. In addition, an overview of methodologies of methods related to precision livestock farming (PLF) technologies was developed (Stachowitz and Umstätter 2021). The machine-learning techniques used to predict farms for risk-based animal welfare inspections reached similar classification performances with sensitivities above 80%. The most important predictors were participation in federal ecological and animal welfare programs, farm demographics and farmers' notification discipline for animal movements.

## Discussion

A common method with individual sets of indicators was developed for each species. The results show that models based on proxy data can achieve high correlations with animal health and welfare. Nevertheless, for sufficient validity, a combination of data-based indicators and on-farm assessments is needed. For a broad implementation of the method, a time-saving on-farm data collection is required whereby PLF technologies have shown to be a promising approach.

## Acknowledgement & Funding

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## **Innovation in broiler housing: Environmental Enrichment and a Farmer Assistance System**

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### **Introduction**

Broiler production has constantly grown over the past years. With higher numbers of animals housed together, new challenges have emerged. Especially the monitoring of the animals and the structuration of the houses to ensure animal welfare have become more and more important. In our study we housed 8,100 birds in a trial group with the provision of three different types of environmental enrichment designed to offer the birds elevated seating positions. The trial was repeated for a total number of six fattening runs. Therefore perches and elevated plains, as well as a combination of both, were placed in the barn since the first day of the fattening period. Additionally we used a real-time monitoring device (Farmer Assistance System FAS), running on rails under the roof, to collect data. It continuously measured climate and air quality parameters and offered an insight into the barn through four different cameras.

### **Material & Methods**

Over the course of six fattening runs a representative number of 50 birds were weighted and examined individually on days 14, 21 and 28. In addition to this both feet were scored regarding Foot Pad Dermatitis and litter samples were taken, to evaluate the influence of the environmental enrichment on the litter but also on the animals themselves. The FAS, as a monitoring device, continuously collected data from the air inside the barn, which was used for evaluating the influence of the environmental enrichment on the air quality.

### **Results**

Our results showed overall no negative influence of the enrichment or the monitoring device (FAS) on the broilers growth performance and health. There were no negative differences in body weight or Feed Conversion Ratio on the birds. Litter and air quality were also not negatively influenced regarding dry matter in the litter or CO<sub>2</sub> and NH<sub>3</sub> in the air of the barn.

In conclusion the environmental enrichment and the real-time monitoring, for example via a Farmer Assistance System, can offer a lot of tools to improve animal health and welfare and in our study we could not confirm any concerns regarding negative influence of the provision of environmental enrichment on the performance of broiler chicken. But further research and practical testing needs to be done to continuously improve these possibilities.

## **Acknowledgement & Funding**

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## Quality of drinking water for cattle.

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### Introduction

In this study (drinking)water preferences of dairy cattle were investigated. This because most welfare assessment protocols assume that the water should be as clean as possible and put a heavy weight on this during an assessment. However, cows might have another preference. In pigs, water uptake rose when the supplied water had a lower pH level compared with tap water (3.7 in comparison with 7.3)<sup>1</sup>. In practice, often cows 'pollute' their drinking water with rests of food. Due to the acidic properties of silage, this might lower the drinking water pH (and change its taste). And thus, indirectly, making it possibly of a better palatability for the cow<sup>2</sup>.

### Material and methods

Nine 15-22 month old Holstein heifers were kept on pasture 24 h a day. Before the start of the experiment, they had a 2 week adjustment period, with free access to all water supplies tested. Four types of drinking water were examined. During the day the water was refilled/added when needed. All water was supplied in 90 L polyethylene containers 67 cm in diameter and 37 cm in height, and the content was completely refreshed every 24 h. The types of water were: D1) Clean tap water and this drinker was also cleaned every 24 h. D2) Tap water mixed with 250 grams of free floating corn silage. The refill consisted of water, mixed 24 h prior to refilling with silage (250grams). D3) Tap water mixed with 1kg of free floating corn silage. The refill consisted of water, mixed 24 h prior to refilling with silage (1 kg). D4) Tap water mixed with 500 grams of manure. Refilling was done with a 24 h earlier mixed refill of tap water and manure. The drinkers were placed 5 meters apart in a random order for 5 days. The number of times a cow came drinking, the amount of water consumed from every drinker, air temperature, minutes of sunshine, and relative humidity (RH) were recorded on a daily basis.

### Results and discussion

The total daily water intake per cow varied from 23.1 to 64L per day. The cows drank in total 508L from D1, 266L from D2, 295L from D3 and 0L from D4.

There were correlations of the amount of water consumed per day with the amount of sunshine ( $r^2$  around 0.5) and RH ( $r^2$  around -0.6). Solar radiation lowers the upper critical temperature and thus increases the need for evaporative heat loss, whereas a higher RH decreases the amount of evaporation and thus the need for extra water.

It appeared that the cows preferred the somewhat dirtier water (total of D2+3 was 561L) over fresh clean water. The water that was contaminated with manure was not consumed at all. So the scores for drinking water quality in the welfare assessment protocols need to be adjusted.

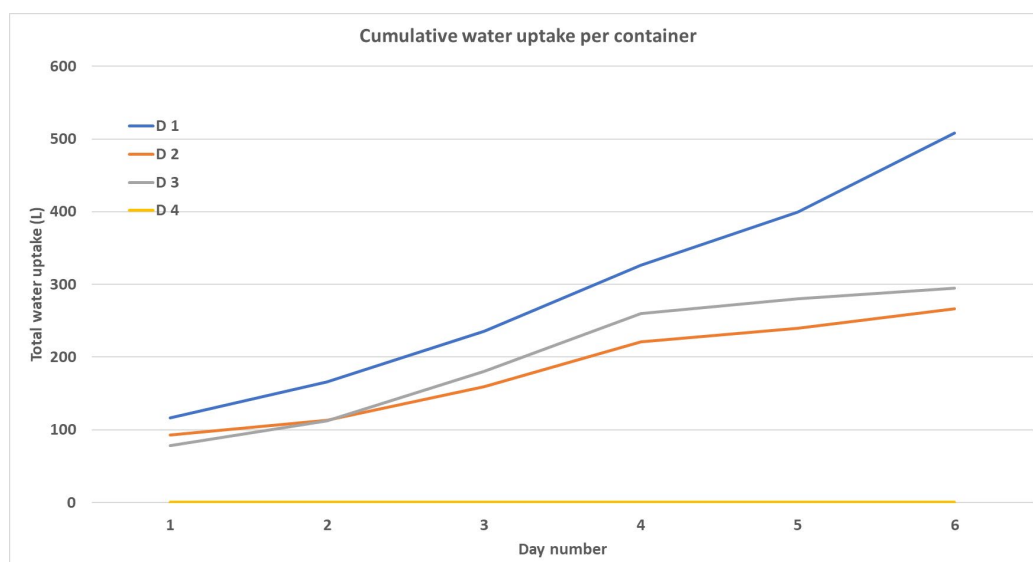


Figure 1: Cumulative amount of water consumed by the 9 cows. Container D 1 had clean tap water, D 2 had tap water mixed with 250 grams of free floating corn silage, D 3 had tap water mixed with 1kg of free floating corn silage and D 4 had tap water mixed with 500 grams of manure. It is clear that the cows preferred the water mixed with corn silage and did not drink the water that was contaminated with manure.

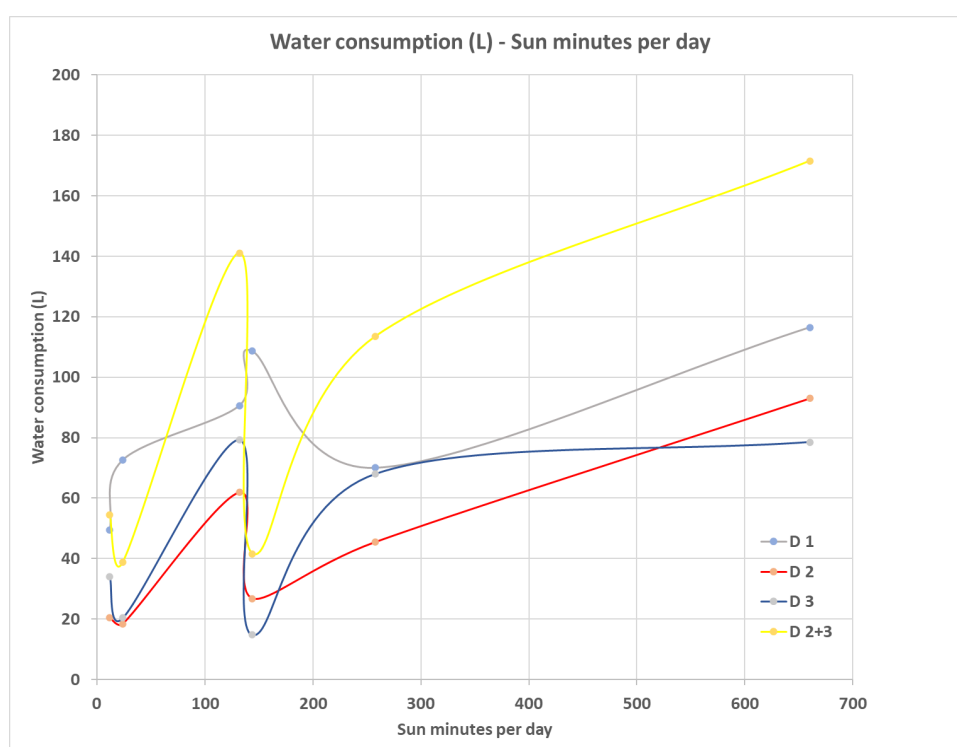


Figure 2: Water consumption per day for the 9 cows in relation to the number of sun minutes of that day. Container D 1 had clean tap water, D 2 had tap water mixed with 250 grams of free floating corn silage, and D 3 had tap water mixed with 1kg of free floating corn silage. Because the cows did not drink anything from the container with the manure contaminated water this was not included in this graph.



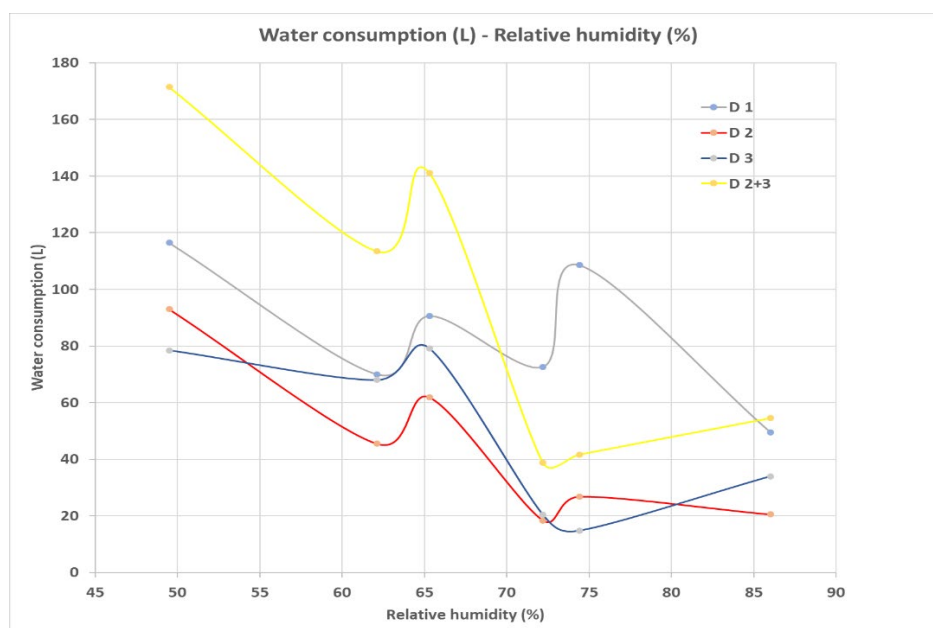


Figure 3: Water consumption per day for the 9 cows in relation to the average relative humidity of that day. Container D 1 had clean tap water, D 2 had tap water mixed with 250 grams of free floating corn silage, and D 3 had tap water mixed with 1kg of free floating corn silage. Because the cows did not drink anything from the container with the manure contaminated water this was not included in this graph.

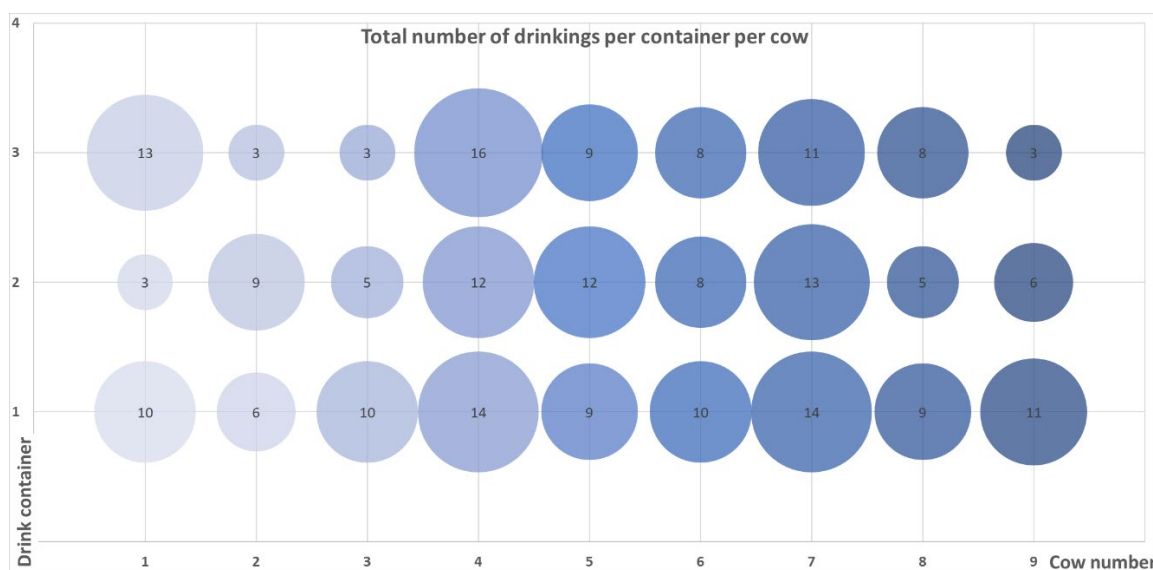


Figure 4: The number of times a cow drank from a certain container. It appeared that there were substantial differences between individual cows. (Container D4 (manure contaminated) was not used at all)

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## Abstracts Session 8

### Animal Welfare (II)

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#### **Expectations and concerns about the use of information and communication technology tools at poultry and pig farm - results of a survey of Polish producers**

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#### **Introduction**

Promoting and adopting ICT tools is a significant challenge in animal production. Although advanced ICT solutions improve animal husbandry's environmental and economic viability, farmers are not open to new technologies due to a lack of in-depth knowledge about the implications of their usage [1, 2]. Management of data collected in real-time by specialized equipment allows control of animal welfare, health, and performance [3]. However, animal husbandry development requires removing socio-economic and cultural barriers preventing the broader adoption of ICT tools [4]. Thus, the main goal of the LivestockSense project, the consortium of which consists of representatives from Poland, Hungary, Denmark, Sweden, Estonia, Israel and Austria, is to identify the barriers to the introduction of modern technologies and to support farmers through education and mentoring, to determine what kind of assistance farmers need in the application of innovative methods.

#### **Material & Methods**

Questionnaires on the current use of PLF tools were developed and completed by respondents running/employed on commercial poultry and pig farms. Survey questionnaires have been designed to be used in the online version. Then, with their use, a survey was carried out on a representative group of Polish farms involved in the production of poultry (22 farms: 12 with broiler chickens and 10 with laying hens) and pigs (23 farms: 13 with a closed cycle, 5 with an open cycle focused on the production of fattening pigs, 5 with an open cycle aimed to the production of piglets). The questionnaires concerned the knowledge and future expectations of farmers towards ICT.

## Results

Among poultry farmers, the average age of the PLF equipment/technologies used on the farm was less than ten years in the case of approximately 43% of the surveyed farms and between 10-19 years in the case of 34% of the monitored entities (Figure 1). On the other hand, over 68% of PLF installations were less than ten years old on pig farms (Figure 2).

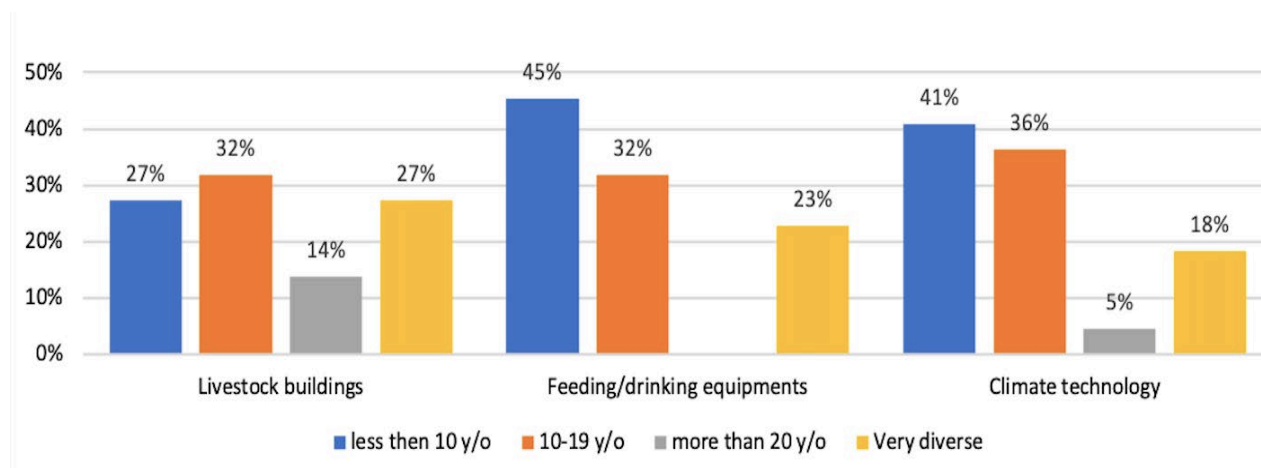


Figure 1. The average age of production technology at responding Polish poultry farms, 2022

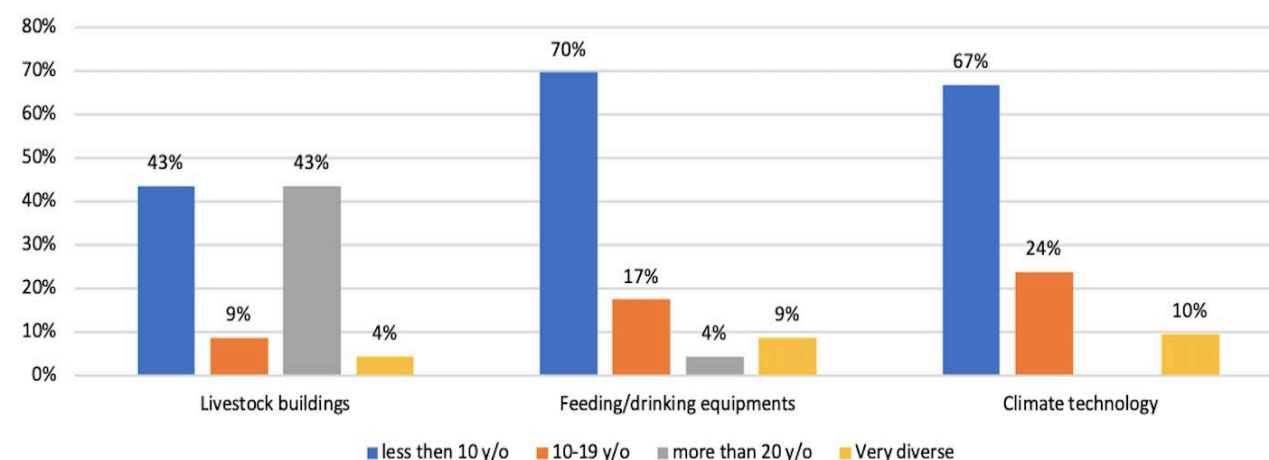


Figure 2. The average age of production technology at responding Polish pig farms, 2022

The majority of the surveyed breeders considered that the so-called smart devices/technologies allow increasing production efficiency (86% of poultry farmers and 83% of pig producers responded positively), and contribute to the early detection of problems in the herd (73 and 79% for poultry and pigs, respectively), provide real-time information (91 and 83%, poultry and pigs, respectively) and provide reliable measurements/results (82 and 78%, poultry and pigs, respectively).

More than half of the respondents (59% for poultry and 57% for pigs) stated that the PLF technology would enable the fulfilment of obligations regarding reducing the environmental pollution. Both

poultry and pigs farmers' level of agreement about general statements on the operation of smart devices is presented in Figures 3 and 4.

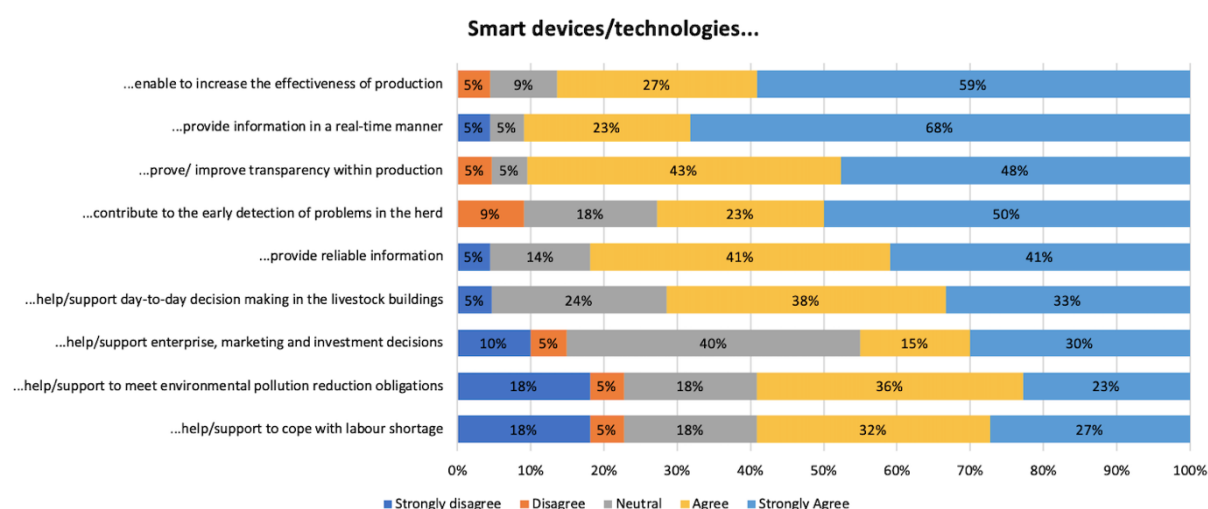


Figure 3. Polish poultry farmers' level of agreement about general statements on the operation of smart devices/technologies, 2022

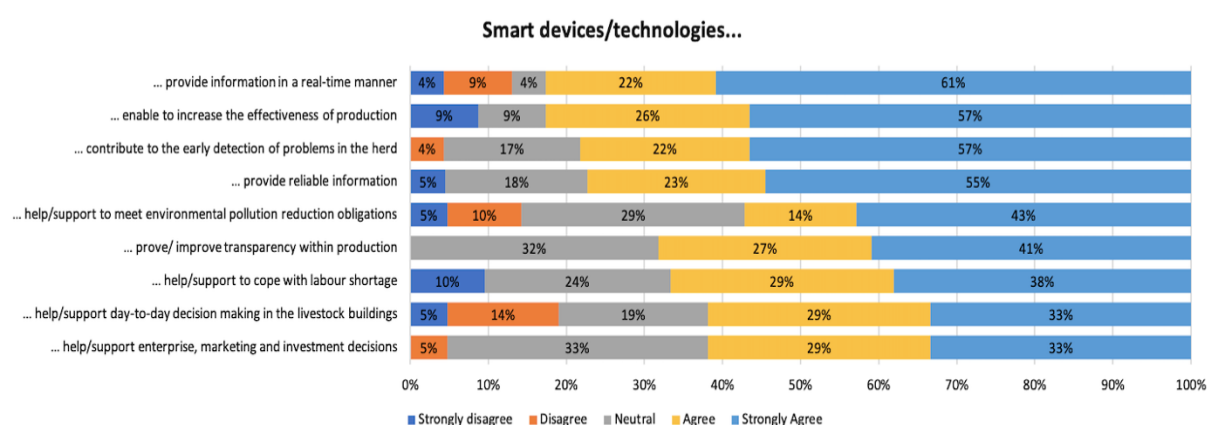


Figure 4. Polish pig farmers' level of agreement about general statements on the operation of smart devices/technologies, 2022

At the same time, around 80% of the respondents from the poultry sector stated that access to smart technologies, information about them and their distribution on the market is easy. The pig breeders were much more critical in this matter (about 60% of positive responses) (Figure 5). Additionally, 57% of pig producers stated that purchasing a PLF solution at a reasonable price was impossible, unlike poultry producers, of which only 21% had such a critical opinion (Figure 6).

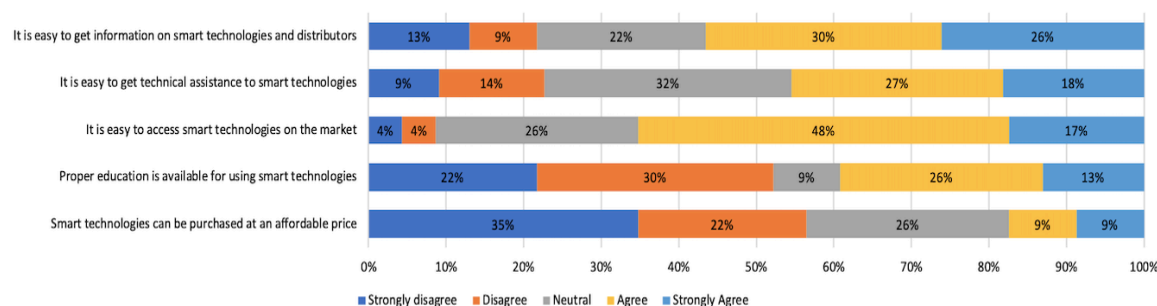


Figure 5. Polish pig farmers' level of agreement about statements on the availability of smart devices/technologies, 2022

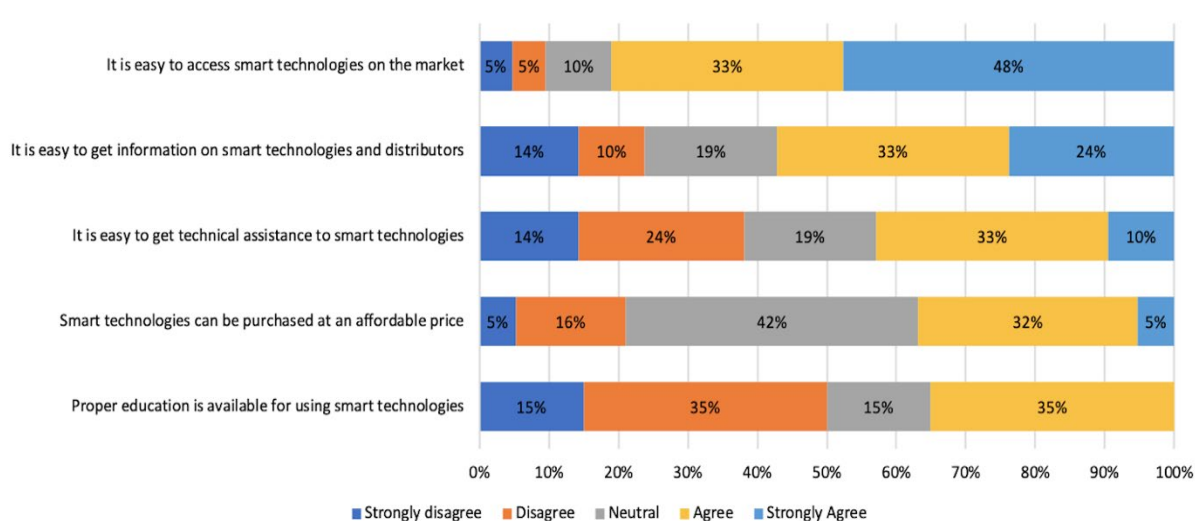


Figure 6. Polish poultry farmers' level of agreement about statements on the availability of smart devices/technologies, 2022

Similar discrepancies between poultry and pig farmers were shown by the survey results regarding the operation and handling of PLF solutions (Figures 7 and 8). Over 55% of pig producers considered that smart technologies do not work reliably (only 25% of poultry farmers). Only 32% of pig farmers declared that PLF could be exploited at a reasonable cost (for poultry, as much as 68% of positive responses), although 42% found that such technologies are easy to use (for poultry, 50%). The respondents indicated microclimate, air quality and feed/water control as the main objectives of the PLF technology application.

Over 60% of poultry producers indicated that the main reasons for dissatisfaction with the operation of the PLF technology were insufficient involvement of people (staff) in solving current problems, the lack of good-quality internet connection and, consequently, delayed data receiving, and over 50% also complained about the lack of sufficient technical support from the companies providing the devices. In the case of pig producers, 60% of the respondents indicated that the equipment was incompatible with the current structure of the livestock buildings and that there were problems in the connection between the equipment within the technology/system used.

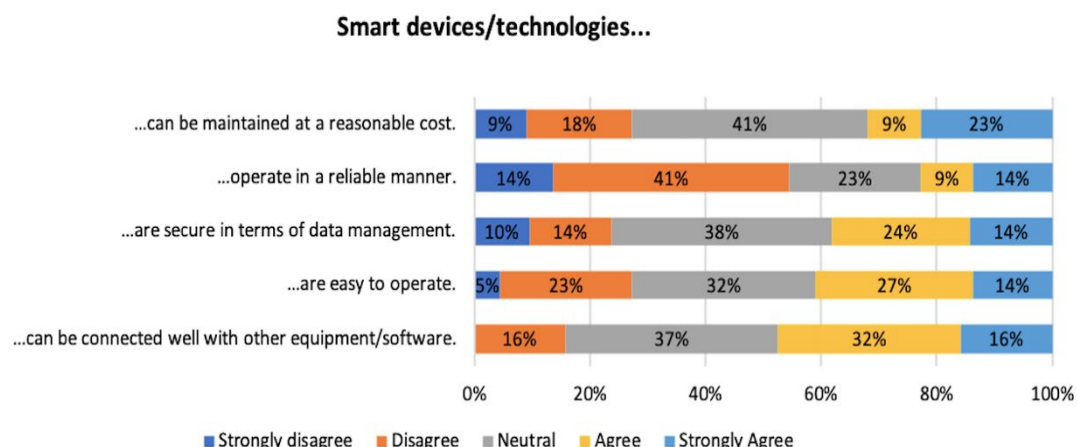


Figure 7. Reasons for polish pig farmers' dissatisfaction with the operation of smart devices/systems, 2022

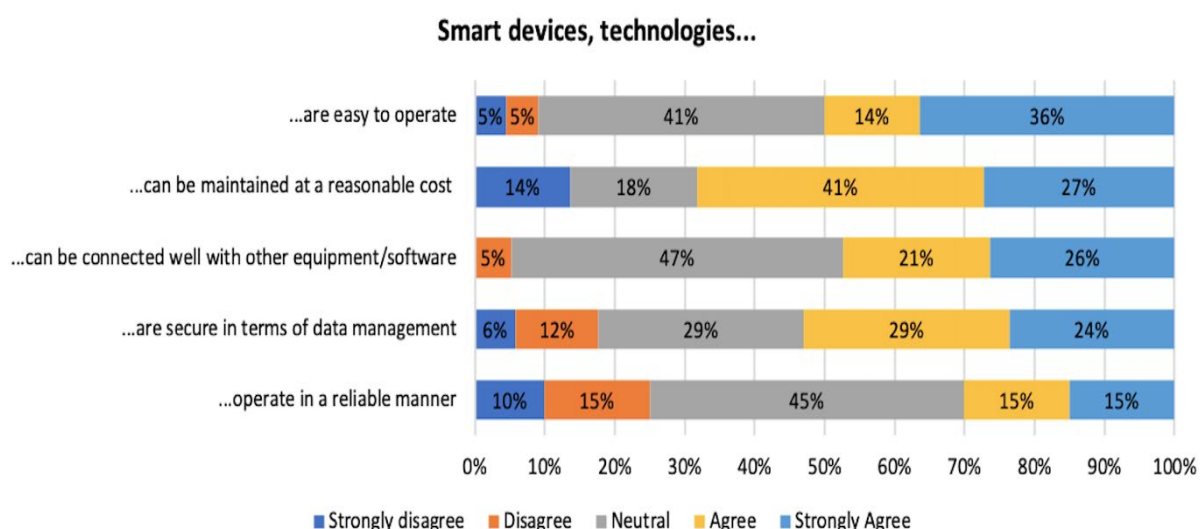


Figure 8. Reasons for polish poultry farmers' dissatisfaction with the operation of smart devices/systems, 2022

All respondents indicated that the most important aspects influencing the decisions of Polish breeders to invest in new PLF technologies are calculated and proven economic and environmental benefits and guarantee safe data management (Figures 9,10, and 11). Notably, the survey process was mainly attended by farm owners (50% poultry and 87% pigs), who have the most significant influence on financial decisions made on the farm.

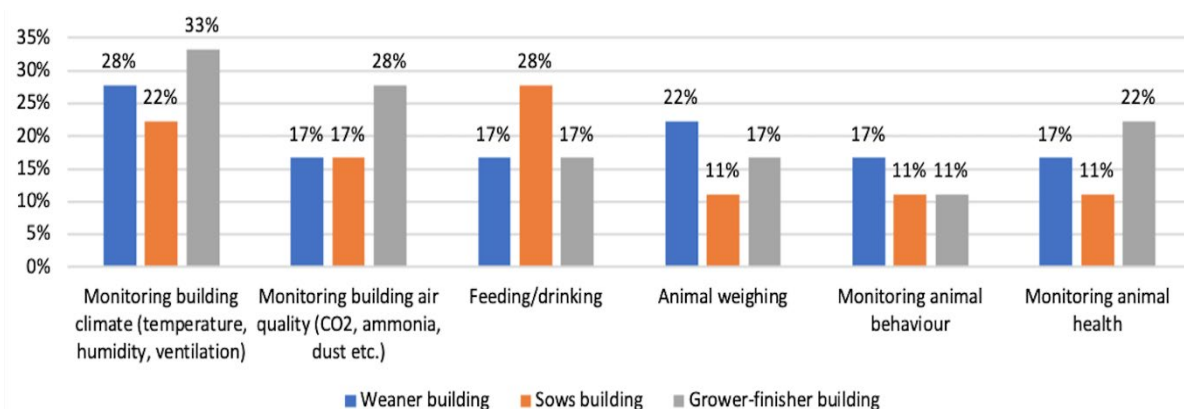


Figure 9. Planned purpose of using smart devices/technologies at Polish pig farms, 2022

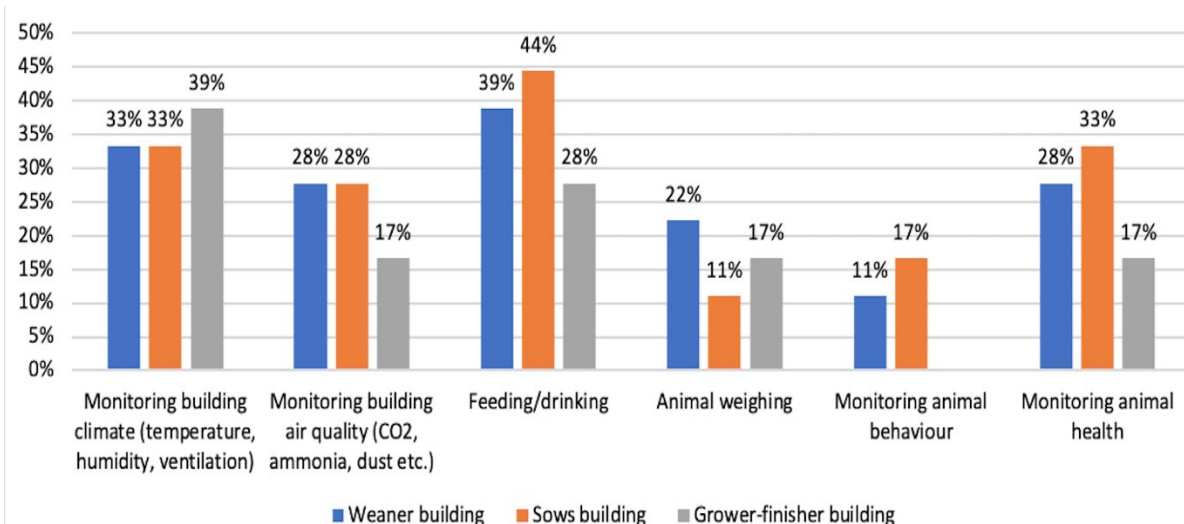


Figure 10. Purpose of using smart devices/technologies at Polish pig farms, 2022

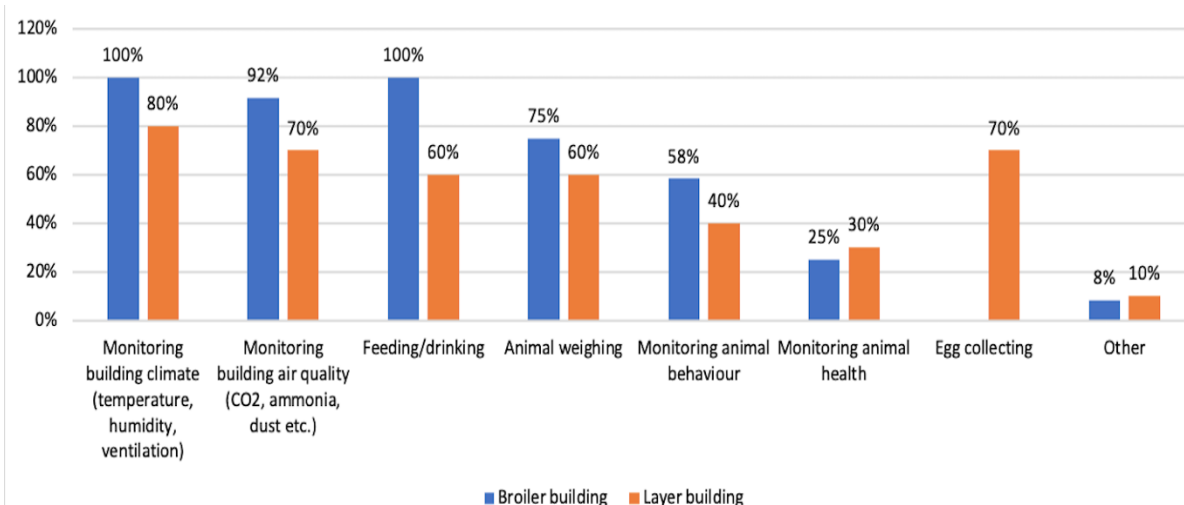


Figure 11. Purpose of using smart devices/technologies at Polish poultry farms, 2022



## Discussion

The introduction of modern technologies is associated not only with an investment but also with a change in the method of farm management. Traditional methods for monitoring production indicators, the microclimate in livestock buildings and widely understood animal welfare is time-consuming and labour-intensive. Thus, farm management with the use of PLF tools is gaining many supporters on the global market, who, thanks to openness to innovative solutions, increase profits, reduce the potentially negative impact of farms on the environment, but above all, ensure an appropriate level of animal welfare.

## Acknowledgement & Funding

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## Assessment of dairy donkey welfare from five farms in Northern Serbia

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### Introduction

Growing interest in donkey milk in recent years has led to the intensification of donkey milk production. There is still no central database of donkeys in Serbia, which makes monitoring the health and welfare of these dairy animals difficult. Therefore, the aim of this study was to determine and compare the welfare, health status and housing conditions of dairy donkeys on five farms in Northern Serbia.

### Material & Methods

Donkeys included in this research originated from five different farms in Northern Serbia visited in March and April 2022. Farm I (n=103) is located in Srem region, farm II (n=19), III (n=17) and IV (n=30) are located in Bačka region, while farm V (n=160) is located in Mačva region. The assessment of donkeys was performed according to the AWIN welfare assessment protocol for donkeys (2015).

### Results

Body Condition Score of the most donkeys from all visited farms ranged from 2 to 3. None of the donkeys on all visited farms showed signs of stereotypes, dehydration, thermal stress, lameness, dyspnea, hot branding, cheek abnormalities, genital discharge and prolapse. Integument alterations were recorded in donkeys from all examined farms, except from farm IV. The highest frequency of integumentum alterations was recorded in donkeys from farm I, where alopecia (90%) and skin lesions (30%) were the most common findings. Hair coat was healthy in most of the assessed donkeys, with an exception of farm I, where all examined animals had poor quality hair coat. Swollen joints were observed on farms II and III, with a frequency of 20%. Ocular and/or nasal discharge were observed in donkeys from all assessed farms, with the highest frequency on farm V, where 90% of individuals had ocular discharge and 50% had nasal discharge. Most of the assessed donkeys, on each visited farm,

showed some signs of hoof neglect, with a highest frequency on farm I (90%). All of the assessed donkeys had social contact with other conspecifics. The human-animal relationship tests showed that most of the donkeys had a positive relationship with humans, with the exception of farm III, where 40% of the donkeys showed avoidance behavior and 20% had a negative reaction to the walk down the side test.

## **Conclusion**

Housing conditions and estimated welfare differed between examined dairy donkey farms. The most acceptable welfare conditions were observed on farm IV, while welfare conditions were the poorest on farm I. The greatest differences were observed in terms of integument alterations and hoof condition. The significance of these parameters lies in their connection with potential health disorders and the pain, which can seriously compromise donkey welfare.

## **Acknowledgement & Funding**

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## **Prevalence of gross lesions and handling practices in pigs and their association with pork quality, Kiambu, Kenya**

Sentamu Derrick Noah<sup>1</sup>, Joshua Orungo Onono, Patrick Muinde, Nicholas Bor, Dorcas Chepyatich, Lian Francesca Thomas.

<sup>1</sup>International Livestock Research, Institute Kampala, Uganda

### **Introduction**

Pre-slaughter treatment of pigs is known to affect the quality of meat, but there are no studies to date investigating this relationship in the Kenyan context. The aim of this study was to determine the prevalence of gross lesions and practices related to sub-optimal welfare in pigs presented for slaughter, and to analyze the relationship between occurrence of these and meat quality at a non-integrated pig slaughterhouse in Kenya.

### **Materials and methods**

Data on welfare-associated lesions and handling practices were obtained by examining 529 pig carcasses. From these, 387 meat samples were obtained to determine meat quality, based on pH<sub>24</sub>, meat color and EZ Drip loss. Pork was then grouped into one of four recognized quality categories: Red, Firm, Non-exudative (RFN), Pale Soft Exudative (PSE) Dark Firm Dry (DFD) and Red Soft Exudative (RSE).

### **Results**

Almost all pigs were insufficiently stunned based on presence of signs of consciousness post-stunning. The majority of pigs had one or more welfare-associated gross lesions. The most prevalent lesions were lacerations made on ears for pig identification through out the slaughter line by owners. Many pigs were transported under conditions of high loading density, under which we also grouped pigs which were transported while tied to motor bikes and bicycles. Approximately half of sampled pigs were slaughtered on the same day on which they were purchased by a trader from the farm of origin and delivered to the slaughterhouse. Over a quarter of the pork meat samples were of sub-optimal quality including: RSE, PSE and DFD. Results from a multinomial logistic model revealed an association between slaughtering pigs on the same day they were purchased from their farm of origin and obtaining RSE pork. There was also an association between transporting pigs at a high loading density and obtaining DFD pork.

### **Conclusion**

All results indicate the need to educate stakeholders in the pork value chains on improved pig handling before and during slaughter to enhance pig welfare. This study also shows that although there is Kenyan animal welfare legislation, it appears to be insufficiently enforced. The economic consequences of the link between poor handling practices and sub-optimal meat quality must be further investigated and utilized as an incentive for behavior change to enhance pig welfare and pork quality along the value chain.

## Danish welfare-label pig production systems: Usage of antibiotics and occurrence of disease

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### Introduction

Reducing antibiotic usage in pig farming is a prerequisite for supporting sustainable and publicly acceptable farming practices. Organic and other types of welfare-label (WL) pig production systems offer an alternative to conventional indoor production with a markedly lower antibiotic usage (Nielsen et al., 2021). Alternative ways of housing and managing pigs in these systems, e.g. a suckling period spent outside, a higher weaning age and a lower stocking density are intended to benefit the welfare and health of the pigs (see Table 1).

Table 1: Rearing conditions in welfare-label (WL) and conventional pig production systems in Denmark. Modified from Kongsted and Sørensen, 2017

	WL ORGANIC	WL NON-ORGANIC	CONVENTIONAL
<b>FARROWING</b>	Outdoor in huts No tail docking	Outdoor in huts No tail docking	Indoor in crates
<b>MINIMUM AGE AT WEANING</b>	7 weeks	5 weeks	3 weeks
<b>HOUSING (SPACE REQUIREMENT AT 100 KG)</b>	2.3 m <sup>2</sup> per pig Outdoor access Access to roughage Bedding material	1.2 m <sup>2</sup> per pig Outdoor access Access to roughage Bedding material	0.65 m <sup>2</sup> per pig
<b>ANTIBIOTIC-TREATMENT</b>	Veterinarian must initiate treatment Double withdrawal time	Farmer can initiate treatment Double withdrawal time	Farmer can initiate Standard treatment withdrawal time

It is disputed whether WL systems are supportive for the health of the pigs, and among veterinarians, very low levels of antibiotic usage are often questioned based on animal welfare concerns (Kruse et al., 2019). Studies on lesions in slaughtered pigs have pointed out some disease challenges in alternative systems, with higher levels of e.g. white liver spots, bone fractures and arthritis compared to conventional systems. Furthermore, as tail docking is forbidden in alternative systems, tail lesions and septicaemias associated with this condition are more common at slaughter in alternative systems (Kongsted and Sørensen, 2017, Alban et al., 2015).

Regulations on antibiotic usage are much more restrictive in WL organic systems than in conventional systems. Thus, Danish organic farmers cannot have medicine on store, but need to call a veterinarian

in cases of disease that needs medical treatment regardless of animal age group. In Denmark, WL non-organic farmers mainly apply to the same rules as conventional farmers and can have medicine on store if a Veterinary Advisory Service Contract\* is agreed. Until June 2022, within all pig production systems in Denmark, pharmaceutical levels of zinc oxide (pZnO) in feed were allowed (max 2500 ppm in feed for max 2 weeks post-weaning) in order to prevent post-weaning diarrhoea.

We previously reported on the usage of antibiotic and pZnO in alternative and conventional Danish systems, based on data from VetStat (the Danish national register on all prescribed farm animal medicine (sales from pharmacies)) (Nielsen et al., 2021). The study showed, that within all age groups, the antibiotic treatment incidence was highest in the conventional system, lower in the WL non-organic system and lowest in the WL organic system. Within all production systems, the treatment incidence was highest in weaners. The levels of treatment were, however, very different between systems. The study showed that conventional-, WL non-organic- and WL organic herds had an average treatment incidence in weaners of 7.20, 3.37 and 0.48 Animal Daily Dosages (ADD)\*\*/100 animals/day, respectively. With respect to the amount of pZnO, we did not find systematic differences between systems, but we saw that a higher proportion of alternative herds did not use pZnO at all during a whole calendar year.

In the current study, we were inspired by the huge differences in antibiotic usage between systems and interested to explore whether the level of clinical disease in WL weaners would appear to be negatively affected by a restrictive use of antibiotics. Furthermore, we were interested to determine risk factors associated with diarrhoea post-weaning in WL systems. We also aimed to explore through interviews, how alternative farmers approach disease challenges and how they consider health to be supported in their systems.

## Materials & Methods

We used the Danish Central Herd Register (CHR) to select WL herds with minimum 50 sows to participate in the study. Our goal was to recruit 10 WL organic- and 10 WL non-organic herds with a minimum of 50 sows, keeping the weaners in-door with outdoor access, which is the common way of housing of weaners in Danish WL systems. Unfortunately, from the CHR gross list of 37 WL organic and 13 WL non-organic herds we were only able to recruit eight and four, respectively, who fulfilled the criteria and were willing to participate.

The study was conducted from August 2019 to March 2021 with three visits in each herd, approximately six months apart. At the first visit, farmers were interviewed about their practices with respect to herd health management and within this, their usage of antibiotics and pZnO. Furthermore, they were asked to consider which factors or practices within their herds, they considered most important in order to achieve a low antibiotic use. During the next two visits, information from the interviews were updated, in order to register eventual changes. The ordained amount of antibiotics for weaners (given in ADD/100 pigs/year) in each of the twelve herds was extracted from VetStat for the calendar year 2020. As organic herds cannot have medicine on store after the end of one treatment period, and therefore have to discard remains, the antibiotic consumption in these herds will be lower than the amount registered in VetStat. Antibiotic remains from organic herds must be delivered to the pharmacy, but the amount of remains is not registered in Vetstat (Kruse et al., 2019).

All visits included individual clinical examinations of 100-200 8-week-old pigs from each herd as well as registration of pen factors like design and stocking density. Due to differences in weaning ages (see Table 1), we examined WL organic pigs one week (4-9 days) post-weaning and WL non-organic pigs three weeks (19-25 days) post-weaning. All pens (except sick pens) with pigs at the age of interest were included for sampling of pigs with an equal number of pigs sampled in each pen. During the study period, use of pZnO was allowed during the first two weeks post-weaning. Thus, if this was applied, organic pigs would be under treatment during the examination, while non-organic would have finished their period of pZnO medication.

Individual clinical examinations included: Rectal diarrhoea (liquid faeces on glove at rectal examination), perianal staining (min 1 cm in diameter of wet faeces around anus or wet faeces in a stripe from anus – if pigs were dirty in a large area of perineum this examination was not carried out), low body condition (spine visible), unthriftiness (dull and bristly haircoat and a big head compared to body size), tail bite (shortened tail/lesion on tail), ear lesions (lesion on one or both ears with a diameter of min 1 cm<sup>2</sup>), leg problem (visible and palpable swelling of joint(s)/stiffness of gait/lameness), hernia (visible and palpable swelling at the navel or groin) and airway problem (belly-breathing/eye discharge/otitis media).

As only four WL non-organic herds participated, we decided to examine 200 rather than 100 pigs in these herds. However, due to time constraints, in all herds, only 100 pigs were rectally examined for diarrhoea. For the analysis, “diarrhoea” was defined as rectal diarrhoea and/or perianal staining. In cases without perianal staining where rectal examination had not been performed, we established a prediction algorithm based on data from pigs with both types of examinations. Thus, despite differences in examination method, we ended up with comparable variables.

For the analysis, we had observations from 2400 WL organic pigs and 1962 WL non-organic pigs (438 pigs from non-organic herds were excluded, as they had been treated with antibiotics). We tested the following risk factors for diarrhoea in 8-week-old weaners: Production system (WL organic or WL non-organic), stocking density (animals per m<sup>2</sup>), number of animals per pen (below or above 100), diurnal temperature variation (max temperature minus min temperature on the day before examination measured at the nearest meteorological station), number of animals per water nipple (below or above the median value 24), feeding space per animal (below or above the median value 4 cm), crude protein in feed (below or above the median value 17.9%) and wash of pens between batches (yes or no). We screened for risk factors with a possible association to diarrhoea using univariable mixed effects logistic regression models with pen (within visit within herd) as random effect. Factors with  $P < 0.25$  in the univariable models were included and tested in a multivariable mixed effects logistic regression model with pen (within visit within herd) as random effect. One of the organic herds differed markedly from all other herds, as it was the only one not using pZnO during the whole study period. We chose to exclude this herd from the risk factor analysis. In four other herds (two organic and two non-organic), some or all of the pigs did not receive pZnO at all visits. These herds were kept in the analysis, and in these cases the potential effect of pZnO was included as part of the random effect. These herds were kept in the analysis, and effect of pZnO may therefore be part of their random effect.

## Results

### *Antibiotic usage*

VetStat data showed that usage of antibiotics in weaners varied considerably between the twelve herds in the study. The total consumption of antibiotics for weaners in the twelve herds during 2020 averaged to 3.24 ADD/100 pigs/day. WL non-organic herds used on average 7.8 ADD/100 pigs/day, whereas WL organic herds on average used 1.0 ADD/100 pigs/day (including for organic herds the amount being discarded at the pharmacy). Figure 1 shows the 12 months running average of antibiotic usage for weaners for each herd against the general average extracted from VetStat in December 2020 (including for organic herds the potential amount discarded at the pharmacy). Statistically, there was a tendency ( $P=0.1$  (Wilcoxon signed-rank test)) for WL organic herds to have a lower antibiotic usage compared to WL non-organic herds. In both organic and non-organic systems, we saw herds with a marginal or no usage of antibiotics during the twelve months period.

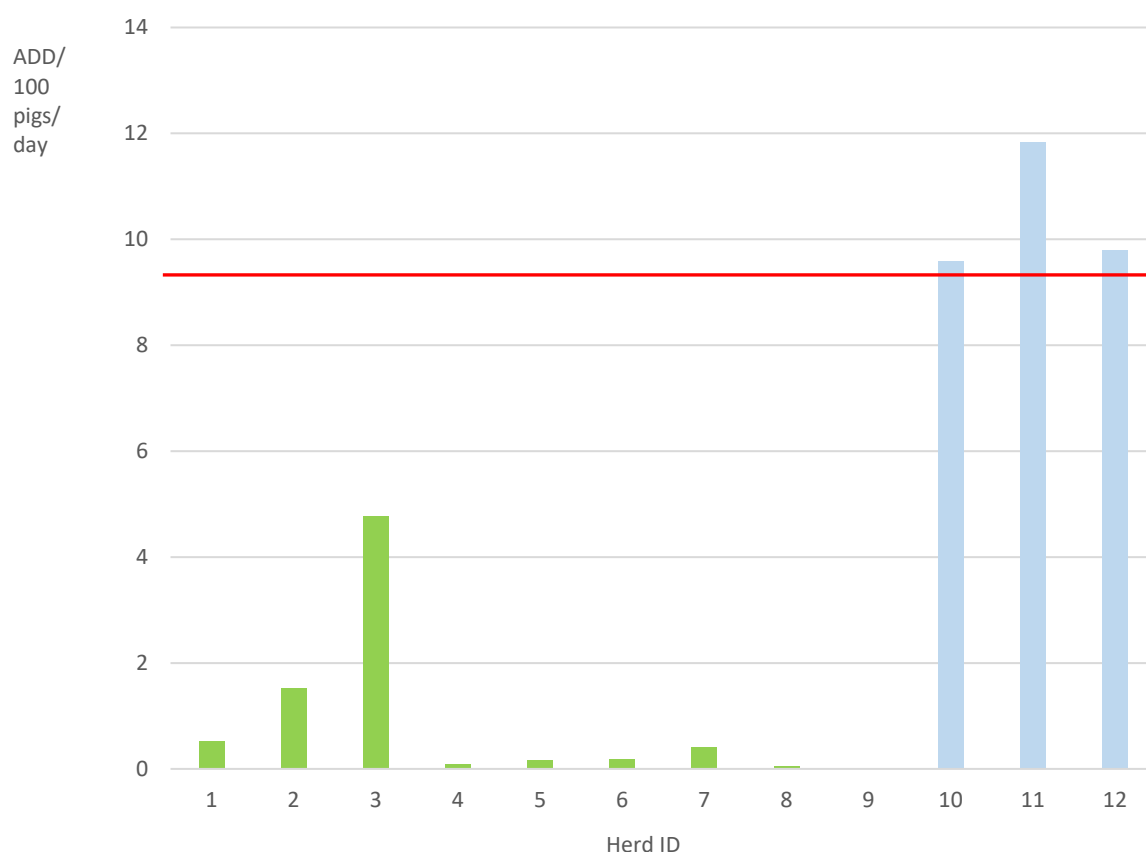


Figure 1. Usage of antibiotics for weaners in the twelve study herds during 2020. Data was extracted from the VetStat database. Organic herds are shown in green colour (Herds 1-8), non-organic in blue (Herds 9-12). The red line depicts the Danish national average for usage in weaners (9.3 ADD/100 pigs/day). Note that in organic herds, pigs are older and therefore heavier at weaning compared to non-organic pigs. Therefore, the same amount of antibiotics can be used for fewer animals in these herds.



## Clinical findings

In both organic and non-organic herds, the most prevalent clinical findings registered during the visits were ear wounds, diarrhoea, and low body condition. Table 2 provides an overview of clinical findings across herds and visits.

Table 2. Clinical findings in 8-week-old weaners in the twelve study herds. The table shows mean herd prevalence as well as minimum and maximum prevalence within herds [in brackets].

	WL organic (N=2400)	WL non-organic (N=1962)
Ear wounds	33.5% [19-54%]	19.0% [12-27%]
Diarrhoea	19.0% [6-53%]	27.3% [11-50%]
Low body condition	7.8% [5-13%]	10.1% [5-19%]
Unthriftiness	2.7% [0- 5%]	4.5% [3-9%]
Tail bite	2.4% [0-5%]	0.8% [0-2%]
Hernia	1.1% [0- 2%]	1.9% [1-3%]
Airway problem	0.6% [0-2%]	1.9% [1-4%]
Leg problem	0.4% [0-1%]	0.8% [0-2%]

Not least for diarrhoea, we saw much variation between visits, pens and herds. On average, 19% of pigs in WL organic herds and 29% of pigs in WL non-organic herds were diarrhoeic at the time of examination. Fig. 2 shows the variation in prevalence of diarrhoea between pens and herds. For simplicity, the variation between visits is not shown explicitly, but lies within the pen variation. As seen from Fig. 2, two herds stood out with a markedly higher prevalence of diarrhoea than the others. One of these herds did not use pZnO throughout the study period.

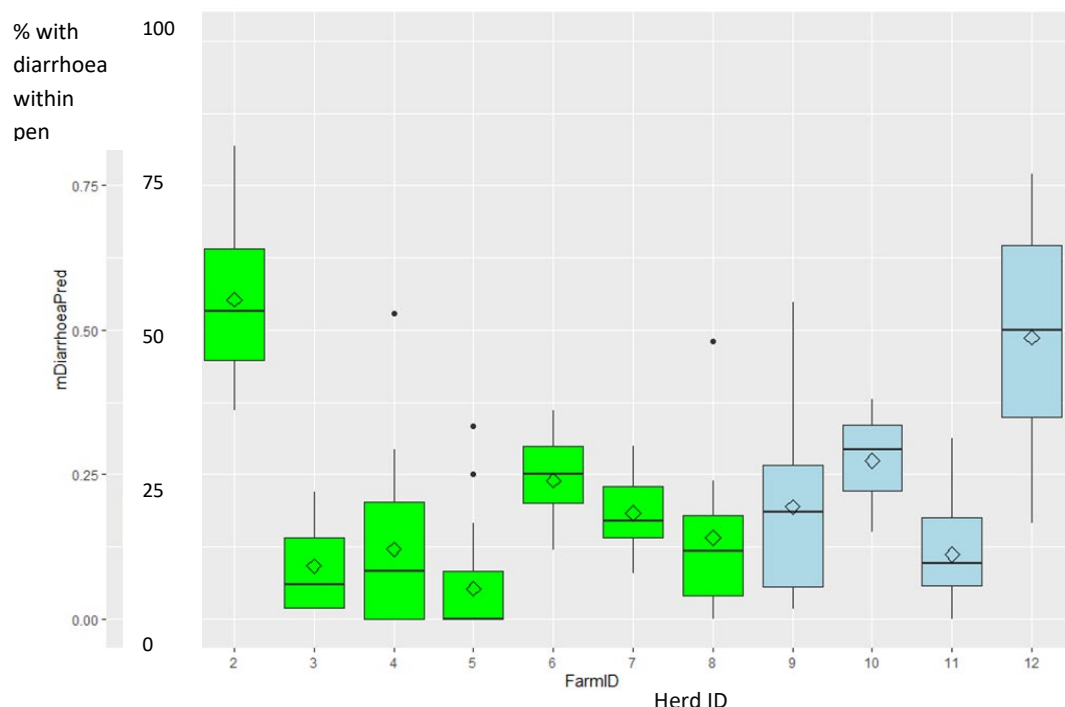


Figure 2. Boxplots depicting the prevalence of diarrhoea within pens during the three visits. Diamonds indicate means. Organic herds are shown in green colour, non-organic in blue. Herd 2 did not use pZnO.

The only risk factor to be associated with diarrhoea in the multivariable model was the production system. Thus, we found an OR of 2.4 for WL non-organic systems compared to WL organic systems ( $P=0.07$  (statistical tendency)). This effect was no longer present when the model was run with Herd 2 (the herd not using pZnO) included ( $P=0.34$ ).

### ***Measures to prevent diarrhoea postweaning***

Around half of the herds in the study occasionally used metaphylactic treatment with coccidiostats (in the farrowing field) and anthelmintics (post-weaning) for either all or a subgroup of pigs. Thus, 38% of WL organic and 50% of WL non-organic herds used coccidiostats for the main period during the study. Fifty percent and 75% of WL organic- and WL non-organic herds, respectively, used anthelmintics in the majority of batches. The farmers considered these treatments protective for post-weaning diarrhoea. The most valued preventive measure, however, was pZnO as an additive in feed. As mentioned, pZnO was used as a routine by 11 of 12 herds in the study. The farmers generally considered pZnO necessary in order to obtain a low usage of antibiotics and a low level of diarrhoea in the post-weaning period. In around half of the herds, less than the allowed dosage or a shorter treatment period than the allowed was applied. Supplements with organic acids in feed or water were used in all of the herds, except in two organic herds.

Management measures considered efficient by the alternative farmers to prevent diarrhoea post-weaning fell in two categories. Many of the farmers mentioned measures that are also routinely applied in conventional systems. This included e.g. cleaning and disinfection of weaner pens between batches. In most cases, the herds used relatively long periods of time (4-7 days) for drying out of pens between batches.

Vaccines against diarrhoea post-weaning were also very often highlighted as important tools by the farmers. During the study period, 50% of the herds (50% of WL organic- and 50% of WL non-organic herds) vaccinated their pigs against *E.coli* using Coliprotec F4/F18®. In total, 58% of the herds (75% of WL organic- and 25% of WL non-organic herds) used vaccines against *Lawsonia intracellularis*. Several farmers highlighted the positive effect of change of vaccine against *Lawsonia intracellularis* from an oral- into an injectable type. One farmer had another example on how elimination of the negative effect of pathogens had been beneficial to pig health. In his case, a complete depopulation-repopulation had been carried out in order to eliminate PRRS and *Actinobacillus pleuropneumoniae* type 2 from his herd. He highlighted, that after repopulation, all types of disease problems, including diarrhoea, were significantly reduced.

Feeding management and high quality feed ingredients were very often mentioned as a prerequisite for avoiding diarrhoea. Within this category, restrictive feeding procedures, customized feed for the specific age group and size of pigs, low level of protein and high levels of fishmeal in the diets were the factors most often mentioned. Other traditional measures considered by the farmers to increase the robustness of the pigs around weaning was floor heating in the lying area, extra supplements with easily accessible water and supplement with electrolytes in the water.

Another category of measures highlighted by farmers was related to the alternative system and the more natural conditions offered to the pigs within these systems. Later weaning (1-4 weeks later than in conventional systems) was by many considered essential to avoid diarrhoea post-weaning. Some of the organic farmers experimented with weaning later than required by the organic regulation (typically, at 8-9 weeks of age) and with weaning outdoor in fields. In both cases, they saw a beneficial effect on the robustness of the pigs in terms of reduced problems with diarrhoea.

Some farmers assumed the uptake of grass, soil and dirt during the suckling period to benefit the intestinal environment and microflora and thereby to be protective against diarrhoea. Some farmers also offered the pigs soil in the weaning stable in order to support the microflora and to entertain the pigs. All herds used silage as roughage. The quality of silage was highlighted as beneficial for health by some of the farmers who stressed that if of good quality, the pigs would eat a significant amount of it and thereby obtain a better gut health. Another highlighted element, related to raising in alternative systems, was the straw bedding in the pens. Some farmers stressed that the warmth and comfort provided by the straw bedding supposedly had a beneficial effect on health, both mentally and physically.

Organic farmers with previous experience from conventional farming, emphasized how the organic regulation on antibiotic usage had changed their way of reacting in cases of disease. As conventional farmers, they had commonly used pen treatments against diarrhoea post-weaning. As organic pigs would lose their organic status after a second treatment with antibiotics, pen treatments are not considered an option in these systems. Thus, regulatory restrictions had had a significant effect on their usage of antibiotics. These farmers also stressed that the restrictive regulation motivated them to look for alternatives (e.g. extra supplement with water and electrolytes) or sometimes to wait and see rather than immediately turn to treatment, as they would previously have done. Their experience was that often it turned out that no treatment seemed necessary when the pigs were examined later in the day or on the following day.

## Discussion

All WL organic and one of the WL non-organic herds in the current study had a markedly lower usage of antibiotics compared to the Danish national average. This is in line with previous register-based studies, showing a generally lower usage of antibiotics in WL systems (Nielsen et al., 2021). The clinical examinations carried out in the current study showed a relatively low level of disease with ear wounds and diarrhoea being the only disease occurring in more than 10% of the examined animals. Thus, since the pigs in the study were generally healthy, the study did not indicate problems with inadequate treatment in alternative systems, despite low usage of antibiotics.

In a roughly comparable Danish study in conventional herds from 2015, on average, 32% of pigs had diarrhoea (Weber et al., 2015). Thus, the alternative herds in the current study generally had a lower level of diarrhoea. In our study, all examined pigs were eight weeks of age, whereas in the study by Weber et al., the range of age was wider, from six to thirteen weeks. This discrepancy may play a role for comparison. In a previous study on alternative pigs of 10 to 50 kg, we also found a very low level of diarrhoea. On average, 1% (0% to 4% within herds) of the 3294 pigs in this study were diarrhoeic at

the time of examination (Kongsted et al., 2018). However, in the study from 2018, rectal examination was not performed.

In the current study, we found a higher prevalence of unthrifty animals (3.3%) than in the previously mentioned study in conventional herds in 2015. In that study, only 0.4% of the animals were considered unthrifty by the examiners. The reason behind this discrepancy may be that suckling pigs in alternative systems live a more unprotected life than indoors. Furthermore, pigs with special needs during suckling are more difficult to detect and eventually treat in outdoor systems. In the study from 2018 on 10-50 kg alternative pigs, we saw a comparable level of unthriftiness of 4% (Kongsted et al., 2018).

The relatively low level of diarrhoea compared to conventionally raised pigs seems to indicate, that pigs in these systems are more resilient to disease than conventional pigs. It is not obvious from the results of the study, which elements in raising contribute to a higher robustness. Nevertheless, there was a tendency for the organic production system to be protective against diarrhoea. Organic pigs are weaned later and are provided more space than non-organic weaners. Both elements could likely play a role as supported by previous studies (Pluske et al., 2003, Madec et al., 1998). In WL systems, pigs have access to soil and grass during early life, and these elements may benefit to a more diverse microflora, as suggested by the farmers. Also, provision of roughage could offer an advantage in relation to intestinal health.

Perhaps surprisingly, pZnO was the most often mentioned preventive measure when farmers were asked about their ways to avoid post-weaning diarrhoea and to achieve a low level of antibiotic usage. The high level of diarrhoea in the herd not using pZnO in the study, supports this hypothesis, though other explanations may exist. Traditional measures like cleaning and disinfection and high hygienic standards were highly valued by the farmers. Vaccines were also very widely used by herds in the study. In total, 4% and 17% of pigs raised in Denmark are vaccinated against *E.coli* and *Lawsonia intracellularis*, respectively (data on vaccine purchase extracted from [www.vetstat.fvst.dk](http://www.vetstat.fvst.dk), data on population size extracted from [www.dst.dk](http://www.dst.dk)). Thus, it seems that the Welfare Label herds have a more extensive use of vaccines against diarrhoea in weaned pigs compared to conventional herds. This may contribute to the relatively low level of diarrhoea in these herds.

The reflections by the farmers on how the restrictive legislation on antibiotics affects their approach towards treatment is interesting and suggests that restrictions can be constructive in processes of change. Overall, the study suggests that the alternative housing and management strategies in Welfare Label herds should be used as an inspiration for change in the conventional usage of antibiotics while at the same time attending to animal health and welfare.

### Acknowledgement & Funding

We would like to thank all farmers who participated in the study as well as technicians and students who assisted during the examinations in the herds. This work was partly funded by the Ministry of Environment and Food of Denmark as part of the “Contract between Aarhus University and Ministry of Environment and Food for the provision of research-based policy advice at Aarhus University”.

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\*: Danish national regulation requires that all pig farmers with 300 sows, 6000 weaners or 3000 finishers have a Veterinary Advisory Service Contract agreement with a veterinarian. The veterinarian consults the farm regularly (monthly in sow herds) and prescribes all medicine needed. The farmer is allowed to store the medicine and use it according to the instructions from the veterinarian.

\*\* : Animal Daily Dosage is a way to generalize different types and concentrations of antibiotics in order to make them comparable. For the calculation, a standard weaner weight of 15 kg is used.

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## Anesthetic incidents, labour time and occupational safety of automated isoflurane anaesthesia during piglet castration

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### Introduction

Since the beginning of 2021 it is prohibited to castrate suckling piglets without anaesthesia in Germany. According to legal regulations the farmers are allowed to perform the anaesthesia by themselves using a certified automated isoflurane device after they completed a two-day course and passed a written and an oral examination. In the present survey the implementation of the automated isoflurane anaesthesia for suckling piglet castration on conventional breeding farms with different anaesthetic devices was evaluated (1). Therefore, the depth of anaesthesia based on defensive movements, anaesthetic incidents, labour time required in contrast to anaesthetic-free castration and occupational safety were investigated.

### Material & Methods

A total of 129 farrowing batches including 11576 under eight days old piglets of 15 farms were investigated concerning various parameters of the castration procedure under field conditions and during routine animal management. On each farm ten batches of castrations under isoflurane anaesthesia (IA) were evaluated and compared to one batch of piglets castrated without anaesthesia (anaesthetic free = AF) before 2021. All male piglets (including AF and IA batches) were administered an analgesic treatment (Meloxicam 0,4 mg/kg, Metacam® 5 mg/ml i. m., Boehringer Ingelheim Vetmedica GmbH, Ingelheim/Rhein) at least 30 min before castration and additionally internal management procedures (e. g. ear tagging, iron supplementation, vaccination programme, tail docking) were carried out. Anaesthesia was performed using the PigNap 4.0 (BEG Schulze Bremer GmbH), PorcAnest® 3000 (Promatec Automation AG) or Anestacia® (GDO Precision Technology GmbH) anaesthetic device on five farms each. The narcotic gas (5 Vol % mixed with room air as carrier gas) was released for 70 seconds over the provided masks. After checking that the interdigital claw reflex was negative to assure sufficient anaesthetic depth, the castration was performed using a scalpel (AF) or emasculator (AI). In case of a positive interdigital claw reflex the anaesthesia was prolonged.

Defensive movements and anaesthetic incidents (apnea, cardiovascular arrest, gasp) during the anaesthesia were documented. The required working time was assessed divided into time for preparation, administration of preoperative analgesic, and post-processing as well as for the complete castration process in three IA batches and one AF batch. Isoflurane concentration in the ambient air was examined at five defined measuring points on each farm twice.

## Results

Anaesthetic incidents occurred in 1.7 % (n=201) of the piglets. The most common incident was apnea in 66.7 % of the cases. The mortality rate of the 129 IA batches was 0.1 % (n=11). In the AF batches the mortality rate was 0.4 % (n=6). The required labour time for the castration process itself did not differ significantly between AF ( $0.9 \pm 0.4$  minutes) and IA ( $1.0 \pm 0.3$ ) batches whereas the required time for the complete working progress differed significantly between  $1.7 \pm 0.8$  minutes in the AF batches and  $2.2 \pm 0.8$  minutes in the IA batches.

Table 1: Mean value of the required working time in minutes for single process steps and complete process.

Required time (in minutes)	AF runs (n = 14)		IA runs (n = 45)	
	mean $\pm$ SD	min/max	mean $\pm$ SD	min/max
<b>Preparation (total)</b>	$2.8 \pm 1.6$	1 - 6	$17.3 \pm 8.9$	5 - 37
<b>Analgesia (per piglet)</b>	$0.7 \pm 0.4$	0.1 - 1.6	$0.5 \pm 0.3$	0.1 - 1.1
<b>Castration (per piglet) *</b>	$0.9 \pm 0.4$	0.4 - 2.0	$1.0 \pm 0.3$	0.3 - 2.0
<b>Post-processing (total)</b>	$4.4 \pm 3.7$	1 - 14	$21.1 \pm 7.4$	5 - 40
<b>Complete process (per piglet)</b>	$1.7 \pm 0.8$	0.9 - 3.6	$2.2 \pm 0.8$	0.8 - 4.6

The mean isoflurane concentration that was measured in the respiratory air of castrating persons and persons transporting the piglets was  $5.8 \pm 5.5$  mg/m<sup>3</sup> and  $4.8 \pm 2.2$  mg/m<sup>3</sup>, respectively. No significant differences between the devices were detected ( $p > 0.05$ ).

## Discussion

The aim of the study was to evaluate the castration of suckling piglets under automated isoflurane anaesthesia on conventional swine farms regarding animal welfare aspects, cost-effectiveness and occupational safety. Even though Härtel et al. (2) and Wenger et al. (3) reported fewer anaesthetic incidents after inhalation anaesthesia, the number of anaesthetic incidents in the present study can be considered low as the castrations were performed under less controlled conditions on conventional

working farms. Moreover, the lower mortality rate after isoflurane anaesthesia compared to the AF batches and the results of the occupational isoflurane exposure below the internationally lowest value of 15 mg/m<sup>3</sup> indicate that the method is safe for the animals and the operator. Though the complete castration process under isoflurane anaesthesia is more time-consuming than the anaesthetic free castration process, the less strongly differing time for the castration itself underlines that it is crucial to consider the number of piglets per farrowing batch when comparing methods. These findings are similar to other studies (4, 5, 6). Still, the improvement in terms of animal welfare and the shown user-safety predominate the evaluation.

### Acknowledgement & Funding

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## Hygienic samples in a mobile house for laying hens

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### Introduction

As an animal friendly and environmental sound alternative to static housing systems, mobile houses for laying hens have gained increasing interest over the last years. However, with regard to biosecurity, uncertainties in the possibilities to establish hygienic measures in mobile houses exist, and hygiene barriers in these housing systems are an exception. Therefore, a concept consisting of a modified mobile house and a management package was developed, and the feasibility of the implementation was investigated. The aim of the study was to evaluate the effect of the hygienic measures on bacteria occurrence.

### Material/Methods

A prototype of a mobile house for 315 laying hens with an integrated hygiene barrier was tested under practical conditions. Samples (sock swabs) of the different stable areas (outside the mobile house, black and white part of the hygiene barrier, inside the mobile house) were taken during two laying periods every four weeks in routine operation. During the period when the hens were kept indoors, samples were taken every two weeks. Furthermore, after each laying period, the mobile house was cleaned and disinfected, and control samples (sock swabs and swabs from the slatted floor) were taken. The total bacteria count (TBC) and *Escherichia coli* (*E. coli*) were determined and evaluated descriptively.

### Results

The course of TBC stayed on the same level during the two laying periods, and was similar for all sampled areas. Hence, no differences were found. Regarding the course of *E. coli*, no differences between the black and white part on each side of the hygiene barrier were determined. When the hens were kept indoors, during both laying periods the amount of *E. coli* outside of the stable decreased, whereas *E. coli* remained on the same level inside the stable. The amount of TBC in the mobile house decreased after cleaning and was reduced further after disinfection in both laying periods. No *E. coli* were detected after cleaning.

### Conclusion/Discussion

In this study, it can be assumed that cleaning and disinfection of the mobile house is relevant to reduce the bacteria occurrence. The course of TBC did not reveal if the implementation of hygienic measures in mobile houses was effective. An indicator bacterium that only occurs outside the stable could be helpful to determine if a hygiene barrier prevents the carry-over of this bacterium into the mobile house.

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## Abstracts Session 9

### Disinfection, Probiotics, ...

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#### Use of probiotics containing lactobacteria to improve the microclimate and foot health of broilers

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Promoting animal welfare is a very important topic in agriculture, especially in production conditions. Current issues in poultry farming are animal density, litter and air quality, which have a direct impact on birds' health and productivity. The aim of the study was to determine the effect of the application of the probiotic *Lactobacillus farciminis* CNCM-I-3699 and *Lactobacillus rhamnosus* CNCM-I-3698 complex (on feed and on litter) on air (ammonia content) and litter quality (volume and humidity) to improve broiler well-being and foot health.

In a trial 260 one day old Ross 308 broiler chicks were randomly divided in two groups – the control group (Con) n=130 and the probiotic group (ProX) n=130. The birds were placed in closed and ventilated identical pens with full microclimate control and ammonia content monitoring. 20 kg of wood shavings with 15% moisture were used for bedding. At the probiotic group the mixture of lactobacteria was manually spread 10g/m<sup>2</sup> before the chickens were placed, and it was spread manually once a week on the litter, each time 5g/m<sup>2</sup>. Bird density, housing and microclimatic conditions were assimilated to production conditions. The amount of water consumed was measured for each group. The dietary treatments was basal diet for the Con group and basal diet + the mixture of lactobacteria 4g/10kg for the ProX group. Broilers were raised till day 35. Litter weight and moisture was determined for each group at the beginning and end of the study. The health status of broiler foot pads was graded in scale from 1 to 3 (0 - no lesions, 1 - superficial lesions, 2 - lesions of the upper layers of the skin, 3 - lesions of the deepest layers of the skin).

The total weight of bedding in the control group reached 157.5 kg, but in the probiotics group 154 kg at the end of the study, which is 2.3% less than in the control group. The moisture content of the litter in the free zone and in the feed zone was similar, ProX 41.4%, 28.3% and Con 40.4%, 26.9%, respectively. The wettest bedding was observed below the water lines, where the moisture of the Con group bedding reached 53.6%, but the ProX group bedding was 11.6% drier, respectively 42%. When analyzing the amount of NH<sub>3</sub> in the exhaust air, the average rank was calculated, accordingly for the ProX group it was 1362.59 and for the Con group 1596.41, which did not differ significantly in the

interpretation of the effect size ( $r = 0.11$ ). The study found that there was no significant difference in the scores of the birds feet between the groups ( $p > 0.05$ ), however, the control group with scores 3 had more foot damage.

Although there was not a significant difference in foot health between the groups, the probiotics group had lower litter moisture below the water lines, the total litter size was reduced, and the average ammonia rank was lower compared to the control group. In industrial production, this difference could be more substantial and improve the welfare of birds in general.

**Key words:** ammonia, *Lactobacillus farciminis*, *Lactobacillus rhamnosus*, litter moisture, foot pad dermatitis

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## Canine protothecosis – a genotyping and meta-analytical study

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### Introduction

The *Prototheca* genus comprises unicellular, non-photosynthetic, saprophytic, microalgae, which are opportunistic pathogens of humans and many animal species, including dogs. Canine protothecosis typically presents as a subcutaneous infection following penetrating trauma, or as colitis, initially localized, which becomes more severe over time and eventually spreads haematogenously to well perfused tissues with vascular tortuosity. The disseminated form involves many organs (central nervous system, eyes, heart, kidneys, liver), causing a variety of symptoms and often life-threatening complications. So far, only one review has been published on canine protothecosis. It provided data on 44 cases, diagnosed in 20 countries, over 36 years. The aim of the present study was to perform a meta-analytical review of the literature on canine protothecosis to provide the most recent and most comprehensive picture of this rare infection. Furthermore, 28 *Prototheca* strains isolated from as many affected dogs were subject to species-level identification using the current standard, cytochrome B gene sequencing method.

### Methods

Five e-databases. i.e. PubMed, Google Scholar, Medline, Web of Science, and Scopus-were searched for literature on canine protothecosis, published over a 60-year period (i.e. between 1963 and 2020). The following keywords, and their combinations, were used: “Prototheca”, “protothecosis”, “dog”, and “canine” with their French, German, Italian, and Spanish equivalents. The articles retrieved were analyzed with regard to clinical, epidemiological, and microbiological features of the disease.

Twenty-eight *Prototheca* canine isolates, genotyped in this study, mostly representing systemic infections (12; 42.9%) came from Germany (8; 28.6%), Brazil (7; 25%), Italy (7; 25%), Australia (3; 10.7%), Japan (2; 7.1%) and the UK (1; 3.6%).

## Results

A total of 69 articles describing 101 cases of canine protothecosis were found and included in our meta-analysis. Most of the cases came from North America (44; 43.6%), followed by Europe (28; 27.7%) and Australia (18; 17.8%), South America (5; 4.9%), Asia (4; 4%), and Africa (1; 1%).

The disease mostly affected pedigree dogs (81; 79%), with a skew towards females (61 or 60.4% vs. 37 or 36.6%). The median age, on diagnosis, was 5 years (range, 3 months to 14 years). The disease occurred chiefly in a systemic form (69; 68%) with manifestations in brain, eyes, heart, kidneys, and gastrointestinal tract. Treatment included, in majority of cases (31; 30.7%), azole drugs, with itraconazole being administered in 19 (18.8%) cases. The overall treatment success rate was only 6.9% (7/101). As many as 76 (75.3%) of the animals did not respond to therapy and ultimately died.

According to literature review, *P. zopfii*, currently known as either *P. bovis* or *P. ciferrii*, was shown as a predominant etiological agent (33 or 32.6% of cases). Of 28 *Prototheca* canine isolates genotyped in this study, 21 (75%) were *P. bovis*, 5 (17.9%) – *P. wickerhamii*, and 2 (7.1%) – *P. ciferrii*.

## Conclusions

This study delivers the most extensive and up-to-date overview of canine protothecosis casuistry. Although rare, the disease has a worldwide distribution and a wide breed coverage. With elusive onset, aggressive clinical behavior, drug refractoriness, poor prognosis, and high mortality rate, canine protothecosis poses a serious veterinary health problem which deserves special attention necessitating search for new methods of treatment and prevention.

## Efficacy of disinfection of various structural timbers in livestock farming

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### Introduction

For decades, wood had been one of the most used building materials in agricultural constructions. Especially in livestock farms, due to hygienic requirements, the disinfectability of wood is a highly debated topic. Former studies evaluated the sanitary characteristics of timber as rather poor and raised concerns about the hygienic safety. More recent studies show an antibacterial effect of wood and even recommend the use in hygienically critical areas. In addition, due to the climate crisis as a highly risk of our society, the use of wood as a renewable raw material for construction trade is becoming increasingly attractive. To evaluate the hygienic aspects of various structural timbers in livestock, this study investigates the sanitization of different structural timber by routinely used chemical disinfectants for large area surface application in animal husbandry.

### Material & Methods

The effect of three different surface disinfectants (peracetic acid, glutaraldehyde, formic acid) each in combination with three different wood types (Spruce (*Picea abies*), Pine (*Pinus sylvestris*), Poplar (*Populus* spp.)) was investigated. Surface test was carried out according to the guidelines of the German Veterinary society (DVG) for efficacy testing of disinfectants in livestock area in accordance with EN 16437. For this purpose, *Enterococcus hirae* (*E. hirae*) was chosen as surrogate for gram-positive and *Pseudomonas aeruginosa* (*P. aeruginosa*) for gram-negative bacteria. The surface tests were performed by +10°C and -10°C, respectively.

### Results

The tests initially performed at +10°C showed that peracetic acid and glutaraldehyde exhibited effective disinfection on all wood substrate types at practical concentrations by 0.5% and 1%. In contrast to this, the disinfection success of formic acid seemed to depend on the type of wooden carrier. An effective disinfection was achieved on pine with a concentration of 4.5%. The other two types of wood needed concentrations higher than suitable for practical use (>7%). At +10°C *E. hirae* required higher disinfectant concentrations than *P. aeruginosa* for successful disinfection. This was the case for all possible combinations of these tests. Subsequent tests at -10°C were conducted with *E. hirae* as the restricted bacteria. Here, only peracetic acid at a concentration of 1.5% showed effective disinfection of all wooden carrier types.

### Discussion

The results suggest that a routine, practical and effective disinfection of wood is feasible. However, a suitable disinfectant requires careful selection. As only peracetic acid was able to achieve a sufficiently disinfecting effect, we conclude that peracetic acid could be successfully used as a disinfectant in

wooden stables. Further investigation concentrate on the effectiveness of wood disinfection under biofilm formation of the investigated bacteria.

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## Development of a rapid molecular assay for validating the efficacy of air purifier in virus inactivation

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### Introduction

The importance of air purifiers has increased in recent years, especially with the COVID-19 pandemic. The air purifiers rely either on the removal of harmful substances from air or the direct inactivation of infectious particles. The efficiency of various systems is usually determined under laboratory condition before widespread application. The standard procedure for testing depends on virus cultivation and titration on cell culture. This, however, requires several days to deliver results. A molecular assay which can differentiate between intact and distorted virus particles can speed up the screening procedure. In this study, the applicability of combining reagents to degrade or eliminate the free nucleic acid and real-time reverse transcription polymerase chain reaction (RT-PCR) or isothermal amplification assay (recombinase aided amplification, RAA) were examined as method of choice for determining virus inactivation. Feline Coronavirus (FCoV) was selected as model for screening.

### Material & Methods

In order to remove any free nucleic acid from inactivated viruses after applying the air purification, the samples were treated with the universal nuclease or propidium monoazide (PMA). Thereafter, nucleic acid extraction from intact viruses was performed by magnetic-beads based method. RNA of FCoV was quantified before and after applying the air purification. Positive control was the original virus stock nebulized to test the efficacy of the air purifier. All experiments were performed in triplicates and compared with the virus infectivity on cell culture.

### Results

The free RNA was completely degraded using the universal nuclease, while PMA efficacy was limited to 40%. Both RAA and real-time RT-PCR produced similar results to the infectivity assay on cell culture. Nevertheless, RAA run time was 15 minutes in comparison to 2 hours in real-time RT-PCR.

### Discussion

To conclude, this study revealed a promising rapid method to differentiate between intact and inactivated FCoV particles based on universal nuclease pretreatment and RAA assay. The method is rapid and produce similar results to the standard infectivity assay on cell culture.

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## Raising awareness and hygiene practices for paratuberculosis in Africa

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### Abstract

*Mycobacterium avium* subsp. *paratuberculosis* (MAP) has been studied extensively in Europe and the United States of America. MAP occurrence, distribution, risk factors and economic impacts have not been fully investigated in Africa. Around 19 African countries have reported the diseases. The few published reports on MAP in sub Saharan Africa came mainly from Sudan, Uganda, Tanzania, Kenya and Ethiopia. However, this does not mean that other countries in the region are free from the disease. We have created a social media initiative to raise awareness about MAP as a neglected disease in Africa. The consortium has established a series of summer schools to train African young scientists on case identification, and laboratory diagnostics as well as hygiene practices. Two MAP molecular detection mobile laboratories have been deployed in Africa to enhance epidemiological studies. Moreover, workshops with decision makers and farmers have been conducted to identify research gaps and a simple web-based platform is being planned to allow community members to report suspected cases in domestic as well as wild animals. Future studies shall focus on creating a disease control strategy suiting the African settings. Zoonotic potential of MAP in Africa is another research gap which needs to be addressed since the relationships between livestock and humans in Africa are very strong, as in some communities in Africa, humans share the dwelling and water sources with their livestock. Furthermore, most of the animal products are consumed untreated or under-cooked by humans. In conclusion, our activities helped in increasing the knowledge about the disease in Africa and would provoke international foundations and local stakeholders to allocate funds for more research on MAP in Africa and on establishing control programmes.

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## Poster Abstracts

### Poster 1

#### **Development of an economic model to assess the cost-effectiveness of biosecurity measures to reduce the burden of *Salmonella* and Hepatitis E virus in the pork production chain**

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#### **Introduction**

The objective of this study is to evaluate the cost-effectiveness of biosecurity measures (BSMs) in the pork production chain to reduce the burden of *Salmonella* spp. and Hepatitis E virus (HEV) in the countries of the European Union (EU). Pork is the second most common source of foodborne *Salmonella* outbreaks, having caused 11 outbreaks in 2020 in the EU alone. In addition, a significant increase in HEV zoonotic cases related to pork consumption in the EU has been observed during the last decade (i.e. >20,000 clinical cases, 28 fatalities). The impact of the infections on humans depends on multiple factors, such as the individual's immune status and age. Nevertheless, the economic consequences on the public health sector in the EU are significant, i.e. salmonellosis cases attributable to pork result in approximately € 90 million annually (FCC Consortium, 2010).

#### **Material & Methods**

The economic approach of this study uses the results of two different quantitative microbial risk assessment (QMRA) simulation models (Snary et al. 2016, Wilkins, 2022, McCarthy et al. 2021) which estimate the prevalence of the pathogens across the entire pork chain with and without implemented BSMs. Depending on the effectiveness of selected BSMs, the numbers of human salmonellosis and HEV cases originating from the consumption of pork are assessed with the epidemiological models. A literature-based meta-analysis within the BIOPIGEE project delivered the effectiveness values for the BSMs (Huber et al. 2021).

To translate exposure into cases, a dose-response model is used. Based on these calculated changes in human incidence rates, economic evaluation including cost-benefit analysis followed by optimization analysis is conducted. The total costs estimated comprise the costs related to the specific BSMs implemented at respective stages along the pork chain. The monetary benefits will result from the costs avoided due to the reduction of human cases and the reduction of prevalence in primary production, which might increase the productivity and/or optimize the market prices. The economic analysis will build on these identified costs and benefits.

## Results

Based on the epidemiological output, the results of the economic model (i.e. net benefit value (NBV) and benefit-cost ratio (BCR)) will suggest the most cost-effective BSMs in terms of the reduction of the above-mentioned pathogens. In the final stage of the project, the economic model will be transformed into a user-friendly web tool (e.g. Shiny App). Taking the national epidemiological situation in the respective EU countries into account, this tool will support the decision-making process of the national authorities regarding the implementation of the economically optimal BSMs.

## Discussion

Since the occurrence of *Salmonella* and Hepatitis E virus is considered a public health problem in the EU having numerous health, social and economic implications, it is of utmost importance that the pathogens are better controlled and considerably reduced through the implementation of cost-effective measures, such as BSMs. This will contribute to consumer protection by lowering the burden of the above-mentioned zoonotic pathogens and increase trust in food safety.

## Acknowledgement & Funding

This study is part of work-package (WP) 4 within the One Health EJP BIOPIGEE project. BIOPIGEE, in specific WP2, WP4, WP5 are acknowledged due to their contributions and support of this study through their acquired data.

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## Vaccination of pigs against *Lawsonia intracellularis* can help to improve efficiency and with this have a positive effect on sustainability

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### Introduction

One of the most important enteric diseases in swine is caused by the bacterium *Lawsonia intracellularis* (*Li*). After entering the gut, the pathogen colonizes the ileal enterocytes. Infected animals react depending on their age at the time of infection as well as influenced by other factors, differently. So that symptoms can vary between subclinical ileitis with reduced growth performance and decreased feed conversion without markedly clinical illness up to diarrhoea and bloody diarrhoea that can peak in sudden death. Regardless of symptoms even subclinical *Li* infections result in economic losses. Furthermore, the reduction in animals' performance has an impact on feed efficiency with resulting ecological consequences. The question appears whether a prophylactic approach of intramuscular vaccination can not only reduce clinical occurrence of the disease but also improve performance and efficiency of pig meat production.

### Material & Methods

Groups of animals were vaccinated with an intramuscular *Li* vaccine on six farms under field conditions. Farms varied in size, clinical signs and strategy of treating *Li* infection and were all detected positive for *Li* before vaccine introduction. In the vaccinated (vac+) and comparison group (vac-) performance parameters (daily gain, total losses, feed conversion ratio, antibiotic use, economic result) were recorded and compared on farm level – detailed results are shown in a poster abstract.

**Table 3: Overview of the farms: structure, clinic, previous measures, details of *Lawsonia intracellularis* intramuscular vaccination (individual / combined; age)**

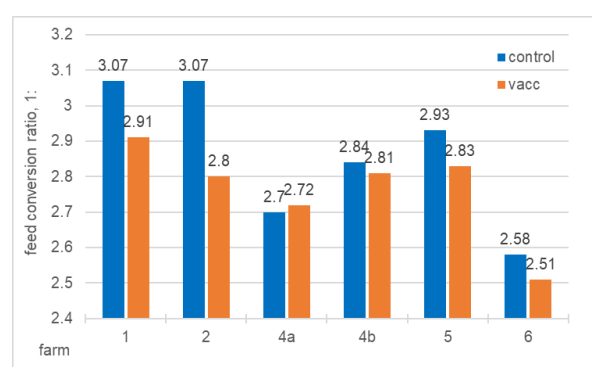
intracellular intramuscular vaccination (individual / combined, age)						
farm	1	2	4a	4b	5	6
fattening places, appr.	3500	1400	11500	5000	1920	1980
clinical expression	acute-chronic	acute	chronic		sub acute	acute
L.i. measure, so far	AB	oral L.i. vaccination			AB	AB
vaccination L.i. im single or combined	mixed in PCV & M. hyo combined vacc.		as single vaccine			
age / weight at vaccination	4. week				appr. 33 kg	appr. 26 kg
stalling / evaluation	time-shifted				at same time	time-shifted



To evaluate the impact on efficiency and sustainability a model calculation on the base of an average standard fattening feed mixture with 13.0 MJ ME, 16.4 % crude protein and 3.4 g phosphorus and an increase of bodyweight during fattening of 94 kg was used. Due to principles of DLG leaflet 418 the N and P excretion was determined on farm base. The CO<sub>2</sub> balance was calculated using the license-free program FeedPrint NL, version 2020.00-18 June 2020 from the University of Wageningen.

## Results

Clinical *Li* situation could be improved in all farms after using the i.m. vaccine. This was seen in the documentation of *Li* related symptoms and in the use of antibiotics particularly oral group medications. The performance data in all farms increased, especially feed conversion ratio was improved. Having a level of feed conversion from 1:2.58 up to 1:3.07 before i.m. vaccination the decrease reached values up to 0.27.



**Figure 3: Feed conversion ratio on the analysed farms before (control) and after (vacc) introducing the intramuscular *Lawsonia* vaccine**

The positive effect of an efficient use of feed (mean improvement of 0.1) resulted in a calculated reduction of nutrient excretions (-230 g N and - 35.1 g P per pig) and an improved CO<sub>2</sub> balance by 5,321 g per pig.

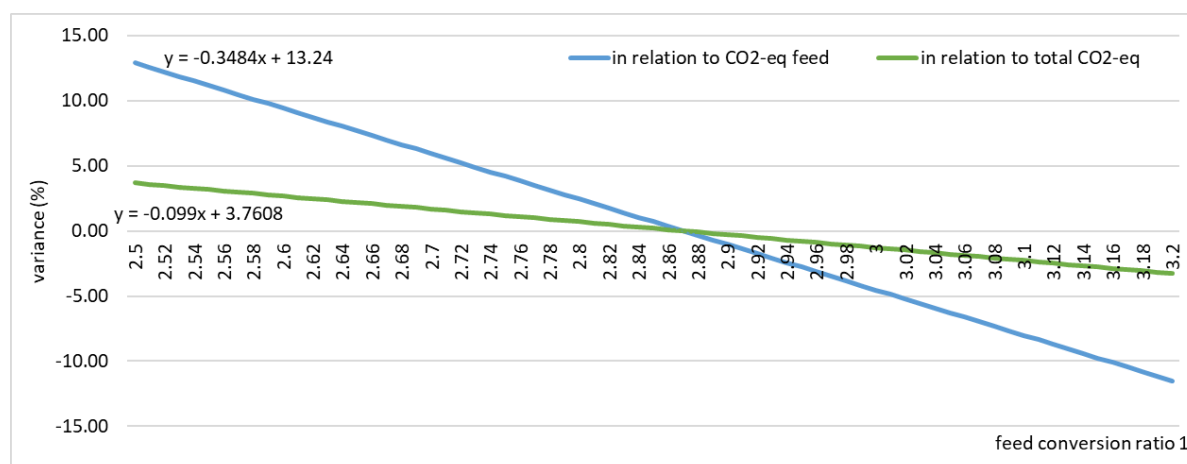
**Table 4: Influence of feed conversion on the evaluated farms and the resulting effects on N / P excretion and hectare (ha) requirement**

farm	number of animals	deviation FCR (%)	deviation N (%)	deviation P (%)	deviation ha-requirement N*	deviation ha-requirement P <sub>2</sub> O <sub>5</sub>
Surveyed from field observation			Calculated values based on standard fattening mixture			
1	2034	-5.21	-7.91	-9.40	-3.52	-3.08
2	419	-8.79	-13.34	-15.86	-1.22	-1.07
4a	11183	+0.74	+1.21	+1.50	+2.42	+2.11
4b	4142	-1.06	-1.67	-2.04	-1.34	-1.17
5	962	-3.41	-5.31	-6.40	-1.04	-0.91
6	1533	-2.71	-4.56	-5.77	-1.16	-1.01

\* incl. 20% stable and storage losses; at 170 kg N / ha and 85 kg P<sub>2</sub>O<sub>5</sub> / ha

## Discussion and Conclusion

The use of the intramuscular Lawsonia vaccine resulted not only in a major improvement of pig health in German fattening farms leading to a significant reduction in the use of antibiotics.



**Figure 4: Influence of feed conversion on the CO<sub>2</sub>-eq (g / kg live weight) compared to the CO<sub>2</sub>-eq feed and the total CO<sub>2</sub>-eq (g / kg live weight) with a feed conversion of 1: 2.87**

The clear positive effects on key production parameters furthermore resulted in a countable economic benefit for each farm. One highlighting parameter in this course is feed conversion. Due to climate changes and reduced availability of nutrients the efficiency of meat production is crucial. Even governmental restrictions are made to reach ecological goals during the next years and decades. The results shown are not a new approach but even more than a positive side effect: fattening of healthier pigs due to efficacious vaccination contributes to a more efficient and sustainable pork production.

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## CRISPR-Cas-based plasmid curing of conjugative AMR plasmids

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### Introduction

Bacterial resistance against antibiotics has become commonplace in medical and environmental settings. This development is particularly driven by conjugative plasmids carrying antibiotics resistance genes, which spread easily in environments of high bacterial density such as the gastrointestinal tract. Understanding the dynamics of spread of AMR genes among commercially raised animals is a crucial component of preventing spread to humans. We therefore sought to better understand the contribution of non-AMR genes on the maintenance of conjugative plasmids by curing environmental bacterial isolates of their conjugative AMR plasmids and comparing the resulting plasmid-cured variants to the parent strains.

### Materials and Methods

Molecular cloning methodologies were employed to generate pCBL from pFREE, a plasmid-borne CRISPR-Cas9-system [1]. Individual DNA fragments were either amplified by PCR or commercially synthesised and assembled into the final plasmid by Gibson assembly. Environmental *E. coli* isolates were transformed with pCBL and assayed for plasmid loss via PCR and phenotypic  $\beta$ -lactam resistance.

### Results

We constructed pCBL to address the unique challenges of curing conjugative AMR plasmids, such as the common presence of toxin-antitoxin systems and broad resistances conferred by the plasmids. We employ pCBL to produce plasmid-cured variants of ESBL/pAmpC-carrying *E. coli* strains isolated from broiler chickens and their environment by targeting the ESBL/pAmpC genes for double-strand break induction. The modularity of plasmid-based delivery enables a wide variety of antibiotics resistance cassettes and crRNA arrays to be used, broadening the potential host and target gene range.

### Discussion

We aim to provide a fast and reliable method of generating plasmid-cured variants to assess the physiological impact of plasmid loss in a broad range of isolates and to investigate non-resistance advantages of conjugative AMR plasmids. The targeted nature of the system presented here does not necessitate further downstream sequencing of plasmid-cured isolates.

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## The importance of prevention, prophylaxis and biosecurity on antimicrobials consumption and the spread of antimicrobial resistance

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### Introduction

Antimicrobial resistance (AMR) is now a major global health problem in human and animal population (Aidara-Kane et al., 2018; Robinson et al., 2016). There has been considerable pressure to reduce overall usage of antibiotics on animals or to cease or minimize using of human critical antibiotics. The most important step in preventing the development of AMR in livestock is to maintain good health in reared animals. At the same time, this doesn't only reduce the amount of antibiotics used, but also creates the preconditions for achieving genetically given production and reproduction indicators of animals that will lead to the farmers' profitability.

### Material/Methods

The aim of our work was to create a complex screening system for assessing influences of prevention, prophylaxis and biosecurity practices on reducing usage of antimicrobial substances and AMR developing. We resulted from a professional literature (PubMed, Web of Science) especially the practical experience of the authors.

### Results

Prevention, prophylaxis and biosecurity measures are critical in the control of all infectious microorganisms as they reduce the need for antimicrobials. Good breeding practice principles play very important role in improving the health status of animals (Postma et al., 2016). The basis of these procedures are, first of all, to ensure an adequate level of immunity after birth, optimization of the breeding environment, including minimization of animal stress, and adhering of the developed herd health plan. The fundamentals of prophylaxis are based on adequate level of innate and acquired immunity and implementation of a vaccination plan. Adherence to the principles of biosecurity significantly reduces the potential risk of infection penetrating on the farm (animals, people, feed and water, means of transport, etc.) and its spread in the farm area (optimization of technological systems, creation of barriers, quality control of raw materials and products, etc.).

### Conclusion/Discussion

Overuse and misuse of antibiotics of animals and humans is contributing to the rising of AMR (WHO, 2017). The decision to use antimicrobials to treat humans and animals must be based on the veterinarian's correct diagnosis, identifying the causative agent and therapy designing based on laboratory confirmation of antimicrobial susceptibility. Under no circumstances may antimicrobials in

livestock farms be used to compensate for low hygiene, shortcomings in the work of management, zootechnics and caretakers.

### **Acknowledgement & Funding**

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## NO HINT OF INTEGRATED IMMUNE EVASION CLUSTERS IN MRSA ISOLATES FROM LIVESTOCK AND HORSES

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### Abstract

Integration of prophages can play an important role in *Staphylococcus aureus* (*S. aureus*) virulence. Genes of the immune evasion cluster (IEC) can be integrated into the *S. aureus* chromosome by  $\beta$ -hemolysin (hly) converting phages. The occurrence of the IEC in clinical isolates from humans is typical. In methicillin resistant *S. aureus* (MRSA) clonal complex (CC) 398 isolates from pigs and pig farmers the frequency seems to be rare. To gain more information on the occurrence of the IEC in MRSA isolates from horses, pig barns and poultry barns a spa typing method and a Microarray-based analysis were conducted. Isolates obtained from different studies lead to a heterogenic origin. Fifty-seven isolates were confirmed as MRSA and five different spa types were detected. Most spa types belonged to MRSA CC398 to11 and to34, the widespread livestock associated MRSA (LA-MRSA). Among the isolates from poultry, two other MRSA types (CC5 and CC9) were identified. All MRSA isolates and the negative control (DSM 1104) shared the lack of the IEC. The genotype of a positive control (DSM 2569) indicated the expected integration of IEC type A, which harbored all genes of the IEC except for *sep*. Virulence characteristics of poultry isolates CC5 and CC9 were different from CC398 isolates. Clonal complex 5 and CC9 belonged to agr-type II and carried clearly more virulence genes. Isolates originated from healthy animals or from the animal environment and their role as potential animal or zoonotic pathogens remained unclear. Further studies are recommended to evaluate the pathogenicity and zoonotic potential of CC5 and CC9 from livestock.

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## Teat disinfection - (Co-)selection of udder pathogens and antibiotic-resistant bacteria? A longitudinal experiment

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### Introduction

Teat disinfection is a common method of hygiene management on dairy farms, which reduces bacterial colonization at the teat orifice, preventing new intramammary infections (Kumar et al. 2012). Resistance to applied concentrations of teat disinfectants has not yet been demonstrated *in vitro*. However, it is under debate whether application of teat disinfectants results in microbial selection, since bacteria differ in their natural susceptibility (Khan et al. 2017; Schwaiger et al. 2014). Such differences between bacterial species or strains could lead to changes in the bovine udder microbiota (Cassir et al. 2015). In addition, lower susceptibility to disinfectants may also be associated with lower susceptibility to antibiotics. Cross-resistance between chlorhexidine gluconate, used for teat disinfection, and several antibiotics has been reported (Köljalg et al. 2002). Considering this issue, the influence of different teat disinfectants on the susceptibility of microbiota and mastitis pathogens was investigated.

### Material & Methods

A 6-day intervention (14 days p.p.) was applied in a split-udder design, using two different postmilking teat treatments (lactic acid (LA) and chlorhexidine (CH) teat disinfectant) and an undipped control group. Quarter milk samples were collected before and after intervention and bacteria were cultured and identified by MALDI-ToF. The minimum inhibitory concentration (MIC) to the applied teat disinfectants was determined by macrodilution. MIC-values against antibiotics related to mastitis therapy were assessed using microdilution. Bacterial composition and changes in MIC-values before and after intervention were compared.

### Results

The proportion of *Corynebacterium* spp. increased significantly when LA was applied. After intervention with CH and LA, the percentage of coagulase-negative staphylococci (CoNS) decreased. The MIC-values of the bacterial community against LA were significantly higher in the LA-group after the intervention. A significant increase was also shown for CH-MIC-values upon LA-treatment. The antibiotic MIC-values of *S. aureus* and CoNS-isolates showed no significant differences before and after the intervention. However, there was a trend for CoNS to increased MIC-values towards penicillin after intervention with LA.



## Discussion

Changes in the bacterial community occurred as a result of the LA-intervention, while CH did not change the teat microbiome. This fact indicates that different bacteria are, *in vivo*, more equally reduced by CH, while LA selects corynebacteria to the disadvantage of others. MIC-values of disinfectants increased following LA-intervention. However, both applied teat disinfectants did not seem to co-select antibiotic-resistant bacteria. Further isolates (*Aerococcus viridans*, *Corynebacterium* spp., and *S. uberis*) will be tested for their antimicrobial susceptibility in order to substantiate the latter statement.

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## Clinical relevance of multi-drug-resistant pathogens in the occurrence of postoperative wound infections in horses

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### Introduction

The influx rate of multi-drug resistant pathogens (MDR) including methicillin resistant *Staphylococcus aureus* (MRSA) and extended-spectrum beta-lactamase(ESBL)-producing Enterobacteriaceae via colonized equine patients is important to know, since colonized patients provide a continuing source of MDR for other patients and the personnel.

### Material & Methods

In this study, 341 horses were screened for MDR-carriage at hospital admission, including patients suffering from colic (n=233) and (open) injuries (n=108).

For this, nostril swabs and fecal samples were taken for microbiological examination.

### Results

The detected introduction rates of MDR by colonized equine patients are alarming (nostril swabs 7.6%; fecal samples 12.3%). 10.7% of the horses tested positive for intestinal colonization with ESBL-producing enterobacteriaceae, while 3.5% were positive for nasal carriage of MRSA.

Nostril swabs were MRSA positive in 75% of the colic horses and only 25% of the injuries, which could be explained by a lack of hygiene in the practice when performing nasogastric intubation in colic patients.

48 of the colic horses and 104 injuries were subjected to (emergency) surgery. In the colic group, 11 horses developed a postoperative wound infection (WI), which was in 91% caused by MDR pathogens (81,8% ESBL-producing enterobacteriaceae). In the injury group 31 horses developed a postoperative wound infection, which was in 61,3% caused by MDR pathogens (41,9% MRSA, 38,7% ESBL-producing enterobacteriaceae). Antibiotically pretreated horses developed significantly more often WI than non-antibiotically pretreated horses ( $p = 0.002$ , OR 12.5, 95 % CI 1.6 – 95.4). Hospitalisation time in horses with WI was significantly longer than in horses without WI ( $p=0.01$ ).

### Discussion

In summary, wound infections were commonly caused by MDR pathogens, but the patients were rarely colonised with the specific pathogen upon admission. Therefore most of the WI were nosocomial. Antibiotic pretreatment in the home stable should be critically discussed.

Hygiene management might help reduce the emergence of antimicrobial resistance and WI.

### Acknowledgement & Funding

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## Report of an outbreak of coccidiosis in lambs on a farm in Morelos, Mexico.

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### Introduction

The genus *Eimeria* is of great transmissibility due to the large production of oocysts. Infection of sheep occurs mainly in intensive production units, in which animals are kept in tight spaces in contact with feces. Lambs are the most receptive to infection and can eliminate amounts greater than 100,000 oocysts/g of feces (Cafrune et al 2009).

After 16 days coccidia develop and invade the intestine. At that time exposure to oocysts is constant, producing subclinical and clinical coccidiosis. The transmission of coccidia occurs horizontally, that is, between animals affected or carriers of the disease and healthy animals. Most adult animals are carriers and, although not sick, excrete the parasite through feces, and can infect lambs. At 21-28 days a large number of oocysts is deposited with feces, which when ingested by other animals begins another cycle of coccidiosis. Coccidias cause damage by reproducing in the cells of the animal's intestine (Khodakaram Tafti, A., & Mansourian, 2008).

### Material & Methods

In this region the rainfall regime corresponds to summer because it is an Aw climate, with rains from May to October. The altitude above sea level is 1927 meters. The production unit where the research was carried out has a population of 64 females and 3 stallions of the breeds East Friesian, Awassi and Laucaune. All lambs took colostrum, weaning is done after 60 days. Substitute, milk is not used. Milk as a is given directly from the mothers who are with their lambs 12 hours a day. In the month of June 2021 there was an outbreak of coccidiosis in 20 Lambs, presented pasty diarrhea, anorexia and decreased growth.

### Results

The prevalence rate was 31% with 2 deaths. Mortality rate was 3.1%. Coproparasitoscopic flotation examinations were carried out on all the lambs. The tests carried out, 80% of the lambs were positive for *Eimeria tenella*. Nitrofurans were administered for 5 days.

### Discussion

Several holes with accumulated water were detected in the corrals. That holes were repaired and there was no longer any presence of coccidias in the lambs. The lambs decreased by 5 % of the weight gain, which was recovered when the disease was controlled. Afterwards, there was no more outbreak of that

disease. Farm staff were trained to improve the handling of lambs and thus decrease immunosuppression caused by stress (Khodakaram-Tafti, A., & Hashemnia, 2017).

After the outbreak a coproparasitoscopic flotation examination was performed and 30% of the adult population tested positive for *Eimeria*. Most animals, particularly adults in good health, have *Eimeria*, but do not show clinical signs, in other words, it is a subclinical coccidiosis.

It is very important to take into account that when an adult animal eliminates oocysts, it does not indicate that it is sick, it is only a healthy carrier and should not receive treatment. The importance of this presentation is based on the fact that they are a continuous source of oocysts for young animals (Souza et al., 2012).

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## Antibodies against leptospirosis in a dairy farm

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### Introduction

Leptospirosis is a zoonotic disease, with worldwide distribution, in Mexico found throughout the national territory. Its importance in livestock lies in the fact that it is a causal agent of the decrease in the productivity, generating infertility, increase between the interval of births, conception, high number of services per year, abortions and decrease in milk production in dairy cows. Tropical and subtropical countries are the most affected, climatic conditions such as precipitation, temperature, relative humidity, pH, structure and soil composition, are factors that favor the presentation of leptospirosis. Leptospirosis is also a significant public health problem. Normally infected people return to work in 3 or 4 weeks, but they can suffer relapses continuously.

Objective this studio: Detect the presence of antibodies against Leptospirosis in cows and their effect in interval between calving (PI), services by conception (S / C) and open days (AD) (Escamilla et al., 2007; Benavides and Marcillo, 2016)

### Material and methods

Blood samples of the coccygeal vein were taken by means of the technique of Ojeda *et al.*, (2016), from 30 cows of the Holstein breed from a dairy farm located in Hidalgo State, Mexico with a history of abortions, mummifications and reabsorption of embryos.

The samples were analyzed in the Leptospira Laboratory of the Autonomous Metropolitan University, using the microscopic agglutination technique, mixing the serum to be studied with leptospirae of different serotypes, and then evaluating the degree of agglutination under the microscope.

Serums that at dilution 1:100 or higher, showed 50% agglutination or disappearance of cells from the field to observation with the dark field microscope were considered positive. The bacterium used was 9 serovarieties of *L. interrogans* (*Icterohaemorrhagiae*, *Pyrogenes*, *Grippotyphosa*, *Canicola*, *Pomona*, *Hardjio*, *Wolffi*, *Tarassovi*, *Bratislava*). Its relationship with the following reproductive parameters was determined: IP, S/C and DA. The results were analyzed by non-parametric statistics.

### Results

The analysis of the 30 serological samples, 73 % (22/30) of positive cows were found, at one or more serovarieties of *Leptospira interrogans*. In the positive serums, three serovarieties of *Leptospira interrogans* were found: *Hardjio*, *Tarassovi* and *Hardjio H89*, 14% of the animals showed positivity to the three serovarieties, 32% to two serovarieties and 55% to a serotype. However, the results suggest an active infection by *Tarassovi* serovariety as it was found in all cases.

## Conclusions and discussion

The presence of antibodies against leptospirosis indicates that the condition is present in the dairy farm of this study, which explains the problems in the reproductive parameters studied IP, S / C and DA.

The realization of a vaccination schedule for prevention is important to avoid reproductive losses and therefore economic losses for the farmer.

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## Multidrug-resistance mediating integrative and conjugative elements in Pasteurellaceae isolates from Germany

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### Introduction

Integrative and conjugative elements (ICEs) are mobile genetic elements, which facilitate the spread of antimicrobial resistance genes among bacteria. In doing so, they are not limited to bacteria of a specific genus, but might be transferred between bacteria of different genera or even beyond bacterial family borders. Pasteurellaceae isolates carrying ICEs have been reported from the USA and Canada [1,2]. Therefore, a *Mannheimia haemolytica* and a *Pasteurella multocida* isolate from Germany, which play important roles in the pathogenesis of the bovine respiratory disease (BRD) complex, with rarely occurring phenotypic multidrug-resistance (MDR) were analysed to determine (i) the antimicrobial resistance genes present, (ii) their potential association with mobile genetic elements and (iii) mutations within the genes of antimicrobial target structures.

### Material & Methods

In November 2019, the isolates *M. haemolytica* IMT47952 and *P. multocida* IMT47951 were identified during routine diagnostics in lung samples taken at necropsy from a severe case of BRD of a three-month-old German calf. The antimicrobial susceptibilities of *M. haemolytica* IMT47952 and *P. multocida* IMT47951 were determined according to CLSI standards. Hybrid assembly of MiSeq and MinION reads resulted in closed genomes of both isolates.

### Results

Both isolates showed MDR phenotypes. Analysis of the *M. haemolytica* IMT47952 sequence revealed the presence of the resistance genes *sul2* [sulfonamides], *catA3* [chloramphenicol], *floR* [chloramphenicol/florfenicol], *tet(Y)* [tetracyclines], *strA-strB* [streptomycin], *mef(C)* and *mph(G)* [macrolides] as part of the novel ICE Tn7406. In addition, nucleotide exchanges leading to amino acid substitutions D87Y in GyrA [nalidixic acid] and W30R in Fola [trimethoprim] were identified. In the sequence of *P. multocida* IMT47951, the resistance genes *sul2*, *strA-strB*, *tet(H)* [tetracyclines] and *aphA1* [kanamycin, neomycin] were located within the novel ICE Tn7407. Furthermore, an A2059C transition in the 23S rRNA of all six rRNA operons might explain the elevated minimal inhibitory concentration values for macrolides and clindamycin.



## Discussion

The resistance genes *tet*(Y), *mef*(C) and *mph*(G) have been identified in bacteria of aquatic origin, but not yet in *M. haemolytica* [3,4]. Moreover, the first identification of multidrug-resistance mediating ICEs in respiratory tract pathogens in Germany is alarming as these elements may diminish treatment options for BRD in the future and pose a risk not only for animal, but also for human health, if transferred to other bacterial pathogens, via the food chain or established in an environmental reservoir.

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## Encapsulating bacteriophages to treat antibiotic-resistant bacteria in livestock

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### Introduction

Increasing multi-drug resistance in bacteria poses a serious threat to livestock, and bacteriophages are considered a promising strategy to reduce microbial contamination in animal production. The antibiotic resistance among natural microflora may pose a greater threat than direct selection pressure from pathogenic strains<sup>1</sup>. In ecosystems such as the animal digestive tract, species diversity and a high concentration of microorganisms favor the diffusion of resistance genes between bacteria, which can also be pathogenic to humans<sup>2</sup>. The antibacterial potential of bacteriophages has been tested at various stages of the "farm to fork" cycle, taking into account different routes of application<sup>3</sup>. It is worth noting that the development of bacteriophage products for future therapeutic applications faces certain limitations. Considering the possibility of using bacteriophage preparations in the field of animal husbandry, the research aimed to create a solid-state product of small dimensions using mild process conditions. In the light of the high sensitivity of bacteriophages to low pH and the presence of digestive enzymes, the activity of immobilized phages was assessed *in vitro* in a model simulating the conditions prevailing in the stomach and the intestine. The work focuses on bacteriophages BF9, BF15 and BF17 characterized by a broad lytic spectrum against *E. coli* producing AmpC-type cephalosporinases (AmpC) and extended-spectrum  $\beta$ -lactamases (ESBL) isolated from pig production. The production of alginate-mannitol macrospheres was preceded by a detailed characterization of viruses for their improved usability in a future target delivery.

### Material & Methods

#### *Bacteriophages and bacteria*

Bacteriophages BF9, BF15 and BF17 and their host strains were obtained from the Department of Biotechnology and Food Microbiology UPWr<sup>4</sup>. Phages were selected due to their broad lytic activity against ESBL/AmpC *E. coli* isolated from various samples associated with pig production<sup>4</sup>. Bacteriophages were amplified following protocols provided by Skaradzińska et al.<sup>5</sup>. The double-agar overlay method was used for plaque formation and determination of bacteriophage titer (PFU/ml)<sup>6</sup>.

#### *Viability of free phages exposed to different pH*

For the pH stability assay, 20  $\mu$ l of phage suspension was incubated for 12 h in LB at pH values from 1 to 13. Pre-incubated phages were loaded into wells of 48-well microtiter plates inoculated with 180  $\mu$ l

of host culture at  $OD_{600}$  of 0.1. The plates were incubated at 37°C with shaking (150 rpm) and bacterial growth was measured for 6 h (Tecan Trading AG., Switzerland).

### *Phage encapsulation*

Encapsulation of the phages was carried out following a previously described protocol with modification<sup>7</sup>. Briefly, 2% (w/v) sodium alginate solution (Sigma-Aldrich, Poland) in distilled water was mixed with phage solution in a ratio of 6:1. Macrospheres (beads) were made by extruding the mixture through the nozzle of 0.8 mm diameter into the 0.05 M  $CaCl_2$  enriched with the addition of 0.3 M mannitol (Sigma-Aldrich, Poland). The formed beads were kept in the solution for 30 min with slow stirring and were allowed to harden. Macrospheres with phages were spread on Petri plates under running laminar flow for 16 h and then stored in conical tubes at 23 °C for 4 weeks.

### *Release of phages from macrospheres*

To determine the efficiency of encapsulation (EE%), 1 g of macrospheres were dissolved in 9 ml of microsphere breaking solution (MBS) (pH 7.5) containing 50 mM sodium citrate, 0.2 M sodium bicarbonate, and 50 mM Tris–HCl for 2 h at 37°C. EE% was calculated as follows: (quantity of phage released from the macrospheres/quantity of phage initially taken to prepare the macrospheres) x 100.

### *In vitro evaluation of encapsulated phages activity along the simulated GIT*

The stability of encapsulated phages was tested in the simulated gastrointestinal tract according to the previously described protocol<sup>7</sup>. Briefly, 1 g of macrospheres was mixed with 9 ml of SGF (pH 2.5) composed of 2.05 g/l NaCl, 0.37 g/l KCl, 0.6 g/l  $KH_2PO_4$ , 0.11 g/l  $CaCl_2$ , 0.05 g/l bile, and 3 g/l pepsin (Sigma-Aldrich) and incubated at 37 °C with shaking (200 rpm). After 60 min macrospheres were rinsed twice with distilled water. Samples already incubated in SGF were suspended in a simulated intestinal fluid (SIF) (pH 7.5) made with 25.2 g/l  $NaHCO_3$ , 40 g/l bile, 3.5 g/l pancreatins (Sigma-Aldrich) and incubated for 180 min at 37 °C. 100 µl aliquots were collected and titrated in triplicate by the double agar layer method<sup>6</sup>.

### *Statistical analysis*

All experiments were repeated at least three times. Statistical analysis was performed using STATISTICA v. 13.3 (TIBCO Software, Poland). One-way ANOVA was used to analyze the differences between samples during the sampling period. The differences were considered significant at a P-value of <0.05.

## **Results**

### *Viability of free phages to different pH*

Phages BF9, BF15, and BF17 were sensitive to acidic environments, being unable to infect their hosts after incubation at pH <3 (Fig. 1). After 6 hours of treatment, the biological activity of the bacteriophage BF15 was demonstrated only after incubation at pH 7 and pH 9 (Fig. 1B). The wide range of pH values did not affect the viability of BF17 being only sensitive to extreme environmental pH values of pH 1 and pH 13 ( $p < 0.05$ ) (Fig. 1C).

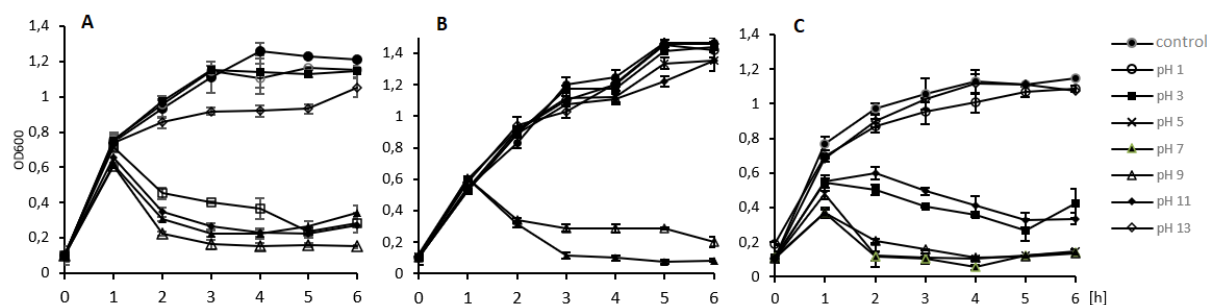


Figure 5. Viability of phages (A) BF9, (B) BF15, and (C) BF17 to infect bacterial host after exposure to different pH values.

### Encapsulation

Figure 2 shows the alginate macrospheres (wet) obtained directly after the extrusion process. The macrospheres represent colourless structures with a spherical and uniform shape. The mean diameter of the beads is  $3.0 \pm 0.2$  mm. After drying, the structure and size of the spheres change significantly (Fig. 3). Macrospheres of irregular shapes and much smaller sizes predominate ( $p < 0.01$ ). The average size of the dry beads is  $1.14 \pm 0.12 \times 0.94 \pm 0.11$  mm.

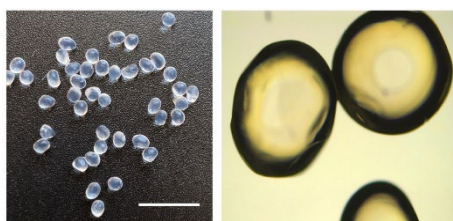


Figure 6. Wet alginate macrospheres

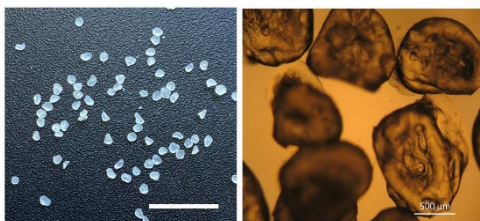
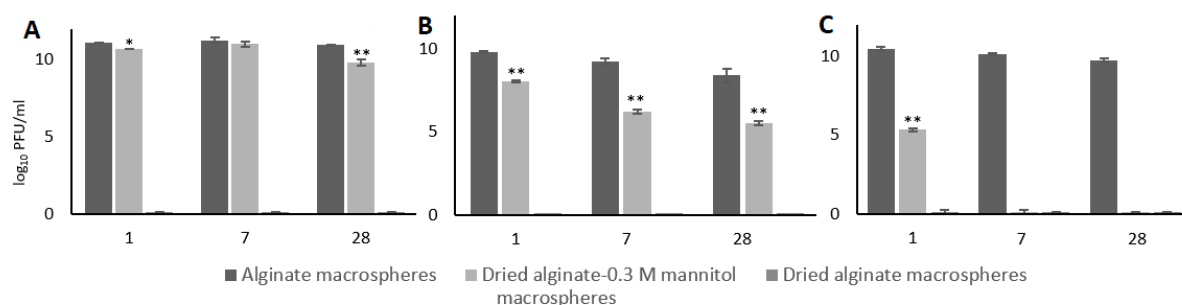


Figure 7. Alginate macrospheres after drying

### Release of the encapsulated phages

The activity of the immobilized phages BF9, BF15 and BF17 was assessed in the MBS buffer to determine the stability of alginate and alginate-0.3 M mannitol macrospheres under mild conditions. Fig. 4 represents the results of the long-term activity of phages released from different forms of macrospheres after 2 h incubation (pH 7.5).

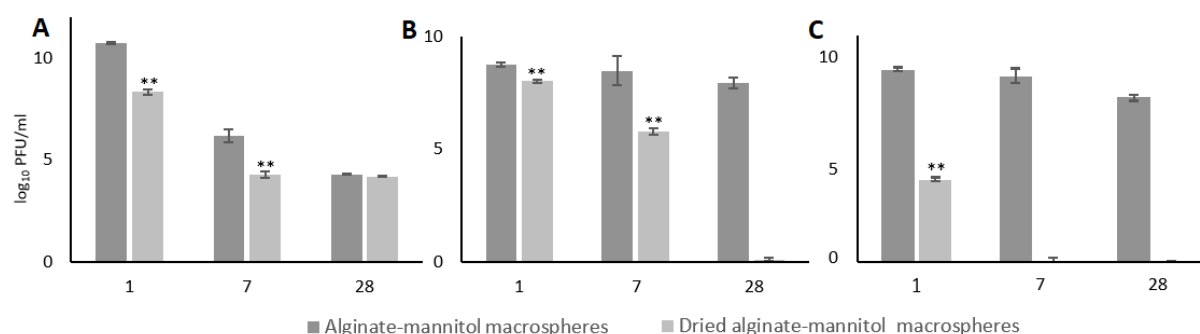


**Figure 8.** Stability of phages (A) BF<sub>9</sub>, (B) BF<sub>15</sub>, and (C) BF<sub>17</sub> released from macrospheres under mild conditions. Data presented as mean±sd. \* $p < 0.05$ , \*\* $p < 0.01$

The encapsulation efficiency of BF<sub>9</sub> phage estimated for both wet alginate macrospheres and macrospheres supplemented with 0.3 M mannitol was >98%. Importantly, the addition of 0.3 M mannitol significantly increased the lytic potential of the BF<sub>9</sub> phage after dehydration ( $p < 0.01$ ). After one week of storage, the phage titer in the dried beads was still high at 10.99 log<sub>10</sub> PFU/ml and comparable to the phage concentration in wet alginate macrospheres ( $p > 0.05$ ). The efficiency of BF<sub>15</sub> phage encapsulation in alginate beads was 97% in variants without mannitol and over 99% in beads enriched with 0.3 M mannitol (Fig. 4B). As a consequence of drying, BF<sub>15</sub> encapsulated bacteriophage completely lost its ability to infect the host. Nevertheless, immobilization in macrospheres with 0.3 M mannitol significantly prolonged the infectious activity of BF<sub>15</sub>. After 7 days of storage, a stabilizing trend was observed and a slight decrease in phage titer by 0.67 log<sub>10</sub> PFU/ml ( $p < 0.01$ ). The number of biologically active phage particles released from the dry spheres after 4 weeks of storage was 5.51 log<sub>10</sub> PFU/ml. The process of BF<sub>17</sub> encapsulation yielded an efficiency greater than 99% (Fig. 4C). However, dehydration significantly reduces encapsulated phage BF<sub>17</sub> activity regardless of the addition of the protecting agent. The lack of mannitol resulted in the complete inactivation of BF<sub>17</sub> phage after drying. After 7 days of storage, the titer of the BF<sub>17</sub> bacteriophage had dropped to values below the detection limit.

#### Viability of encapsulated phages under simulated GIT

The results presented in Fig. 5 illustrate the long-term survival of encapsulated phages in the presence of simulated gastrointestinal conditions. The lack of the stabilizing effect of dry alginate beads on phages prompted the evaluation of the activity of phages immobilized only in alginate macrospheres supplemented with 0.3 M mannitol.



**Figure 9** Stability of phages (A) BF<sub>9</sub>, (B) BF<sub>15</sub>, and (C) BF<sub>17</sub> released from macrospheres in simulated gastrointestinal conditions. Data presented as mean±sd. \* $p < 0.05$ , \*\* $p < 0.01$

Directly after the encapsulation process, a decrease in active BF9 phages was demonstrated in dry beads relative to the BF9 titer determined after incubation of the wet form ( $p < 0.01$ ) (Fig. 5A). Nevertheless, the number of phage particles released after depolymerization of the dry beads that remain biologically active was  $8.33 \log_{10}$  PFU/ml, suggesting high protective properties of the carrier in a low pH environment. The potential stabilizing function was observed also after 28 days in both forms ( $p > 0.05$ ). After incubation in the SGF-SIF system, more than  $4 \log_{10}$  PFU/ml of active BF9 particles remained. Contrary, bacteriophage BF15 completely loses its lytic activity in dry macrospheres during storage, as a result, the titer of BF15 falls below the level of detection (Fig. 5B). After four weeks we observed high titer BF15 phage in wet beads at  $7.96 \log_{10}$  PFU/ml, which poses sufficient stability of the phage in the above variant. Immobilization of phage BF17 in wet capsules with 0.3 M mannitol allowed to maintain a high degree of phage activity after incubation in the SGF-SIF model (Fig. 5C). However, dehydration of the environment of B17 is the factor determining its sensitivity to the conditions prevailing in the simulated environment of the digestive system. The decrease in the activity of the phage released from the dry beads was over  $5.7 \log_{10}$  PFU/ml ( $p < 0.01$ ) but another measurement of the phage activity revealed its complete inactivation.

## Discussion

The limited activity of phages exposed to an acidic environment suggests the need to develop solutions to protect them during their passage through the digestive tract of animals. In phage therapy, *per os* to increase the phage load in the intestinal reservoir of bacteria, preparations that neutralize gastric acid are commonly used<sup>8</sup>. Nevertheless, this strategy might be difficult for the commercial use of phage preparations in animals. Encapsulation of bacteriophages is one of the most promising methods that allow for increasing the stability of phages intended for oral application. The results of studies on animal models published so far provide ambiguous conclusions, indicating that the proposed techniques require further optimization<sup>9-11</sup>.

Phage BF9, BF15 and BF17 retained lytic activity in wet alginate macrospheres and high encapsulation efficiency indicates that phages are effectively immobilized in the alginate matrix. The activity of immobilized was found both during long-term storage and after incubation in the simulated gastric and intestinal environments, which suggests a high potential for the application of the carrier. The beads were found to be acid-stable, enabling phage survival at a low acidic pH value of 2.5. Storage assay confirmed high levels of phage viability four weeks after encapsulation. Nevertheless, our research confirmed that drying is the decisive factor in the activity of encapsulated phages BF9, BF15 and BF17. Contrary to the results of the analogous tests with phage T4, as a result of dehydration of the surroundings of the alginate-only beads, all tested phages lost their lytic activity when it was determined under the control conditions of the MBS buffer<sup>7</sup>. The incorporation of 0.3 M mannitol into the polymer structure played a significant role in maintaining the lytic potential of the phages after drying but data on the protective effect of polyhydric alcohols, to which mannitol belongs, are limited<sup>12</sup>. Worth noting, that phages were characterized by different susceptibility to drying and significantly various sensitivity *in vitro* to the gastric and intestinal juices which suggests that the method of bacteriophage preservation for practical applications must take into account the individual properties of bacteriophages. The obtained results confirm the high sensitivity of phages regardless of their taxonomic position and morphological structure<sup>13</sup>. The lowest stability of the BF17 phage may be due to the specific structure of this virus, which, like other jumbo phages, has a large capsid and a genome encoding numerous proteins (manuscript submitted for publication). Nevertheless, the



results obtained indicate that the immobilization phages in alginate beads enable the preservation of the activity of a large part of bacteriophages in a simulated environment of the digestive system.

Further work is needed to optimize beads formulations considering individual characteristics of bacteriophages and the economic profitability of implementing the proposed solution. Furthermore, *in vivo* evaluation of the gastrointestinal survival of encapsulated phages is needed including reduction of ESBL *E. coli* carriage in animals, including pigs.

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## Genetic insight into a *Salmonella enterica* serovar Enteritidis strain circulating in a rabbit farm reveals IncFIB-IncHI2 and IncN plasmids mediated hybrid virulence resistance mechanism

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### Introduction

Rabbit *Salmonella* infections are not often met with but have been responsible for outbreaks of disease-causing considerable loss. *Salmonella enterica* serovar Enteritidis (SE) is a major cause of food-borne Salmonellosis in China. SE strains harboring plasmids with multidrug resistance and virulence factors are of especial interest.

### Material & Methods

*Salmonella* strain were recovered from rabbit livers in a farm. Antigenic formula, antimicrobial susceptibilities were characterized. Gentamicin protection assay was performed to phenotype its association, invasion, and replication in Caco2, hBMEC and activated peritoneal macrophage. Dissemination capability of SE JL228 was evaluated. Whole genome was then resolved using Unicycler hybrid assembly pipeline with PacBio and Illumina sequencing platform. Molecular typing was performed on the PubMLST database. Antimicrobial resistance and virulence factor were predicted using ResFinder4.0 and VFDB database.

### Results

All eight *S. enterica* serovar Enteritidis isolates showed the same antimicrobial resistance profile and were all resistant to Ampicillin (AMP), Piperacillin (PIP), Cephalexin (CL), Cefovecin (CEF), Ceftiofur (EFT), Amikacin (AMK), Gentamicin (GEN), Tobramycin (TOB), Trimethoprim/ Sulfamethoxazole (SXT), Chloramphenicol (CHL), Enrofloxacin (ENR), Tetracycline (TET), and Nitrofurantoin (NIT). Similarly, these isolates were all intermediately resistant to Amoxicillin/Clavulanic acid (AMC), Cefpodoxime (CPD), and Marbofloxacin (MAR), and all susceptible to Imipenem (IPM). According to an international expert proposal for interim standard definitions for acquired resistance, all these isolates are multidrug-resistant (MDR).

The potential virulence of JL228 to non-phagocytic intestinal epithelial cell Caco2, brain microvascular endothelial cell hBMEC and activated peritoneal macrophages (APM) were also evaluated using Gentamicin protection invasion assay in this study. Results revealed JL228 had demonstrated higher invasion and replication capabilities over the strain LN248 in these three cell types. High intestinal epithelial invasion and intracellular survival capacities within phagocytic cell (APM) enable the strain JL228 to traverse the intestinal barrier and gain access to systemic sites, suggesting the high *in vivo* virulence potential of this strain.

The LD<sub>50</sub> of JL228 in the orally infected mice model was calculated to be  $5 \times 10^6$  CFU, which was approximately 68 times lower than that of LN248. Similarly, in the streptomycin-pretreated infection model, JL228 was lethal in 100% of BALB/c mice. All mice succumbed to  $10^8$  inoculums by day 14 post-infection. Of the mice infected with LN248, however, 40% survived the infection. Significant body mass changes were also observed in both infection groups. whilst, mice infected with JL228 had sharper body mass decrease in the first two days post infection than LN248. Interestingly, 50% (3 of 6 mice) JL228 infected mice developed overt neurological signs that resembled meningitis with balance defect and ataxia, including unsteadiness, unilateral rotation, lateral roll, head and neck torsion, and muscle tremors. Taken together, the rabbit-originated clinical strain JL228 exhibited higher virulence potential in mouse model.

Virulence potential of a *Salmonella* strain is associated with its ability to access intestinal epithelial cells and disseminate to systemic organs. Therefore, bacterial loads of JL228 and LN248 were enumerated in livers, spleens, brains, and mesenteric lymph nodes (MLN). Results showed that significant higher bacteria could be recovered from organs in JL228 infected-mice than that of LN248, suggesting JL228 had aggressive dissemination within the host upon orally infection. In accordance with the findings in neurological signs, JL228 was more frequently recovered as well. Furthermore, bacterial load was also significantly higher in JL228 infected mouse brains than that recovered from the LN248.

The genetic makeup of *S. enterica* serovar Enteritidis str. JL228 was determined to comprise a 4.7-megabases (Mb) chromosome and two plasmids with the genomic size of 211.4 kilobase (kb) and 54.6 kb respectively using Unicycler assembly pipeline. The two plasmids were then designated pSE228A and pSE228B. The GC-content of the chromosome, plasmid pSE228A and plasmid pSE228B are 52.17%, 46.28% and 47.51% respectively. A total of 4896 genes were annotated by NCBI's Prokaryotic Genome Annotation Pipeline (PGAP). Among them, 4612 genes were predicted in chromosome, 223 genes in pSE228A and 61 genes in pSE228B (GenBank accession No. CP094269 to CP094271). Molecular typing using Achtman 7-gene MLST and salmcbgMLST schemes suggested chromosome of strain JL228 match the profiles of ST11 and cgST-7883 respectively. *In silico* replicon-based typing suggested plasmid pSE228A match the loci of *FIB* (allele 22), *smr0018* (allele 1), and *smr0199* (allele 2). according to the Plasmid MLST schemes, therefore, plasmid pSE228A is a multiple-replicon plasmid with the incompatibility group of IncFIB and IncHI2. Further subtyping by IncHI2 double locus sequence typing (DLST) scheme classified the plasmid into ST14 group. The same typing scheme suggested the plasmid pSE228B belongs to IncN group, one of the most prevalent drug-resistance plasmid types in *Enterobacteriaceae*. The pSE228A is a large plasmid encoding 223 genes, among which, two backbone replication *repB* gene identified in the region 146725-147714 and region 86251-87000 (complementary) in the opposite direction. The two RepB family plasmid replication initiator proteins share low amino acid sequence identity (42.17%), whereas the former shared 100% identity with the RepB protein encoded by a replicon FIB plasmid pS82/10 from *S. enterica* serovar Enteritidis. Besides, genes involving in IncHI-type plasmid conjugal transfer, plasmid maintenance were also identified in this region, constituting a *repB-traE-traK-trhB-trhV-traC-parA-parM-htdF-rsp-trhF-trhU-trhN-trhI* loci. In addition, two related genes encoding pili assembly chaperone and plasmid transfer protein, corresponding to gene *MPK88\_23540* and *MPK88\_23545* were also found between *repB* and *traE*. Another *repB* (complement to 86251-87000), *MPK88\_23790* (encoding DNA replication terminus site-binding protein), *ccdA*, *ccdB*, *trhH* and *trhR* might involve in pSE228A replication, maintenance, and conjugal transfer. The 54.6-kb pSE228B belongs to IncN replicon group plasmids with backbone loci of eight replication and stabilization protein-encoding genes locate within the region of 30357-43644, corresponding to the locus tag of *MPK88\_24360* to

MPK88\_24425 (GenBank accession No. CP094271). These include four putative replication initiation genes: *repM*, and three unassigned replication initiation protein-encoding genes, *MPK88\_24365*, *MPK88\_24370* and *MPK88\_24425*, four plasmid maintenance genes, *parA*, *parG*, *MPK88\_24355* (encoding RelE/ParE toxin), and *MPK88\_24360* (encoding a plasmid stabilization protein), a DNA distortion polypeptide gene, *ddp3*.

A total of fifteen antimicrobial resistance (AMR) genes were identified in genome of JL228 using ResFinder4.0 database. These include *aac(6')-Iaa* gene found in bacterial chromosome, three genes [*aac(6')-IIC*, *sul1* and *ere(A)*] in plasmid pSE228A and ten genes [*armA*, *aac(6')-Ib-cr*, *aac(3)-IIId*, *aph(6)-Id*, *aph(3'')-Ib*, *aph(3')-Ia*, *blaTEM-1B*, *mph(E)*, *sul1*, *sul2*] in plasmid pSE228B. AMR genes in pSE228A are clustered in the region 201393-208693, flanked by two identical IS6-like element IS26 family transposase genes and a class 1 integron integrase gene (*intI1*), constituting a gene cluster of *IS6-sul1-qacE-ere(A)-MPK88\_24140-aac(3)-IIg-ereA-aac(6')-IIC-intI1-IS6*. The unassigned gene locus *MPK88\_24140* encodes an NAD(+)-rifampin ADP-ribosyltransferase, suggesting the rifampin resistance potential of this plasmid. AMR genes in pSE228B scattered in four regions among the plasmid (Figure). These AMR genes in chromosome and plasmids are predicted to confer resistance to six class of tetracycline antibiotics (Table x). Besides, quaternary ammonium compound-resistance gene *qacE* were also found in both plasmids, with the predicted phenotype of Benzyltrimethylammonium Chloride, Ethidium Bromide, Chlorhexidine and Cetylpyridinium Chloride resistance. In addition, chromosomal point mutations analysis using ResFinder also identified a nonsynonymous substitution in DNA gyrase subunit A (GyrA: D87Y), which might have a potential influence on the antibiotic susceptibility to nalidixic acid and ciprofloxacin (Table). Interestingly, three heavy metal resistance operons including a copper resistance locus *copESDBAC* (region 15559-20810), a bidirectional silver resistance locus *silPABFCRE* (region 22094-34545) and a tellurite resistance locus *terEDCBZWXYZ* (region 62141-75496) were also identified in plasmid pSE228A

## Discussion

High virulent and MDR *Salmonella enterica* serovar Enteritidis strain contributed to the prevalence of the disease in the rabbit farm. Genomic analysis revealed that SE clonal strain JL228 co-harboring two plasmids, a hybrid virulence resistance IncFIB-IncHI2 plasmid and a drug-resistance IncN plasmid. Multi-drug and metal resistance gene loci and bacteremia-associated locus of *spvRABCD* were identified, emphasizing the potential risk of bacterial and/or plasmid dissemination among animal population and food chain.

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## Air purifier screening revealed the efficacy of HEPA-filter and UVC-light in the inactivation of coronavirus

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### Introduction

The value of air purifiers for livestock and public health is increasing as a result of the SARS-CoV-2 epidemic. Variety of tools to reduce transmission in enclosed rooms e.g. livestock farms as well as classrooms and offices are commercially available. The mode of actions is either filter particles out of the air or the direct inactivation of airborne pathogens. Each method has its own advantages and disadvantages. The potency of the air purifier is greatly affected by the ambient condition as well as differences in the biophysical properties of pathogens. The suitability of any particular device should be determined on an individual basis under strict laboratory conditions, especially for SARS-CoV-2, where a high level of biosafety (BSL-3) is necessary. This, however, is not widely available and of high maintenance cost. Therefore, in this study, feline coronavirus (FCoV) was used as a surrogate for SARS-CoV-2 in order to determine the effectiveness of seven air purifiers based on different inactivation and filtering principles.

### Material & Methods

A workflow for evaluating the efficacy of seven devices with different air disinfection principles was established. FCoV (approx.  $1 \times 10^7$  TCID<sub>50</sub>/m<sup>3</sup> air) was nebulized in a closed container. Air was drawn through a gelatin membrane filter at two sampling points, one at the virus entry site and one after filtration and/or inactivation. The Spearman–Kärber method was used to determine reduction in infectivity (TCID<sub>50</sub>) of FCoV. The following air purifiers were screened: an ionization system, Ultraviolet C (UVC) rays, electro-, HEPA- and moss-based-filters as well as UVC/electro filter with or without HEPA-filter.

### Results

The reduction in FCoV infectivity due to handling and nebulization as measured at the point of virus entry was around 2 log<sub>10</sub>. The systems including HEPA-filter and/or UVC were the ones with the highest reduction in infectivity (approx. 100%). The efficacy of air ionization system varied greatly depending on the number of emitters and the point of measurement (0-78%). With electro- or the moss-based filter, virus reductions of 80 and 20% respectively, were reached.

### Discussion

HEPA-filters and UVC rays are extraordinarily effective. A combination of filter systems with simultaneous inactivation of the virus seems to be ideal.

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## Investigation of the effectiveness of cleaning and hygiene management and their impact on the development of nosocomial wound infections in an equine clinic

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### Introduction

Hygiene management in equine clinics is challenging. The conditions in equine medicine are different from those in human medicine. Open treatment rooms and horse stables harbor many sources of risk for nosocomial wound infections. In addition horse bedding and the resulting dust pollution make cleaning and disinfection difficult. On the other hand, the patients bring a diverse, constantly changing spectrum of bacteria into the clinic. Frequency of nosocomial infections in equine clinics have increased in recent years. Hence, hygiene measures are of central importance in the context of biosafety.

### Material & Methods

From December 2021 - February 2022 we analysed various clinical surfaces in an intensive care unit for the presence of bacteria possibly involved in nosocomial infections. The areas were selected according to frequency of contact and transmission potential Tryptic Soy Contact Agar + LTH - RT plates were used for determination of total aerobic microbial count. Bacteria were subsequently identified by MALDI-TOF. Following cleaning using biocides, bacterial contamination was reassessed.

### Results

We analysed a total of 88 clinical surfaces, assessing 304 samples (76 before and 2 times 76 after cleaning). Surfaces of two treatment rooms (internal medicine, orthopedics), one operating room, and of various horse stalls were tested. Sampling areas included walls, floor, operating table, surgical pillow, anesthesia machine, clipper, drinking basin, feed trough, door handle, examination stand, ultrasound probe, bronchoscope, stethoscope, shelf, and table each; before and after cleaning.

Bacterial contamination was detectable in 96,79 % (n=152) of uncleaned surfaces and 50, 54 % (n= 91) of biocide treated surfaces, respectively. Overall, cleaning and disinfection was successful in 21.7% (n= 33), partial decontamination was observed for 21 % (n= 32) of the specimen and unsuccessful management in 57.15% (n= 87). Among the bacteria identified, we mainly identified biofilm-forming bacteria that permeate the entire clinic. Most frequent bacteria detected were different species of *Acinetobacter*.



## **Discussion**

The aim of this study was to identify sources of potential nosocomial bacteria and to subsequently develop an improved hygiene management. Clinical work processes are subject to many factors which influence the hygienic environment. The evaluation of our sampling data showed in parts lack of sufficient effectivity of cleaning and disinfection measures which could be optimized to reduce the risk of consecutive wound infection.

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## Rapid molecular approaches to monitor microbial pathogens in wastewater

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### Introduction

Early detection of pathogens is crucial to prevent disease outbreaks. Wastewater monitoring is a promising solution in this regard. This is particularly important for hazard prevention in low resource settings or by geohazard, where contaminated drinking water is a major problem. A fast and simple screening approach is needed for accurate identification of pathogens in wastewater. The aim of this study was to develop and optimize a rapid nucleic acid extraction method which can be applied in the field.

### Methods

Various sample extraction procedures were evaluated. Sample were subjected to either alkaline lysis, proteinase K digestion and/or bead-beating followed by a heating step in lysis buffer. Impurities were removed using magnetic beads – a technology named “reverse purification”. Surrogates for Gram-positive (*S. aureus*), Gram-negative (*E. coli*) bacteria and protozoa (*C. parvum*) were included in this study. The performance of the different extraction approaches was evaluated under lab conditions by spiking wastewater samples. The effectiveness of nucleic acid release by each protocol was examined using real-time PCR. All results were compared with a gold standard protocol comprising of specific pre-treatments followed by silica-based DNA purification.

### Results

The effect of nucleic acid extraction varied strongly between the different pathogen groups. Bead-beating was particularly successful for Gram-positive bacteria (DNA yield was increased by 800%), whereas excessive bead-beating had a negative effect on the DNA yield of Gram-negative bacteria and parasites (decreased by 200%). Interestingly, alkaline treatment for *C. parvum* produced the maximum yield of DNA (increased by 700%).

### Discussion

Overall, a rapid and simple extraction method was established. The extraction reagents are cold chain independent and no centrifuge or other large lab equipment is required to perform the DNA extraction. A controlled validation trial is needed to test the effectiveness at field level.

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## **New approaches to the reduction of antibiotic resistance by feed additives in broiler chicken diet**

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### **Introduction**

Antibiotics combined with strict biosecurity and hygiene measures has helped the poultry industry to grow by preventing the negative impacts of many avian diseases (Bermudez, 2003). In livestock farming, antibiotics are administered mainly for the treatment and prevention of diseases and, in the past, as a growth stimulator (Kmet' and Kmet'ová, 2010).

The routine massive use of antibiotics in animal production has led to increased problem of antibiotic resistance, and presence of antibiotics residues in feed and environment), compromises human and animal health (Mehdi et al., 2018)

A significant component in the circulation of antibiotic resistance in the environment is also the livestock farming (Cheng et al., 2016).

Intensive poultry farming is connected with many adverse influences like antibiotic resistance and adverse effect the environment.

Hence, there is a growing need to find effective alternatives to control infectious diseases and limit the spread of resistant bacteria, but more importantly, keep antibiotics a useful tool for the future.

Poultry nutritionists are trying to substitute the antibiotics with different natural feed additives, such as organic acids and probiotics that can be beneficial similar in control of infectious diseases and ultimately improve feed efficiency (Abd El-Hack et al., 2022).

By using different feed additives growth and feed conversion ratio will be improved and also result in disease prevention. Nowadays organic acids, plant extracts, enzymes, probiotics and prebiotics are used as a growth promoter. The use of organic acids has gained importance as a growth promoter and as a substitute of antibiotics to promote poultry performance (Nagaraju et al., 2014).

Organic acids have been practiced for some decades as feed preservatives for protecting the destruction of feed from microbes and fungus. Acidification of diet has resulted in improved digestibility of protein and absorption of amino acids. It also proved that digestibility and utilization of mineral elements also improved through acidification of diet which ultimately improved the growth performance (Mirza and Mukhtar, 2016).

Humic substances are important natural chelating agents with antidiarrheal, antitoxic, immunostimulatory, and antimicrobial effects.

The aim of the study was to assess the influence of humic substances in the broiler chicken diet on the reduction of antibiotic-resistance in *E. coli* isolates from excrements.

## Material & Methods

In the experiment, we used two flocks of 40 one-day-old broiler chickens of the ROSS 308 breed. They were reared on the litter of wood shavings for 42 days in an environment with a controlled microclimate (natural light regime, temperature, humidity, and vacuum ventilation). The room temperature was regulated from 32°C at the beginning to 22°C at the end of chicken rearing.

The chickens were fed ad libitum with classic feed mixtures (BR<sub>1</sub>, BR<sub>2</sub>, BR<sub>3</sub>) in a calculated dose from 295 to 1588 g of feed per day. Chickens in the control group were fed traditional feed mixtures, while chickens in the experimental group were fed humic acid-enriched feed mixtures (Humac natura AFM Mycosorb in concentration 0,7%).

Samples of excrement were taken weekly from each experimental group. Samples of excrements were diluted and inoculated on meat pepton agar, Endoagar, and Slanetz-Bartley Agar. Inoculated plates were incubated in specific condition (Table 1). Total count of bacteria, coliform bacteria, faecal coliform bacteria, and faecal enterococci were counted and recalculated per gram.

Table 1 Incubation conditions for some bacteria

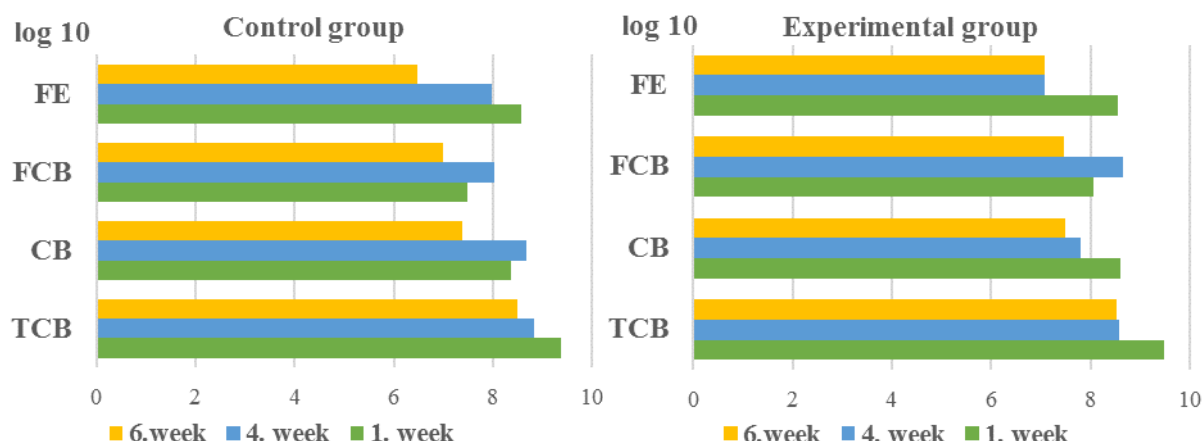
Microorganisms	Culture media	Incubation conditions
Total count of bacteria (TCB)	Meat pepton Agar	37°C / 24 h
Coliforms bacteria (CB)	Endo Agar	37°C / 24 h
Faecal coliform bacteria (FCB)	Endo Agar	43°C / 24 h
Faecal enterococci (FE)	Slanetz-Bartley Agar	37°C / 48 h

The suspected *E. coli* colonies were identified by Enterotest (Erba Lachema) and antibiotic-resistance (minimal inhibitory concentrations - MIC) of *E. coli* isolates were performed by the microdilution method by Miditech plates according to CLSI (Gattringer et al., 2002). The antibiotics used in the presented study were as follows: ampicillin (AMP), ampicillin with sulbactam (SAM), ceftazidime (CAZ), ceftazidime with clavulanic acid (CAC), ceftriaxon (CTR), ceftiofur (CFF), cefquinome (CFQ), ertapenem (ETP), gentamicin (GEN), streptomycin (STM), nalidixic acid (NAL), ciprofloxacin (CIP), enrofloxacin (ENR), chloramphenicol (CMP), florfenicol (FLO), tetracycline (TET), cotrimoxazol (COT), colistin (COL).

## Results

There was no significant difference of the results of detected microorganisms. Lower numbers of faecal coliforms were found in the samples from the group of chicken with feed additives (by one logarithmic order).

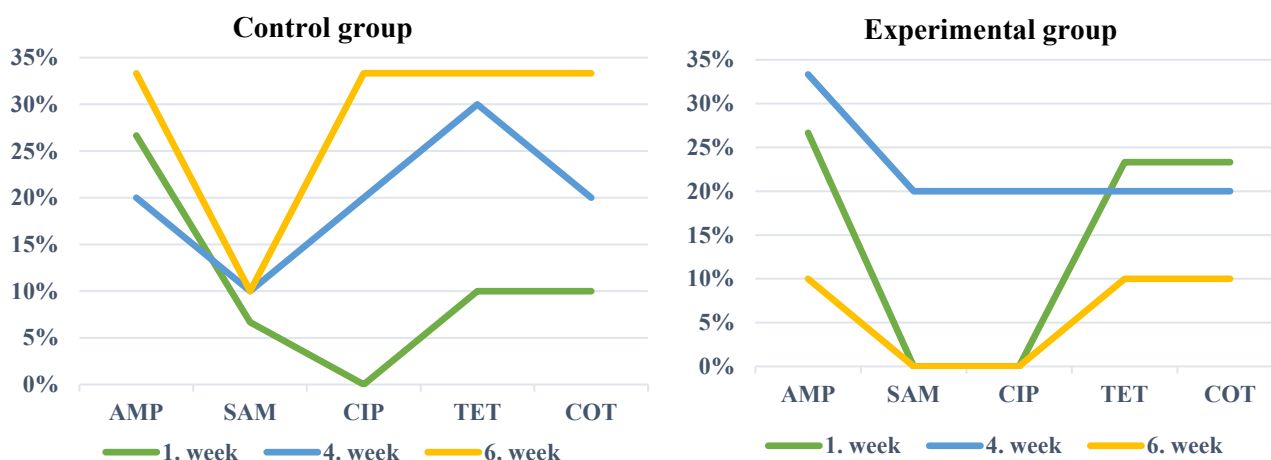
**Graph 1** The comparison of microbial contamination in excrement sample in control and experimental group



We analysed the antimicrobial resistance of 180 *E. coli* strains isolated from fecal samples of both groups during rearing. We detected resistance to ampicillin, ampicillin with sulbactam, ciprofloxacin, tetracycline, and cotrimoxazol in *E. coli* isolates. Resistance to other researched antibiotics was not found (they are not depicted in graph 2).

Decreasing resistance to ampicillin, ampicillin with sulbactam, tetracycline, and cotrimoxazol was observed in *E. coli* isolated from chickens fed with feed additives.

**Graph 2** Percentage of antimicrobial resistance in control and experimental group



## Discussion and conclusion

Humic acid can be used in poultry production as a growth promoter (Šamudovská and Demeterová, 2010), reducing stress, enhancing immunity of birds, improving the protein digestibility of animals, as well as reducing bacterial and mold growth, and reducing toxin levels (Nagaraju et al., 2014). Humic acid can have a favorable impact on poultry performance and improve weight gain via stabilization of gut microflora and increased nutrient absorption (Taklimi et al., 2012).

Humic acid plays a vital role in the protection of gut against infections and favourably affect its functions. Humic substances affect the microbes' carbohydrates and proteins metabolism destroying the pathogenic viruses and bacteria. Humic substances have good buffering capacity and can modulate

the gut pH (Rath et al., 2005, Arpášová et al., 2016). Humic acid has ability to alter the intestinal microflora by increasing the counts of beneficial bacteria (Schepetkin et al., 2003).

Humic derivatives have been recognized to show anti-microbial aspects. In the body, humates suppress the bad microbes but stimulate the good microbes. Shermer et al. (1998) stated that humic acid stabilizes the intestinal microbiota and consequently ensures enhanced nutrient utilization in poultry feed.

We also found a decrease of fecal coliform microorganisms in more than one logarithmic order, which can be caused by competition and changes in pH in feces. A slight decrease in antibiotic resistance is also visible, but it should be tested again.

Humic substances in poultry feed can be important for improving the health of poultry and in balancing the proportion of these substances in reducing the consumption of antibiotics in farm animals. However, it is still necessary to examine the impact of individual doses of humic supplements administered on optimizing the economic benefits of administered preparations usable in intensive farming (Bezuglova, and Klimenko, 2022).

It is necessary more research of using humic acid in animal nutrition. But it should be combined with good nutrition, biosecurity and prevention to achieve intensification of livestock production.

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## **Provision of oat dispensers as enrichment in turkey herds of hens with intact and trimmed beaks**

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### **Introduction**

Pecking injuries occur in turkey herds with intact and with trimmed beaks. The provision of environmental enrichment is one approach of preventing severe pecking outbreaks, because the animals can express their exploratory and foraging behavior. Manipulable material which has to be pecked out of a device seems to be particularly interesting to turkeys. However, it is not known to what extent the condition of the beak shows an effect on the usage of available material. Therefore, a modified oat dispenser was tested during fattening in turkey hens with untrimmed (UT) and trimmed (T) beaks.

### **Material & Methods**

Hens with untrimmed beaks (UT) and those with trimmed beaks (T) were housed in four compartments each (3.5 m x 10 m, each counting 143 B.U.T. 6 hens) evenly distributed over two barns. In the 8<sup>th</sup> week of life one oat dispenser per compartment was installed at the height of the hens' backs and remained until the end (16<sup>th</sup> week of life).

The oat dispenser consisted of a modified 12l bell drinker drilled with 8 holes (13mm diameter), so that the oat grains, stored inside, had to be actively pecked out. It was refilled daily, recording the amount added, and a backweighing of the remaining grains took place once a week. The average daily oat consumption was calculated for each compartment, and the consumed amounts were compared between the UT- and T-group.

### **Results**

Already in the first week after installation, the mean oat consumption differed between the two groups (UT: 9.87 g/animal/day; T: 4.42 g/animal/day). Over the following weeks, the difference between compartments increased, but group differences were also seen over the entire observation period (UT: mean 17.21 g/animal/day; T: mean 4.59 g/animal/day).

### **Discussion**

In this study, a difference of oat consumption, provided by dispensers, was observed between UT and T turkey hens. Whether UT-hens engaged with the dispensers for longer periods of time or perhaps T hens had less success in removing the grains in the same time needs to be validated by behavioral

observation. At this point, it can be assumed that beak condition should be considered when selecting appropriate enrichment material.

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This work (Model- and Demonstration Project for animal welfare: #Pute@Praxis) is financially supported by the Federal Ministry of Food and Agriculture based on a decision of the Parliament of the Federal Republic of Germany, granted by the Federal Office for Agriculture and Food.

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## ***Salmonella* biofilm in poultry housing can be effectively controlled by UPWr\_S134 bacteriophage cocktail**

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### **Introduction**

*Salmonella* can be disseminated to poultry flocks through several sources such as drinking water, feed, equipment and wildlife. Since *Salmonella* occurs in the flock, it can be easily transmitted between birds via the fecal-oral route with water and drinkers as the main points of horizontal transmission. Water contaminated with feces containing organic material and intestinal microflora contributes to the development of multispecies bacterial communities on the poultry drinker surface and may contain pathogens such as *Salmonella* (Maes et al., 2019). There is a striking paucity of data on the impact of phages on the biofilm developed in the poultry farm environment. The interest in *Salmonella* control in the poultry industry leads to renewed consideration of the use of bacteriophages. Bacteriophages are self-replicating and self-limiting as they multiply only at the site of infection when the host bacteria is located and they are eliminated from individual gradually when bacteria is cleared. Low inherent toxicity, lack of cross-resistance with antibiotics and formulation and application versatility are also mentioned among the advantages of phage treatment. Bacteriophages are host specific and often infect only one bacterial species or only one serotype of bacteria within a bacterial species. Since biofilms act as important environmental reservoirs for *Salmonella*, phages have been considered as an effective tool in inhibiting the formation and eradication of its biofilm developed under laboratory conditions on surfaces constituting part of the equipment elements commonly found in the poultry industry such as plastic and stainless steel (Gutiérrez et al., 2016).

*Salmonella* can reach poultry farms with contaminated water, animal feed, and raw materials of animal and plant origin, which are also related to their ability to invade plant tissues and to survive on abiotic surfaces in facilities where these raw materials were processed (Bailey, 1988; Nissen et al., 2001; Chia et al., 2009; Fatica and Schneider, 2011). The presence of *Salmonella* has been detected in mills for chopping and mixing food, on conveyor belts, packaging machines, storage places and other surfaces with which animal feed comes into contact. Thus, it is estimated that equipment and all surfaces on poultry farms and in animal feed production sites made of plastic, stainless steel, wood, and glass can be a substrate for the development of *Salmonella* biofilms (Nesse et al., 2003; Vestby et al., 2009). Biofilms are extremely difficult to prevent and eradicate, because, in biofilms, bacteria show increased resistance to various stressors (drying, antibiotic action, disinfectants, heavy metal ions, UV radiation).

Consequently, modern strategies to combat pathogenic bacteria are insufficient to control pathogens organized into biofilms (Maciorowski et al., 2006; Burmølle et al., 2010).

We established a new research model useful in phage ability to eradicate *Salmonella* from poultry drinkers as the main point in pathogen horizontal transmission within a poultry flock applying phages *in vivo* with an engineered *S. Enteritidis* strain. Further, in this study we report for the first time the UPWr\_S134 phage cocktail anti-biofilm activity in an experimental chicken model. The anti-biofilm activity of single phages UPWr\_S1, UPWr\_S3, UPWr\_S4 and phage cocktail UPWr\_S134 was also indicated in 96-well microtiter plates and in contaminated stainless still washers in *in vitro* models.

## Material & Methods

### Bacteriophages and bacterial strains

UPWr\_S phages were isolated from samples including feces, litter and manure from poultry farms, drainage ditches located near poultry farms or treatment plants. Taxonomically, UPWr\_S phages belong to the genus *Jerseyvirus* within the *Guernseyvirinae* and were described by Kuźmińska-Bajor and colleagues (2021). *S. Enteritidis* A41 was used as host for phage UPWr\_S1, whereas *S. Enteritidis* A36 was employed as a host for UPWr\_S3 and UPWr\_S4. The propagation of isolated bacteriophages was performed on the respective host strain. For biofilm reduction ability estimation two *S. Enteritidis* 327 lux and ATCC 13076 were utilised. The *S. Enteritidis* 327 lux strain exhibits a high ability to cause salmonellosis in chickens and contains markers such as erythromycin resistance and light production, which apparently allows this strain to be easily distinguished from chicken microflora (Kuźmińska-Bajor 2015). The biofilm production ability of *S. Enteritidis* 327 lux was categorized as a moderate biofilm producer. *S. Enteritidis* ATCC 13076, which was categorized as a strong biofilm producer, was used as a control in this study.

### Investigation of biofilm reduction with bacteriophages in 96-well microplate assay

The 96-well microplate assay method was applied to quantitatively determine the phage efficacy in *Salmonella* biofilm reduction. For this purpose, *S. Enteritidis* were cultured in 96-well microtiter plates to produce mature biofilm. Following removal of planktonic cells for biofilm degradation, UPWr\_S1, UPWr\_S3, and UPWr\_S4 phages alone and the phage cocktail UPWr\_S134 were used. After incubation and washing with PBS the remaining biofilms were quantified by staining with 0.5% crystal violet and the absorbance of the released color was measured. Biofilms not incubated with bacteriophages were used as positive controls. All experiments were performed in triplicate.

### Effectiveness of Bacteriophages in Reducing *S. Enteritidis* on the Stainless Steel Surface

To quantitatively determine the phage efficacy against *Salmonella* biofilm formed on a stainless steel surface, the modified method using 10 mm diameter stainless steel washers was applied. Overnight *S. Enteritidis* 327 lux and ATCC 13076 were cultured in Erlenmeyer flasks with LB medium containing washers to produce mature biofilm on the stainless steel. Each washer was transferred to a well of a 24-well plate containing different phage titers of UPWr\_S phages alone and the phage cocktail UPWr\_S134. The biofilm level was estimated by staining with 0.5% crystal violet.

### *Salmonella* eradication from the poultry drinker surface in the experimental chicken model

To determine the effect of the UPWr\_S134 phage cocktail on artificially contaminated poultry drinkers, an experimental chicken model was used. Fourteen day-of-hatch healthy broiler chickens were obtained from a local farm and randomly divided into four groups of ten birds. Chicken groups are presented in Table 1. Within 9 days of the experiment, the total number of attached *Salmonella* on the walls of the drinkers was calculated. To determine the number of *Salmonella* as well as the total viable count (TVC), both expressed as CFU/cm<sup>2</sup>, swabs were taken daily from 1 cm<sup>2</sup> of the surface of five individual drinkers, and bacterial counts were established.

**Table 1.** Chicken experimental design. UPWr\_S134 phage cocktail and *S. Enteritidis* 327 lux were constantly administered via drinking water.

Group	No. of chickens	No of. drinkers	Concentration		Water change schedule [days]
			UPWr_S134	<i>S. Enteritidis</i> 327 lux	
1	10	5	-	1 x 10 <sup>5</sup> CFU/ml	2, 4, 6, 8
2	10	5	1 x 10 <sup>7</sup> PFU/ml	-	2, 4, 6, 8
3 <sup>a</sup>	10	5	3 x 10 <sup>7</sup> PFU/ml	1 x 10 <sup>5</sup> CFU/ml	2, 4, 6, 8
4	10	5	-	-	2, 4, 6, 8

1 - *S. Enteritidis* 327 lux treated positive control

2 - UPWr\_S134 treated negative control

3 - inoculated with *S. Enteritidis* 327 lux and UPWr\_S134 treated

4 - untreated control

<sup>a</sup>UPWr\_S134 phage cocktail was administered together with *S. Enteritidis* 327 lux inoculation

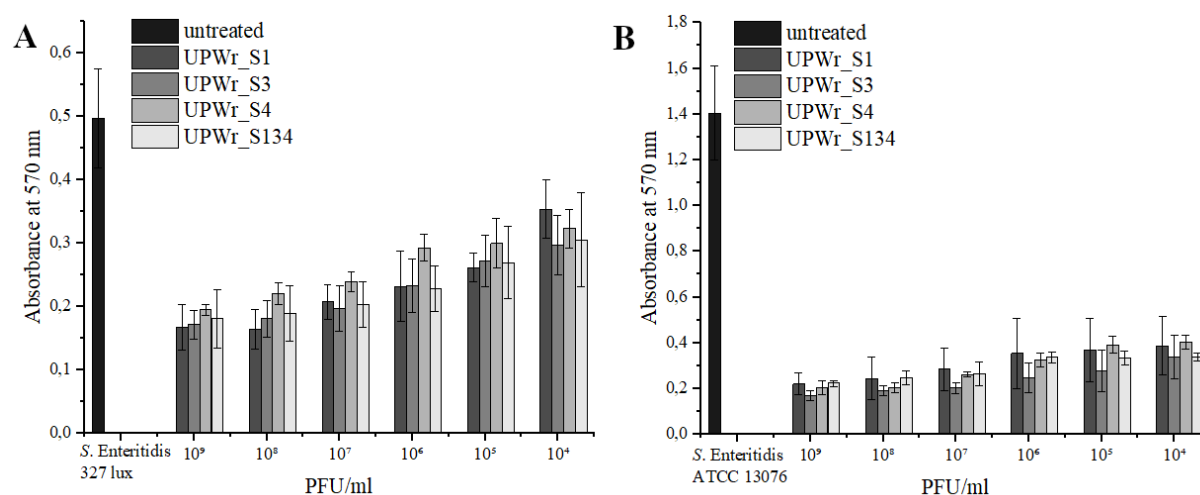
## Results

### Phage activity against *Salmonella* biofilms *in vitro*

The activity of phages UPWr\_S1, UPWr\_S3, and UPWr\_S4 and the phage cocktail UPWr\_S134 in biofilm degradation was determined by crystal violet staining in 96-well plates. The *S. Enteritidis* 327 lux and ATCC 13076 strains were classified as moderate and strong biofilm producers, respectively, according to the classification. The control strain *S. Enteritidis* ATCC 13076 was found to have ~2.8 times greater ability to form a biofilm than *S. Enteritidis* 327 lux and exhibited higher sensitivity to treatment with UPWr\_S1, UPWr\_S3, and UPWr\_S4 phages alone and the phage cocktail UPWr\_S134 (Figure 1). Biofilm biomass degradation compared to the control observed for all tested phage titers of single-phage lysates and the UPWr\_S134 cocktail against *S. Enteritidis* ATCC 13076 was highly significant ( $p < 0.01$ ). For the highest phage titer 10<sup>9</sup> PFU/ml, the reduction level of biofilm produced by *S. Enteritidis* 327 lux did not differ between treatments and was estimated to be 69, 63, 65, and 65% for phages UPWr\_S1, UPWr\_S3, and UPWr\_S4 and the phage cocktail UPWr\_S134, respectively. For treatment with the

lowest titer of  $10^4$  PFU/ml, degradation was estimated to be 32, 41, 34, and 37% for phages UPWr\_S1, UPWr\_S3, and UPWr\_S4 and the phage cocktail UPWr\_S134, respectively, and did not differ between treatments (Figure 1A). Treatment of *S. Enteritidis* ATCC 13076 biofilm in a 96-well microtiter plate with the titer of  $10^9$  PFU/ml of phages UPWr\_S1, UPWr\_S3, and UPWr\_S4 and the phage cocktail UPWr\_S134 resulted in a drastic decrease of biofilm biomass of 84, 87, 82, and 83%, respectively. Applying single phages and the phage cocktail at a low titer of  $10^4$  PFU/ml affected efficient biofilm removal activity, which was still great and estimated to be 72, 75, 65, and 76% for UPWr\_S1, UPWr\_S3, and UPWr\_S4 and the phage cocktail UPWr\_S134, respectively (Figure 1B). No significant differences were found for *S. Enteritidis* 327 lux and ATCC 13076 biofilm treatment with phages alone and in a mixture for all tested titers of  $10^4$ - $10^9$  PFU/ml ( $p > 0.05$ ).

**Figure 1.** Effect of UPWr\_S1, UPWr\_S3 and UPWr\_S4 phages and phage cocktail UPWr\_S134 on biofilm in 96-well microplate. (a) Effect of UPWr\_S1, UPWr\_S3 and UPWr\_S4 phages and phage cocktail UPWr\_S134 on reduction of biofilm formed by *S. Enteritidis* 327 lux, and (b) Effect of UPWr\_S1, UPWr\_S3 and UPWr\_S4 phages and phage cocktail UPWr\_S134 on reduction of biofilm formed by *S. Enteritidis* ATCC 13076. Values represent the mean with a standard deviation of three replicates.

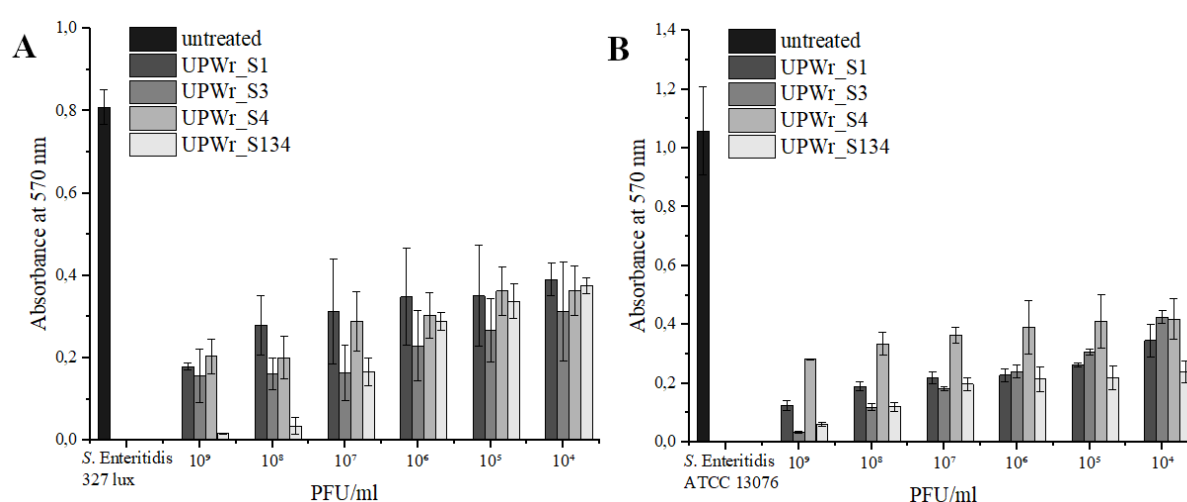


### Degradation of *S. Enteritidis* biofilm on stainless steel

Stainless steel washers used in this study were evaluated for phage lytic activity against strains of *S. Enteritidis* that are able to produce biofilm. The strong biofilm producer *S. Enteritidis* ATCC 13076 showed ~1.4 times higher ability to form biofilm on a stainless steel surface than *S. Enteritidis* 327 lux, and biofilms produced by each strain were effectively destroyed by monophage lysates and the phage cocktail ( $p < 0.01$ ; Figure 2). Statistically significant biofilm removal compared to the control was observed for all tested titers of UPWr\_S1, UPWr\_S3, and UPWr\_S4 phages and the UPWr\_S134 phage cocktail against both *S. Enteritidis* strains ( $p < 0.01$ ). A dose-dependent effect of reduction of pre-formed *S. Enteritidis* 327 lux biofilm was observed for the UPWr\_S134 phage cocktail between titers  $10^5$  and  $10^8$  PFU/ml. For this strain, the UPWr\_S134 phage cocktail exhibited greater efficiency in biofilm degradation for high titers of  $10^8$  and  $10^9$  PFU/ml (96 and 98%) in comparison to monophage lysates ( $p < 0.01$ ). In total, 54% of biofilm removal for a titer of  $10^4$  PFU/ml of the UPWr\_S134 phage cocktail was observed. A low titer of  $10^4$  PFU/ml of single phages UPWr\_S1, UPWr\_S3, and UPWr\_S4 showed the ability to lyse biofilm-based *S. Enteritidis* 327 lux at levels of 52, 61, and 55%, respectively,

whereas these phages at the highest titer of  $10^9$  PFU/ml caused biofilm degradation estimated to be 78, 81, and 75%, respectively. No significant differences were found for treatments against *S. Enteritidis* 327 lux with phages alone and in a mixture for titers between  $10^4$  and  $10^7$  PFU/ml ( $p > 0.05$ ) (Figure 2A). In the case of strain, *S. Enteritidis* ATCC 13076, UPWr\_S1, UPWr\_S3, and UPWr\_S4 phages and the UPWr\_S134 phage cocktail treatments with the highest titer of  $10^9$  PFU/ml reached effectiveness up to 88, 97, 73, and 94%, respectively (Figure 2B). The high effectiveness to reduce *S. Enteritidis* ATCC 13076 of 67, 60, 60, and 77% was observed for the low titer of  $10^4$  PFU/ml of phages UPWr\_S1, UPWr\_S3, and UPWr\_S4 and the UPWr\_S134 phage cocktail, respectively. A dose-dependent effect was found for the phage UPWr\_S3. Phage UPWr\_S4 exhibited significantly lower activity in the elimination of *S. Enteritidis* ATCC 13076 cells from biofilm structure for titers of  $10^5$ - $10^9$  PFU/ml in comparison to other treatments. It was observed that the phage cocktail UPWr\_S134 was significantly more effective in reducing biofilm formed by *S. Enteritidis* ATCC 13076 for low titers of  $10^4$  and  $10^5$  PFU/ml in comparison to monophage lysates ( $p < 0.01$ ).

**Figure 2.** Effect of UPWr\_S1, UPWr\_S3 and UPWr\_S4 phages and phage cocktail UPWr\_S134 on biofilm on stainless steel washers. (a) Effect of UPWr\_S1, UPWr\_S3 and UPWr\_S4 phages and phage cocktail UPWr\_S134 on reduction of biofilm formed by *S. Enteritidis* 327 lux, and (b) Effect of UPWr\_S1, UPWr\_S3 and UPWr\_S4 phages and phage cocktail UPWr\_S134 on reduction of biofilm formed by *S. Enteritidis* ATCC 13076. Values represent the mean with a standard deviation of three replicates.



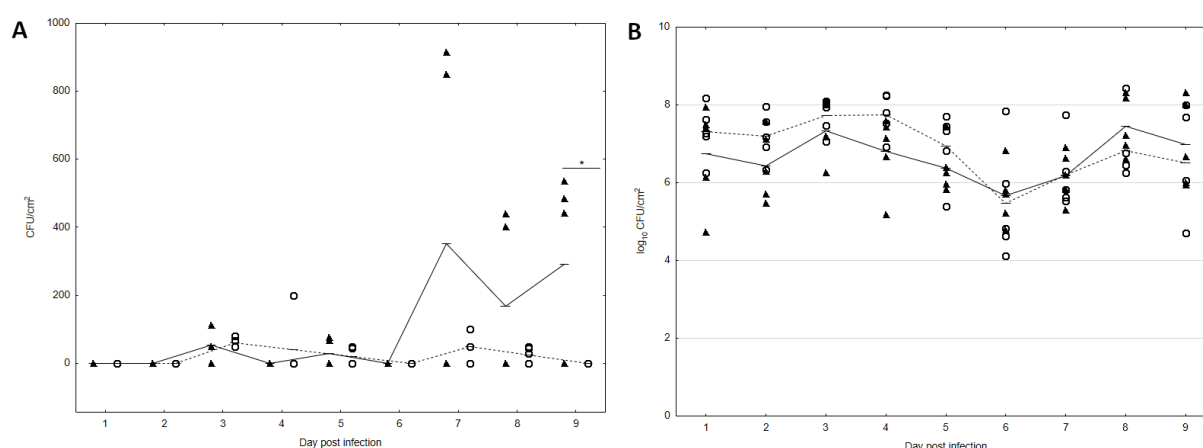
UPWr\_S134 phage cocktail effectively reduced the number of *Salmonella* from the poultry drinker surface in the experimental chicken model

For evaluation of the UPWr\_S134 phage cocktail's ability to eliminate *S. Enteritidis* 327 lux from the multispecies community formed on the poultry drinker surface, analysis with an experimental chicken model was performed. In drinkers from all experimental groups, a high number of TVC was detected. The TVC on the poultry drinker surface did not change significantly during the whole experimental period, with the range of 5–8 log<sub>10</sub> CFU/cm<sup>2</sup> between the untreated group 1 (black lines with black triangles) and group 3 treated with the UPWr\_S134 phage cocktail (dashed lines with empty circles), respectively ( $p < 0.05$ ) (Figure 3B). Treatment of poultry drinkers filled with drinking water contaminated with *Salmonella* and the phage cocktail UPWr\_S134 resulted in inhibition of *S. Enteritidis* 327 lux multiplication, contrary to phage-untreated drinkers ( $p < 0.01$ ). In drinkers from



group 3 treated with UPWr\_S134 phages, the mean number of *Salmonella* did not exceed  $10^2$  CFU/cm<sup>2</sup> during the experiment and on the last day (9 days after infection) *S. Enteritidis* 327 lux were not detected in swabs (empty circles) (Figure 3A). In drinkers from group 1 contaminated with *Salmonella* and not treated with phages, the detected number of *Salmonella* starting on day 7 was higher than in group 3 and ranged between  $1.5$  and  $3.5 \times 10^2$  CFU/cm<sup>2</sup> ( $p < 0.05$ ; black triangles).

**Figure 3.** Effect of phage cocktail UPWr\_S134 on bacteria attached to the poultry drinker surface in an experimental chicken model. (a) Effect of phage cocktail UPWr\_S134 on the number of *S. Enteritidis* 327 lux. (b) Effect of phage cocktail UPWr\_S134 on the total viable count. Results for bacterial load show counts for individual drinker plus the mean ( $n=5$  per group). \* $p < 0.05$  indicates a significant difference between control and treatment groups. Black triangles indicate control group 1 infected with *S. Enteritidis* 327 lux and empty circles indicate group 3 treated with both *S. Enteritidis* 327 lux and phage cocktail UPWr\_S134.



## Discussion

In this study, we investigated phage ability to reduce *Salmonella* in poultry drinkers. We indicated the high potential of UPWr\_S1, UPWr\_S3, and UPWr\_S4 bacteriophages and UPWr\_S134 phage cocktail to reduce biofilm formed by *S. Enteritidis* *in vitro*. Our results revealed that UPWr\_S134 phage cocktail treatment of artificially contaminated poultry drinkers resulted in a decreased number of *S. Enteritidis* attached to the drinker surface in laboratory conditions. Moreover, the study showed for the first time that the UPWr\_S134 phage cocktail specifically and effectively eradicated *S. Enteritidis* from bacterial load located on the poultry drinker surface in an experimental chicken model, indicating that this phage cocktail is a promising candidate for *Salmonella* biocontrol at the farm level.

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## Influence of Using a narrow strip of Perforated Plastic Flooring below the drinking lines on Growth Performance, Litter Quality, and Footpad Health of Broilers

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### Introduction

In today's poultry farming, housing conditions and management significantly influence animal health and welfare [1]. In Germany's commercial chicken farms, broilers are kept on a littered concrete floor [2]. The litter not only serves as a bedding material [3], but also should allow the animals with the essential species-specific behavior of pecking, scratching and sandbathing, thus improving animal welfare [4,5]. The aim of this study was to implement a narrow strip of a slatted floor beneath waterlines in broiler barns in order to evaluate its influence on litter quality and subsequently foot pad health. The hypothesis tested is that application of slatted flooring underneath of waterlines helps improving litter quality and thus reduces foot pad diseases in order to enhance animal welfare.

### Material & Methods

The broiler chickens in this study were raised under standardized husbandry conditions and subjected to a standard fattening procedure on the Farm for Education and Research Ruthe, University of Veterinary Medicine Hannover, Foundation, Sarstedt, Germany.

The study was performed in five consecutive runs with approximately 15,000 broilers (Ross 308) of both sexes per run. Each run was defined as one fattening period of 32 days. One-day-old chicks were divided into two groups per run: the control group (CON) and the experimental group (EG). Every run was carried out in two barns: 8000 animals in the CON group ( $29.5 \text{ m} \times 15.9 \text{ m} = 469 \text{ m}^2 = 17.05 \text{ animals/m}^2$ ) and 7000 animals in the EG group ( $35.6 \text{ m} \times 11.3 \text{ m} = 402.3 \text{ m}^2 = 17.4 \text{ animals/m}^2$ ). The experimental barn was equipped with 80 slatted floors below the drinking lines. Each level segment was formed from a slatted floor consisted of holes ( $15 \times 10 \text{ mm}$ ) and bridges (plastic covered steel; width 3.5 mm) to a trapezoid, which has a dimension of 70 cm base area, 19 cm leg length, 36 cm roof area and a height of 10 cm. According to the European Council, the slatted floor is not a "useable area" for broilers, as only a littered area that is accessible to the birds at all times can be considered a "useable area". [6].

In each fattening run, the individual body weight (BW) and FPD of 50 randomly selected broilers per barn was recorded weekly and every barn was divided for weekly sampling (DM, Nitrogen, calorific

value and starch) into three areas (drinkers, feeders and comfort area). The foot pads were scored on a scale from 0 to 7 in accordance with Mayne et al. [7] and at the end of the fattening period, a camera-based footpad scoring (QS Fachgesellschaft Geflügel GmbH Germany) [8], with a scale of 0, 1, 2a, 2b, which is commonly used at the slaughterhouse, was performed.

## Results

In group comparison, no influence on growth performance was noted. The slatted floor influenced the litter quality by preventing the litter in EG from getting wet until day 14 ( $690 \pm 167$  g/kg DM;  $p = 0.00139$ ) of the fattening period. The first two weeks the CON group showed significantly lower FPD scores compared to those reared in EG group (Table 1.). However, at days 21 and 28 of life, birds housed in the experimental barn (EG) had significantly lower FPD scores in comparison to those in the CON

Day of Life	Groups		<i>p</i> -Value
	CON	EG	
7	$0.73^b \pm 0.84$	$1.47^a \pm 1.17$	0.0001
14	$1.46^b \pm 1.23$	$2.31^a \pm 1.32$	0.0001
21	$2.56^a \pm 1.68$	$1.91^b \pm 1.48$	0.0001
28	$2.88^a \pm 1.82$	$2.11^b \pm 1.51$	0.0001

group. Similarly, on d 32 of life and according to slaughterhouse data (five runs), the FPD scores for broilers housed in the CON group showed significantly higher scores (22.2) than those housed in the EG group (8.80).

**Table 1.** Footpad dermatitis score of broilers ( $n = 250$ ) from d 7 to d 28 (mean  $\pm$  SD).

CON = control group; EG = experimental group with slatted flooring beneath the waterline.

<sup>a, b</sup> = Means within the same row with different superscripts differ significantly ( $p < 0.05$ ).

## Discussion

These study shows that slatted floors, beneath the waterline, have no significant negative influence on the BW of the Broilers, but a significant positive effects on footpad health. The condition of the litter, in terms of infiltration and its quality, were mainly reflected by the DM values. Therefore, the moisture of the litter is in most parts of the barn significantly dryer, especially in the drinker area. In this study, a perforated area below the watering lines seems favorable to influence the moisture of the litter and with it the foot pad health of the broiler.

## Acknowledgement & Funding

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## The brood behavior of the domestic duck

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### Introduction

Poultry is the only group of livestock that has almost completely lost control of their reproduction. Hatching takes place in incubators that allow for the simultaneous incubation of several hundred thousand eggs. The technique of incubating duck eggs is not yet well developed. Understanding the brood behavior of the domestic duck of should help to develop an optimal incubation program for their eggs.

### Material & Methods

A dummy eggs equipped with 5 temperature sensors and one omnidirectional motion sensor were inserted into 5 nests. Temperature measurements were performed in continuous operation every 1 minute, while the direction and angle of movement were recorded when the position of the egg was changed  $\geq 20^\circ$  every 1 second. Measurements were started after the eggs had been inserted into the nests. The registration period was 13 weeks

### Results

In the initial period of hatching, the ducks spent the time spent in the nest ranged from 4 to 15 hours a day. During this period, the ducks heated the eggs from 1 to 5 times. The average time a duck stayed in the nest depended on the number of bird entries to the nest during the day. When the duck stayed in the nest more often, the single time of heating the eggs was from 30 to 45 minutes, and with less frequent it was up to 4 hours. During their stay in the nest, the ducks heated the eggs to a temperature of 20-25°C. In the second period of hatching the total time of heating the eggs during the day increased to about 13 hours. The number of individual inputs per socket has also increased. During the day, the ducks heated the eggs 6 to 12 times, with the average time spent in the nest from 30 minutes to 1.5 hours. There was also an increase in the temperature to which the eggs were heated (24-34 °C)

### Acknowledgement & Funding

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## Development of new methods to reduce microbial contaminants during chicken slaughtering

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### Introduction

Intervention measures to prevent humans from foodborne illnesses are implemented in the entire broiler production chain. As these measures are primarily taken in the pre-harvest production, cross contamination at slaughterhouse level is commonly detected. We aimed to optimize existing procedures and develop new methods to reduce microbial contaminants in chicken slaughtering. For this, we focused on (I) process water and (II) ambient air in the slaughterhouse and their role in terms of cross contamination during the slaughter process.

### Material & Methods

I) The efficacy of organic acids (formic acid and lactic acid) and oxygen releasing agents (peracetic acid) was tested in suspension (DIN EN 1276) on different bacterial pathogens (*Enterococcus hirae*, *Staphylococcus aureus*, *Salmonella* Typhimurium) using autoclaved scalding water from a broiler slaughterhouse as organic soiling. Based on the scalding process, a testing temperature of 52°C and an exposure time of 3 minutes was chosen.

II) We evaluated the bacterial load in the ambient air by taking samples at different process steps and at multiple times in a broiler slaughterhouse using two Coriolis µ cyclonic air sampler. We tested the efficacy of the preinstalled measures ozonation, UV-C irradiation and ionization to reduce airborne pathogens by taking samples over the course of a day with and without these measures in operation.

### Results

I) The tested disinfectants showed a 5-log reduction of the investigated bacterial pathogens using concentrations ranging of 0.001% to 0.004% for peracetic acid, 0.04% to 0.5% for formic acid and 0.1% to 2% for lactic acid. *E. hirae* was the limiting pathogen in all experimental setups, requiring the highest concentrations of disinfectants for a 5-log reduction.

II) The investigated air samples from the slaughterhouse showed a bacterial load in the processing area with quantities ranging from  $< 1 \times 10^1$  cfu/m<sup>3</sup> to  $3.3 \times 10^3$  cfu/m<sup>3</sup>. During the course of a day, bacterial load increased significantly in the air. No differences in airborne bacterial load were shown using ozonation, UVC irradiation or ionization in the processing area of the slaughterhouse.

### Discussion

I) Our results demonstrate that an effective disinfection in suspension is possible with practical relevant very low concentrations for the three tested disinfectants. For an implementation of adding these

disinfectants to the scalding water during slaughter process, the impact of disinfectants on carcasses needs to be evaluated subsequently.

II) The results of our ambient air samplings indicate a low bacterial load in the processing area of broiler slaughterhouses. The rise of airborne bacteria during the course of the day was to be expected due to the continuous slaughter process and increasing number of passing carcasses. However, ozonation, UVC irradiation and ionization are non-effective measures to decrease bacterial load in this setup.

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## Potential efficacy of combined non-biosafety-based intervention measures to reduce *C. jejuni* colonization in broiler chickens at slaughter age

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### Introduction

Campylobacteriosis was the most commonly reported foodborne gastrointestinal infection in the European Union (EU) in 2020. Therefore, reducing *Campylobacter* colonization in broilers at slaughter age is considered an important key step. Since previous measures are still insufficient on their own, we aimed to evaluate the efficacy of combined non-biosafety measures. Based on previous studies, we selected and combined four non-biosecurity-based interventions that were individually successful in reducing *C. jejuni* colonization. In fact, we combined a CE culture with bacteriophages and an essential oil (carvacrol) with organic acids.

### Material & Methods

Per experiment, 58 newly hatched broiler chickens of breed Ross 308 were raised in the experimental animal facility on floor housing with litter. On day 10 of age, 12 broiler chickens (seeder) were orally inoculated with  $10^4$  cfu/500 µl *C. jejuni*. Thereafter, *Campylobacter* colonization and load was determined weekly by taking cloacal swabs of 23 randomly selected untreated broilers (sentinels). At the end of each experiment (33 days post hatch), sentinels were dissected and cecum and colon contents were collected for *C. jejuni* count determination. To examine the effect of a combination of carvacrol and organic acids, broilers were fed daily with 120 mg/kg feed of carvacrol and a mixture of four acids in their drinking water. Moreover, to evaluate the efficacy of the combination of a CE-culture with bacteriophages, broiler chickens were treated with the CE-culture twice (day 1 using spray application, day 25 via drinking water application) and received a phage combination of two phages continuously via drinking water four, three, and two days prior to necropsy. Broilers were provided free access to commercial broiler feed and filtered water from the municipal water supply ad libitum during the entire study.

### Results

Cecal count enumeration demonstrated that the *C. jejuni* load was significantly reduced for the group receiving a combination of the CE-culture and bacteriophages compared with the control group (log reduction of  $1.0 \log_{10}$  MPN/g). Likewise, colon counts were significantly decreased for the group receiving a combination of bacteriophages and the CE-culture (log reduction of  $1.0 \log_{10}$  MPN/g). In contrast, although we observed a log reduction of  $1.0 \log_{10}$  MPN/g in *C. jejuni* cecal counts in the group receiving a combination of carvacrol and organic acids, this reduction was, however,

nonsignificant compared with the control group. Likewise, there was no significant difference in *C. jejuni* counts in the colon.

### **Discussion**

We conclude that a combination of this particular CE-culture and bacteriophages may be a promising practical approach in broiler production to reduce *C. jejuni* colonization in broiler chickens at slaughter age. However, why the combination of carvacrol and organic acids failed to reduce *C. jejuni* intestinal colonization is unclear and remains to be investigated.

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## Quality of thawed semen from meat bulls

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### Introduction

The characteristics of sperm, such as motility or the percentage of normal sperm, form a positive relationship with fertility, and when obtained more than 70% increases the fertility rate. It is important to have high quality semen because this positively correlates with fertility in cattle. For a sperm to be able to fertilize an oocyte it must meet a series of requirements. The objective was to assess the effect of the bull breed on sperm quality after thawing of semen (Aitken and Baker, 2004; Amann AND Pickett, 1987).

### Material & Methods

The bull breeds used were Charolais, Brahman and Simbrah. 10 plastic straws of 0.5 ml of frozen semen from three different breeds of bull were used, which were acquired in a commercial company. The straws were thawed at 37°C for 40 seconds. Immediately afterwards, motility, viability and acrosomal integrity (NAR) were assessed.

The evaluated macroscopic evaluation consisted of: pH, color, odor volume, consistency. Microscopic evaluation included progressive motility, viability, sperm agglutination, sperm concentration, and morphology. Motility and viability were assessed by observing a drop of semen under the microscope and the result was reported in percentage. For NAR the Giemsa technique was used, and for the analysis of the results the ANDEVA was used.

### Results

The results were for motility 75.0, 87.5 and 85.0 %; for viability 74.5, 74.5 and 72.5 %; and for NAR 97.1, 96.9 and 96.9% for Charolais, Brahman and Simbrah breeds, respectively.

Motility varied according to race, Charolais had a lower percentage with 75%; Brahman and Simbrah had 87.5% and 85% respectively. These results are similar to those reported by Cabrera and Pantoja (2012), who found a motility of 77 to 85% when working with dual-purpose bulls. In terms of feasibility, the results were superior to those reported by Ribeiro et al. (2014), who indicated a viability of 60% when working with thawed semen from epididymis. The present NAR results were higher than those reported by Cabrera and Pantoja (2012), who reported between 60 and 73% of NAR when working with national bulls of Peru.

## Discussion

Three breeds of bulls showed good sperm quality after thawing, which can be recommended to be used in artificial insemination without any problem (Bucak, 2007).

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## **The effect of the calves' age on moving to group housing on their performance, health and welfare**

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### **Introduction**

The success of calf group housing depends on the age at which the calves will be grouped together. It is not recommended to create a group between the 14th and 21st days after birth, when the level of passive antibodies from mother's colostrum decreases to minimum and calves begin to generate their own antibodies (Hulber and Moisé, 2016). The aim of this study was to determine the age suitable for moving calves from individual to a group housing with regard to their performance, health and welfare.

### **Material & Methods**

The two-year study was performed in 48 calf-bulls (from birth to 60 days of age) which were placed in the individual hutches immediately after their birth. After then 16 calf-bulls were moved to group hutches in fours at the age of 15, 20 and 31 days. The calves were being weighed in weekly intervals as well as the starter intake and health were recorded once a day. The behavioural activities of calves (lying, standing and cross-sucking, etc.) were analysed in 24-hour cycles every week. All statistical analyses were performed in TIBCO Statistica.

### **Results**

The calves, of all ages placed into the group hutches, spent most of day time by lying and resting (62.9-66.4%). The calf-bulls reared in the group from 20th day of age sucked the body parts of other calves ( $p < 0.05$ ) for a longer period of time (by +0.5%) in compared to the calf-bulls reared in the group from 15th day of age. Calf-bulls housed in group from 15th day of age had a significantly higher ( $p < 0.05$ ) weight gain by +4.4 kg in consequence higher intake of starter by +1.3 kg than calf-bulls housed in group from 31st day of age. Calf-bulls reared in the group from the 31st day of age had a significantly lower ( $p < 0.01$ ) occurrence of diarrhoea and a non-significantly lower occurrence of respiratory diseases.

### **Discussion**

The length of resting and sleeping of the calves was shorter than found by Hänninen (2007) who report that calves spend lying down about 70 to 80% of the day. Cross-sucking is most common at calves which drink milk from open buckets (Lidfors and Isberg, 2003). Group housing soon after birth can increase weight gains and intake of solid feed (Costa et al., 2015). However, the risk of spreading enteric disease in the first few weeks of life and increased in respiratory disease during the first grouping are the primary factors for delaying group housing (Svensson et al., 2003). The placement of

the calves from the individual to the group housing is appropriate to take place from the 31st day of age from the point of their health and welfare.

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## Acclimatization to moderate altitude in ewes having low or high hematocrit levels

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### Abstract

The object of the present study was to investigate the acclimatization strategy to mild hypoxia in ewes having low or high hematocrit levels. Ile De France ewes were selected according to their levels of hematocrit and then were allocated into two groups. Group I comprised ewes with low hematocrit level (n=10) and group II comprised ewes with high level of hematocrit (n=10). Immediately after the shearing, performed at the experimental unit of the Institute of Animal Science, Kostinbrod, (altitude of 500 meters) the ewes were transported to a mountain pasture at altitude of 1440 m. where they were raised for 4 months (June-September). Blood samples were collected via venipuncture at the following time points: before shearing, immediately after the transport, on day 5, 10, 30 and 60 after the transport. The following indices were measured: plasma cortisol, hematocrit, total leukocyte count, erythrocyte count and reticulocyte count. Adrenal response to transport and exposure to altitude was significantly higher in high-hematocrit ewes relative to low hematocrit ewes. The higher hematocrit level in the ewes of group II persisted throughout the experiment. The ewes having high hematocrit level had higher cortisol concentration immediately after transport and during the rest of the experimental period as compared to low-hematocrit ewes. Erythrocyte count tended to be higher in high-hematocrit ewes throughout the experimental period. Reticulocyte count increased significantly as compared to basal count and remained elevated in both groups at the mountain pasture. There was distinct difference in the reticulocyte number dynamics among the groups. Leukocyte count tended to be higher in the high-hematocrit ewes immediately after transport and on day 5 and 10 following exposure to altitude. The observed trend reached significance on day 30 and 60. It was concluded that basal hematocrit level is related with the pattern of adrenal and hematological adjustments of newly shorn ewes to moderate altitude. Acknowledgement: This work was supported by Bulgarian Science Fund (grant KP-06-H26/2, 04.12.2018).

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## **Analysis of the legislation on the welfare of the dairy herd in the EU and Ukraine.**

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### **Introduction**

Over the past 40 years, the European Union has adopted a number of other laws, directives, and regulations on animal welfare (Simonin, Gavinelli, 2019) Undoubtedly, changes in legislation have a positive impact. The European Union has high regulatory standards for animal welfare (Broom, 2018) EU animal welfare legislation is quite extensive. The latest EU legislation now focuses on the welfare of farmed animals, but EU legislation also covers animals in zoos, animals used for scientific purposes and in other areas.

In 2014, Ukraine signed an agreement with the European Union on the creation of a deep and comprehensive free trade zone within the framework of the association agreement. This greatly expands the horizons in terms of trading opportunities, but also imposes certain obligations that must be fulfilled. First of all, this is a change in legislation, its implementation to European standards, the introduction of stricter compliance with requirements and laws. On February 18, 2021, more than 5 years after the entry into force of the agreement, a ministry order was issued in Ukraine to bring its animal welfare legislation closer to EU requirements. One of the biggest problems of this legislation is the extended terms of implementation, the inconsistency of references to the regulatory and legal framework, the permission to use antibiotics without strict testing, and the lack of mechanisms for monitoring the fulfillment of the requirements of this law (Eurogroup for Animals, 2021)

That is why research, analysis and understanding of European legislation is now so important for Ukraine. The experience of the European Union in ensuring the welfare of animals can become the basis for the development of sustainable animal husbandry in Ukraine.

### **Material & Methods**

In this article, we used methods of analysis and generalization. We have researched the legislation, directives, standards and regulations of the European Union regarding the welfare of the dairy herd. At the same time, we analyzed the current and new law on veterinary medicine in Ukraine.

### **Results**

EU legislation on the protection of animal welfare covers welfare during the keeping of animals, their transport and slaughter. Despite the wide range of legislative provisions, it is not sufficient. This is worrying, especially in the light of the findings of a report (European Parliament, 2017) on animal welfare prepared by Parliament for the European Parliament's Committee on Petitions of the Directorate-General for Internal Affairs that "the welfare of dairy cows may be considered one of the biggest animal welfare concerns in the EU" (Nalon., Stevenson., 2019)

Addressing welfare issues in the EU dairy sector is somewhat hampered by the lack of species-specific legislation on dairy cow welfare. As a first step, the European Commission should develop a formal recommendation on the steps dairy farmers should take to prevent and treat lameness or mastitis as



part of their the legal obligation to "take all reasonable measures to ensure the well-being" and avoid unnecessary pain, suffering or injury, as referred to in Directive 98/58 (Nalon., Stevenson., 2019)

In 2020, the "Farm to Fork" strategy announced the intention to review EU legislation on animal protection. The commission plans to review the following legislative acts: the Directive on the protection of animals kept for agricultural purposes, four directives establishing minimum standards for the protection of hens - laying hens, broilers, pigs, calves, Regulations on the protection of animals during transportation and slaughter.

This initiative is very thorough and will include analysis of current legislation, open public discussions, development of scientific conclusions on animal welfare and, as a result, updated and more effective legislation in the field of welfare. The revision of the legislation will broaden its scope, simplify its implementation and ultimately ensure a higher level of animal welfare in the EU.

In June 2014, the EU and Ukraine signed an agreement on the creation of a deep and comprehensive free trade area within the framework of the association agreement (Eurogroup for Animals, 2021) This agreement provides for the maximum approximation of the legislative norms of Ukraine to the EU legislation in the field of animal welfare. On February 18, 2021, more than 5 years after the entry into force of the agreement, Ukraine finally issued a ministerial order to approximate its animal welfare legislation to the EU. However, there are obvious weaknesses in the Ukrainian legislation, and first, this concerns the fact that new rules will be introduced only in 2026.

Other shortcomings include lack of control mechanisms and updating of legislation, extension of legislation to farms with more than 10 animals, authorization of medicinal products without the need for rigorous testing, lack of detailed description of standards for electrical stunning equipment, insufficient awareness, and training of farmers to best comply with legislation, and, as a result, improve the level of animal welfare.

Considering all the facts, by 2023 the EU will change the legislation in the field of animal welfare, which in 2026 Ukraine is only planning to approach. Based on this, Ukraine now needs to take radical steps, namely: to implement the law on veterinary medicine as soon as possible, to conduct trainings for farmers in order to teach and explain the new norms, to develop a specific provision with minimum standards to ensure the well-being of cattle and cattle, review shortcomings and organize discussions with scientists in the field of animal welfare to improve the legislation, create a clear road map for this law.

## Discussion

Analyzing EU animal welfare legislation, it can be conditionally divided into three groups: a) animal welfare during their life on the farm, b) welfare during transportation, and c) welfare during slaughter. In general, agricultural activity is regulated by one general directive, which in turn covers all types of animals, and four directives that are specific to a certain type of animal (calves, pigs, laying hens, broilers). These directives establish minimum standards for keeping, feeding, reproduction. In turn, transportation and slaughter are covered by rules that are common to all EU member states. This complex was created gradually and was aimed at solving the biggest problems of intensive animal husbandry.

The purpose of Directive 98/58/EC (protection of animals kept for agricultural purposes) is to establish general rules for the protection of farm animals, regardless of species. This directive is based on the concept of the 5 freedoms of animals, which was developed by the British Farm Animal Welfare Council

(FAWC) in 1979, and was originally applied to assess the welfare of farm animals in intensive livestock systems.

In the EU, the welfare of dairy cows is covered only by this directive. In particular, it provides that "Member States must ensure that owners or keepers take all reasonable measures to ensure the welfare of animals in their care and to ensure that no pain, suffering or injury is caused to that animal." The wording imposes legal obligations on the member states and, in turn, on the owners and keepers of dairy cows. Ultimately, better dairy cow welfare in the EU will require species-specific legislation (Nalon., Stevenson., 2019). Also in the European legislation on the welfare of cows on dairy farms, there are: WOA standards for dairy herds, recommendations of the Council of Europe, a directive on the protection of calves, regulations on transportation and slaughter of animals.

In the Farm Animal Protection Index, in which countries are evaluated according to animal welfare criteria, Ukraine is assigned an "E" rating (API, 2020). This is a very low rating, on the same level as countries such as China, Nigeria, Argentina, Indonesia, and Venezuela.

In Ukraine, legal reform innovations began on February 21, 2006 with the adoption of the Law "On the Protection of Animals from Cruelty". Despite this positive legal framework, the authorities have still not adopted the by-laws necessary to fulfill their duties. Thus, the adoption of this law did not cause significant changes in the field of animal welfare (Eurogroup for Animals, 2021). The Animal Cruelty Act covers farm, domestic and wild animals, as well as laboratory, zoo and circus animals. This law aims not only to protect against cruelty, but also obliges owners to provide animals with their biological and species-specific needs. Norms and requirements of the law are not enough to ensure the establishment and compliance of animal welfare. Unfortunately, no changes are made to the law from 2017 to 2020, despite rapid changes in social relations, the environment, and the animal world (Liubchych et., al 2020).

The international legal framework for animal welfare is based on the principles of animal welfare and protection (Voelkl et., al 2020). However, there is no such definition in the general provisions of this law.

In the Law "On Veterinary Medicine", which was adopted in 1994, there is only one article that highlights the requirements regarding the actual issue of animal welfare, namely: providing animals with high-quality and safe feed and water; application of preventive veterinary and sanitary measures regarding animal health; use of veterinary drugs according to the instructions of a veterinary medicine doctor; prevention of cruelty to animals; provision of appropriate means of transport for moving animals. However, these requirements are very unclear and can be interpreted in different ways. This law has not been updated for many years, therefore it is maximally not adapted to modern conditions.

In June 2014, the EU and Ukraine signed an agreement on the creation of a deep and comprehensive free trade area (DCFTA) within the framework of the association agreement. This agreement contains an important provision on cooperation in the field of agriculture and rural development, which encourages the use of sustainable methods that take into account the welfare of animals. This is a recognition of the strong link between sustainable development and farm animal welfare. The agreement requires Ukraine to bring its animal welfare legislation closer to EU legislation. It also requires that the EU and Ukraine agree on a schedule for such approximation of legislation no later than 3 months after the date of entry into force of this agreement. The parties formally agreed to this document only in October 2019, more than three years after the entry into force of this agreement. This schedule only includes the timelines for the adoption of specific legislative instruments, not their implementation. According to this document, Ukraine had to harmonize the entire scope of animal welfare laws by 2019. These deadlines were not met (Eurogroup for Animals, 2021).

Only in February 2021, the new Law "On Veterinary Medicine" was adopted. The document aims to improve the legislation of Ukraine in the field of veterinary medicine and defines the basic principles of ensuring the well-being of animals during keeping, provides for the responsibility of market operators for violating the requirements of legislation on veterinary medicine and animal well-being. The articles of this law contain requirements for keeping, breeding, feeding, transporting and slaughtering animals, thereby bringing the legislation of Ukraine closer to the legislation of the EU.

In the official accompanying document, it is noted that the law will enter into force two years after its entry into force. But according to the Eurogroup for animals, this text provides for the extension of the implementation period until 2026

Also, in 2021, the Ministry of Economic Development, Trade and Agriculture of Ukraine developed requirements for the maintenance of certain types of animals (broilers, courses - laying hens, pigs and calves). These requirements actually duplicate the requirements of the EU and are very important within the framework of fulfilling the terms of the Deep and Comprehensive Free Trade Agreement.

Requirements for the well-being of calves during their maintenance establish minimum requirements for the conditions of maintenance of calves, including fattening and care for them. The weakness of the requirements, as well as the law, is that they do not apply to farms with fewer than 10 animals. That is, the well-being of animals on such farms is still under threat.

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## Animal welfare and lameness in donkeys

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### Introduction

There are 3.25 million donkeys in Mexico (FAO, 2017), placing it immediately after countries such as Ethiopia, Pakistan, China, and Egypt. The modern technology that gets the job done more efficiently has decreased the donkey population in several states. (INEGI, 2007).

In Mexico the population of donkeys is decreasing due to the export of their skins to China. In this country, donkey skins are used in the manufacture of a traditional medicine called *ejiao*, made with extracts of the gelatin found in the skin, mixed with herbs and other ingredients. It is attributed properties for the treatment of diseases related to blood nutrition and reaches 375 USD / kg. In 2020 Mexico exported to China the amount of 1,689 salted skins, with an export cost exempt from tariffs and free of value-added taxes. The demand in China reaching ten million skins a year and a global supply estimated at 1.8 million skins annually, it is evident that the world population of donkeys is in great vulnerability due to this market (The Donkey Sanctuary, 2019).

The donkey hooves have a complex three-dimensional structure consisting of a horny capsule containing bones, joints, blood vessels, nerves, ligaments, tendons, connective and adipose tissue; they are anatomically different from those of other equids, with a more vertical axis of the pastern, and a more open tubular structure than of the horse's hoof; this means it absorbs and retains more moisture, which can predispose them to abscesses (Fortini Toro, G. 2011).

Lameness is a clinical sign of structural or functional alterations, in one or more limbs, or in the spine, noticeable when the equine is in movement (dynamic examination), and even when it's standing. Its etiology includes other signs such as inflammation and pain (Iranzo 2020). Lameness in donkeys is considered the main discard factor (Broster et al., 2009).

Two institutions participated in this research. One of them is in Otumba, Hidalgo State, where Burrolandia de México A.C. was founded in 2006 to provide asylum and protection to donkeys that arrive from various regions for different reasons: donation, abandonment and rescue for abuse. The other is ASINUS an Eco didactic farm called where it is a donkey rescue and preservation center, belong to the UAS in Sinaloa state.

### Material & Methods

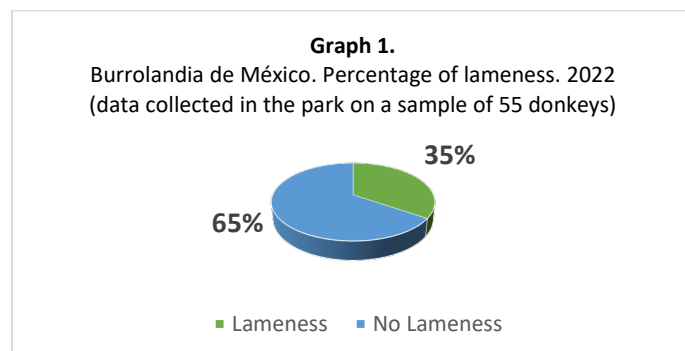
With the aim of diagnosing claudications in "Burrolandia de México", of a total population of 60 donkeys, we worked with a population sample of 55 animals, without distinction of race, sex or age, which were evaluated in January 2022: through a general diagnostic table and the determination and processing of statistical values.

A general clinical examination was performed for detects whether the lameness is due to a primary musculoskeletal condition, or a secondary clinical sign of a systemic disease Musculoskeletal examination relies on a set of maneuvers that locate the exact source of the lameness pain. This

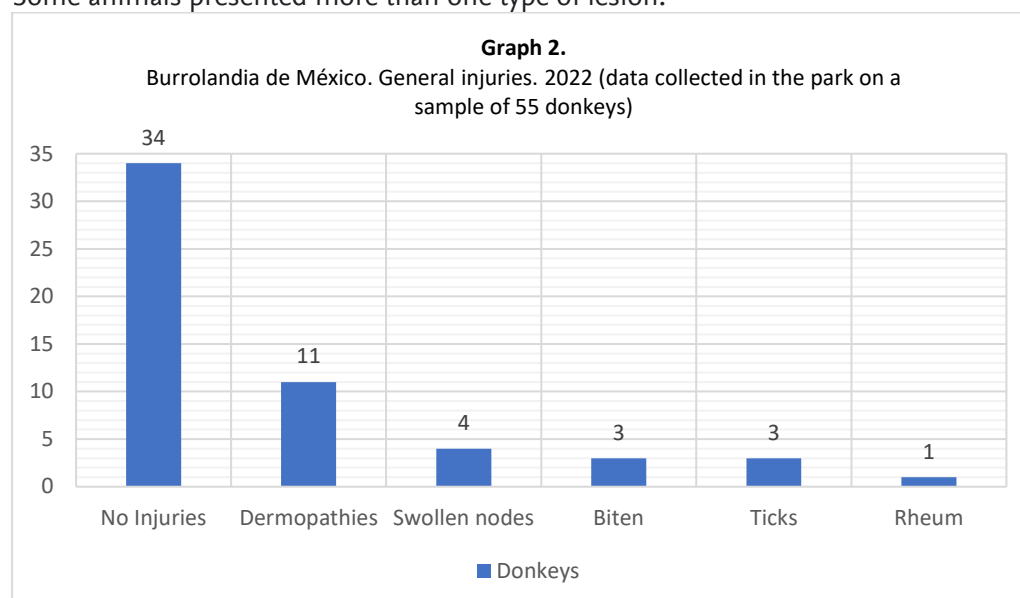
evaluation identifies the affected limb(s) through inspection, palpation, and sensitivity tests, considering the aspects of inflammation or poise that affect the movements of the limbs.

## Results

Morbidity rate was 35% of lameness at "Burrolandia de México".

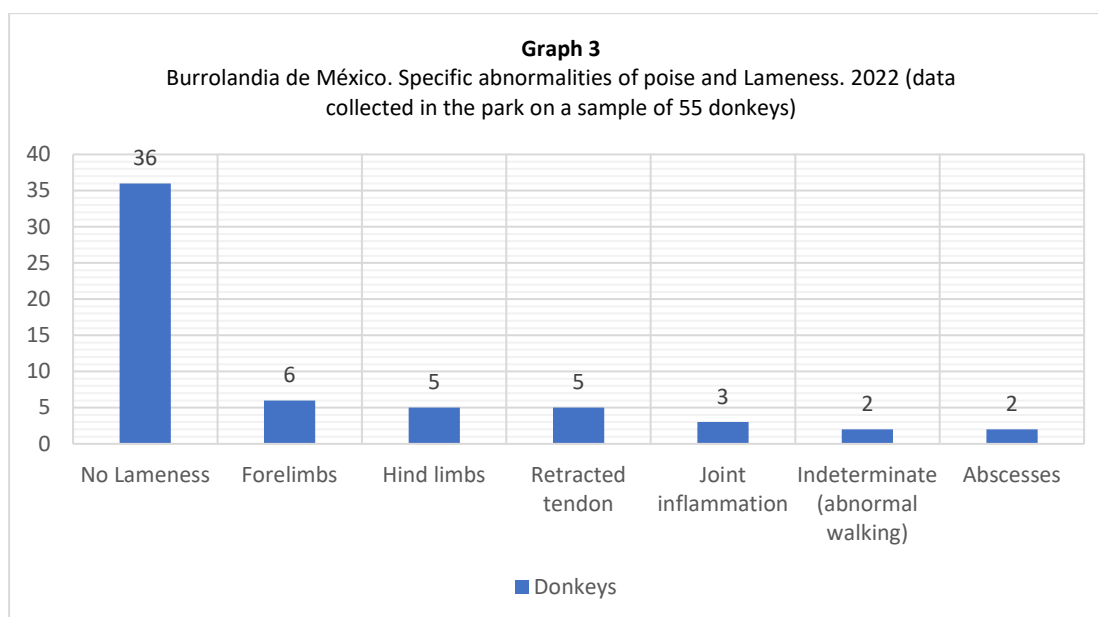


61.8% of the donkeys had no macroscopical injuries; 20% presented dermatopathies (mostly in legs); 7.2% had inflammation in some of the nodes; 5.4% had bites; another 5.4% had ticks; 1.8% had rheum. Some animals presented more than one type of lesion.



High and positive correlation (of 0.7) was detected between the problems of lameness and claudications, and the degrees of lameness on the scale of the AAEP (The American Association of Equine Practitioners).

Of the 41.8% of animals with lameness, 11% suffered it in the forelimbs and 9% in the hindlimbs. "Pie topino" caused by tendon retraction, occupies 9% of the sample, while 5.4% presented joint inflammation; 3.6% presented walking problems without a specific cause (which indicated that more in-depth observations and studies should be carried out); 3.6% had abscesses, although this percentage is low, those are considered serious injuries and therefore should not be taken lightly. Immediate attention was suggested.



High and positive correlation ( $r = +0.7$ ) was detected between the limb structure, lameness, and the degrees of lameness on the scale of the AAEP (The American Association of Equine Practitioners). This indicates that an increase in the problems of poise and lameness, implies a greater degree of lameness in donkeys.

Correlations			
		Poise and lameness	Lameness degrees
Poise and Lameness	Pearson's correlation	1	.692**
	Sig. (bilateral)		.000
	N	55	55
Lameness degrees	Pearson's correlation	.692**	1
	Sig. (bilateral)	.000	
	N	55	55

\*\* . La correlation is significant at 0,7 level (bilateral).

## Discussion

The percentage of animals with claudications was higher than expected (34%), and their severity scale was correlated to factors such as lack of attention to aplomb and claudication review protocols, lack of orderly scheduled cuts, and cleaning of pens. Only one of the animals presented claudication in grade 4 and none of grade 5 on the AAEP scale, which establishes the percentage to be improved in future evaluations. The constant measurement of indicators and diagnoses in the park will prevent bad practices from being normalized.

The existence of this park can help protect donkeys and, in some way, contribute to curbing the depopulation of this important animal species. The small budget is a limitation to address this problem, it does not receive any government support and institutions such as the UAM and UAS provide their services without remuneration. Is important to spread the work of rescue and protection that Burrolandia and UAS does.

## Acknowledgement & Funding

We thank Germán Flores (founder) and Raúl Flores (park administrator), as well as the entire Burrolandia de México A.C. team. for the facilities provided to carry out this review. We also thank the UAS for the facilities granted to carry out this research.

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## The concentrations of gaseous mixtures in broiler houses misted with essential oils

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### Introduction

Numerous reports indicate that essential oils have antimicrobial properties (Reddy et al. 2019; Sim et al. 2019; Witkowska et al. 2016; Witkowska and Sowińska 2013). Therefore, their compounds may have a potential to reduce gas production, as a result of decreasing the metabolic activity of microorganisms, which decompose organic matter into greenhouse and toxic gases and aroma compounds. The aim of our study was to determine if essential oil mist might reduce the concentrations of volatile compounds (GHG<sub>s</sub>, NH<sub>3</sub>, VOC<sub>s</sub>) in broiler houses.

### Material & Methods

The experiment was carried out in 3 broiler rooms (each for 120 birds) during 42 d rearing period. Every 3 d the rooms were misted with pure water (C-control) or emulsified aqueous solutions of 100% natural peppermint oil (PO) and thyme oil (TO). 1 d after EO treating the concentrations of gases were measured by FTIR spectroscopy (Gasmeter DX3040) and analysed using CalcmetPro and Statistica 13.

### Results

The prevalent gases were carbon dioxide, ammonia, methyl mercaptan, o-cresol and aniline, and their concentrations in PO room were statistically lower ( $P < 0.01$ ) than in C and TO houses. The highest NH<sub>3</sub> level was observed in TO room. Trace amounts of volatile gases, such as nitrogen oxides, hydrocarbons, alcohols, amines, aldehydes, phenols, ethers and others organic compounds were also determined, and they did not exceed 3 ppm. The concentrations of the above gases, excluding nitrogen dioxide, were also the lowest in PO group. The level of some aldehydes and phenols was the highest in room misted with thyme oil ( $P < 0.05$ ). In conclusion, peppermint oil has refreshing properties which might improve air quality in broiler houses. The poorer effect of thyme oil in gases reduction could result from inadequate dose, therefore further investigation is needed to its improvement.

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## Pig health in alternative farms: an online survey of 102 farmers

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### Introduction

Organic pig farms and other non-conventional farms are gaining ground with consumers demand. However, critical points may exist in these systems regarding pig welfare, health and biosecurity<sup>1</sup>. Thus this survey is the first part of a French research programme (PIGAL) jointly studying animal welfare, health, biosecurity, and veterinary public health in alternative pig farms. The aim of this online survey was to identify pig health problems in this type of farms.

### Material & Methods

An alternative farm was defined as having at least one housing system different from closed confined building with fully slatted floor in at least one part of the production stage or with at least 30% of the pigs housed in alternative system. An e-questionnaire was sent to 446 French farmers to collect data on the main health disorders observed with regard to the age of the animal (type, occurrence, percentage of affected pigs). The Sphinx survey Software was used to send the questionnaire in an electronic version to the farmers and to collect the data. Some farmers were also contacted by telephone to take part in the e-questionnaire to increase participation rate. The answers of farmers were converted into variables with several modalities. Data were then analysed by multiple correspondence analysis followed by hierarchical clustering<sup>2</sup> (FactoMineR package).

### Results

The questionnaire was filled in by 102 farmers: 65 had farrowing buildings, 82 had weaners and 94 had fatteners. In breeding herds, four health clusters were found ranging from a cluster of herds without frequent health troubles (n=22 farms; 33.8%) to a cluster of herds with sows having reproductive disorders, gilts suffering from respiratory troubles and sows and sucklers suffering from leg disorders (n=17 herds; 26.2%). After weaning, four health clusters were also found from herds without frequent health problems (27 farms; 34.6%), herds reporting lameness and respiratory diseases in finishers (18 farms; 23.1%), herds experiencing diarrhoea in weaners and respiratory troubles in finishers (16 farms; 20.5%) to herds facing leg disorders in weaners and respiratory diseases as well as bites in weaners and finishers 17 farms; 21.8%).

### Discussion

This is the first study considering a wide range of alternative farms and collecting descriptive information about their strength and weaknesses with a specific focus on health situation. The results need to be considered carefully, because data were collected on a voluntary basis, based on spontaneous declaration and only a part of the contacted farmers took part. Farmers may also have under- or overestimated the health levels of their herd according to their perception level. However,

these findings indicate a diversity of health troubles in alternative systems and that room for improvement need to be considered in some herds. The next part of our research project consists in an on farm study which should provide objective data to identify the best facilities and practices related to significant improvements.

### **Acknowledgement & Funding**

The authors are grateful to the farmers and related farm organizations for their contribution to the survey.

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## ***Salmonella* excretion level in pig farms and impact of quaternary ammonium compounds based disinfectants on *Escherichia coli* antibiotic resistance**

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### **Introduction**

The control of contamination of food by *Salmonella* and the reduction of antibiotic resistance are two major public health issues. *Salmonella* is the second foodborne pathogen responsible for gastroenteritis in the European Union (EFSA and ECDC, 2021). Ten to 20% of human salmonellosis in the EU are attributed to the consumption of pork. Meat pork ranks second among the meat associated with human salmonellosis outbreaks (Bonardi, 2017). There is little data on the level of *Salmonella* in the different steps of the pork production from farm to retail whereas these data are important to assess the risk along the food chain. Therefore, in order to estimate what can potentially happen in the food chain, it is important to acquire data on the level of *Salmonella* shedding in pigs at the farm level (Anses opinion 2016-SA-037). Moreover, *Salmonella* resistance to antimicrobials in food-producing animals can spread to humans and consequently infections by multi-antimicrobial resistant *Salmonella* may result in treatment failures. On the other hand, *Escherichia coli* (a well-distributed microorganism on farms) can also be a reservoir of resistance genes, which may be transferred between bacterial species, including organisms able of causing human and animal diseases.

One way to control *Salmonella* and other bacteria all along the food chain is to clean and disinfect the equipment and surfaces of empty premises. However, bacteria are able to adapt to repeated biocides exposure and may sometimes develop cross-resistance to biocide and antimicrobials (Soumet et al., 2012; FAO, 2018). A large amount of in vitro assays supports the role of some disinfectants in the selection or induction of antibiotic resistance in bacteria (Brannen and Davidson 2004, Karatzas et al. 2008, Curiao et al. 2016) but field data are very limited (Anses opinion 2016-SA-238). Since disinfectants are regularly used on farms to break down transmission of infectious pathogens, it is relevant to examine these disinfectant and antimicrobial resistances at this first step in the food chain. This study therefore aimed at acquiring data on the level of *Salmonella* excretion by pigs on infected farms and assessing the impact of disinfectants used in these farms on the evolution of antibiotic resistance in *Escherichia coli* strains.

### **Material & Methods**

Four French farrow-to-finish pig farms deemed to be *Salmonella* positive were visited 3 times from May to November 2019. At each visit, individual faeces from 10 lactating sows located in the same farrowing room and individual faeces from 20 finishing pigs from the same batch were collected. The detection of *Salmonella* was carried out according to the standardized NF-U47-102 method and

enumeration using a method based on the most probable number (MPN) (ISO/TS 6579-2-2012). Isolates were serotyped by agglutination according to the Kauffmann-White scheme and ISO/TR 6579-3:2014 method.

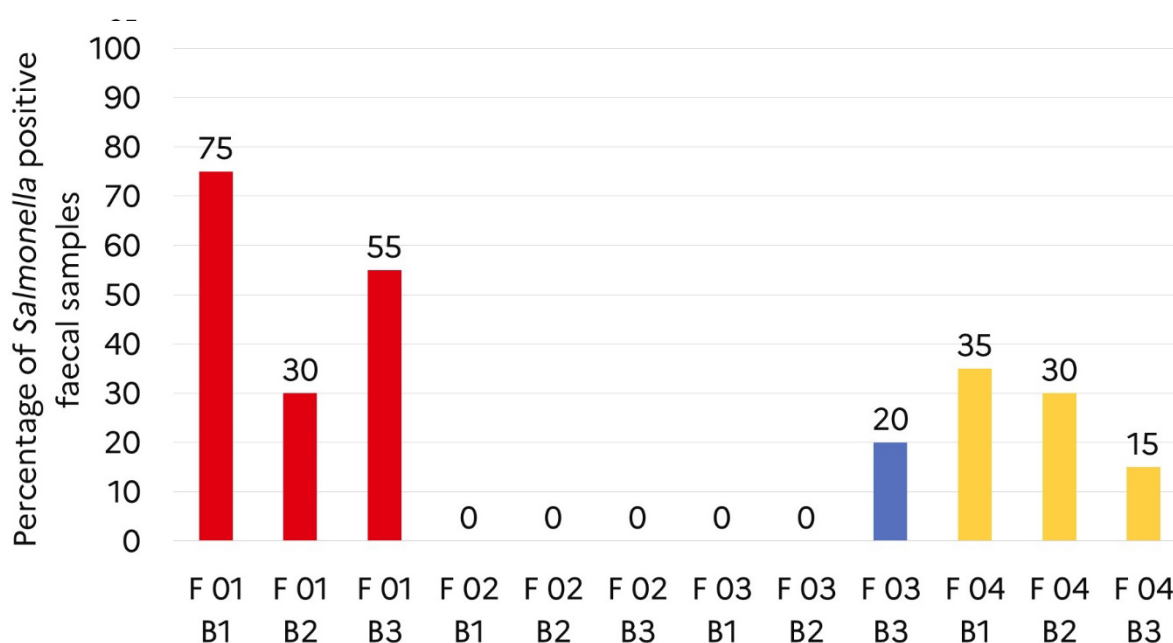
In addition, on three farms, the pen partitions and floor surfaces of 4 pens of the farrowing room (where faeces were collected) were swabbed with sterile gauze swabs (one swab = 1 m<sup>2</sup>) in presence of the pigs and then after cleaning and disinfection (C&D) procedures of the rooms (3 rooms/farm before CD procedures and the same 3 rooms/farm after CD procedures; n=36 samples before CD procedures and n=36 samples after CD procedures). All these farms used quaternary ammonium compounds (QAC) based disinfectants. Total *E. coli* and total *E. coli* resistant to antibiotic were respectively enumerated on Petrifilm™ Select *E. coli* from swab samples without and with a concentration of one of the four antibiotics considered: 16µg/ml for Ampicillin (AMP), Tetracycline (TET) and for Florfenicol (FFN), and 0.125 µg/ml for Ciprofloxacin (CIP). Incubation of petrifilms was performed at 44°C ±1°C for 20h ± 2h. Total bacteria were also enumerated on non-selective media (Plate Count Agar) at 37°C for 20h ± 2h.

Moreover, Minimum Inhibitory Concentration (MIC) for antimicrobials and for biocides were obtained for pools of *E. coli*. The pools of *E. coli* were recovered from Petrifilms™ and were tested for their sensibility to 14 antimicrobials according the Sensititre™ method. They were also tested using a home-made protocol for their sensibility to 4 active substances of biocides among those most commonly used in swine farms in France (BTC50, CDDA, H<sub>2</sub>O<sub>2</sub> and NaClO).

## Results

No sow tested positive for *Salmonella*. For finishing pigs, 52 of the 240 samples of faeces (21.6%) were positive and 44 were enumerable, of which 63.5% were below 10 MPN/g. A low *Salmonella* excretion level was measured on most of the positive samples (figure 1). However, the excretion level was variable between farms and between pigs within a farm (figure 2). *Salmonella* strains were mainly the monophasic variant of *S. Typhimurium* (mVST) (83/97 strains). The second most frequent serotype was *S. Derby* (14/97 strains). Before C&D procedures, the total bacteria and total *E. coli* were detected in all samples. The mean counts of total bacteria and total *E. coli* were 10<sup>9.4</sup> UFC/m<sup>2</sup> and 10<sup>6.6</sup> UFC/m<sup>2</sup>, respectively. The mean reductions between before C&D procedures and after C&D procedures were around 10<sup>2.8</sup> and 10<sup>3.9</sup> UFC/m<sup>2</sup> for total bacteria and total *E. coli*, respectively. After C&D procedures, 10 of the 36 swab samples were found positive for *E. coli*. Levels of antibiotic resistance in *E. coli* strains from these 10 samples were globally lower than those before C&D procedures. The mean resistance in *E. coli* strains was around 50 % for Tetracycline and Ampicillin and about 8% for Ciprofloxacin and Florfenicol.

The results of MIC on pools of *E. coli* showed that the MIC for antimicrobials and for biocides do not change following repeated exposure to biocides.



**Figure 2 : Percentage of *Salmonella* positive faecal samples from finishing pigs, for each farm (F) and batch (B)**

## Conclusion & discussion

This study enabled to collect, for the first time in France, quantitative data on *Salmonella* excretion by pigs under field conditions. These data are required to build accurate risk assessment models and ultimately allow better control of the risk associated with *Salmonella* contamination of food. In our study, *Salmonella* was excreted by finishing pigs in all farms but was not detected in any of the sow faeces samples. Although *Salmonella* excretion by lactating sows was not measured in our study, a previous study showed that sows could shed *Salmonella* (Denis et al., 2013).

The level of *Salmonella* excretion by naturally infected pigs estimated in this study was low for a majority of the samples in contrast to the excretion levels observed in experimental infections (Cevallos-Almeida et al., 2019).

The main serotypes identified in this study were mVST and S. Derby, which are currently the most reported serotypes in Europe in the pig industry (Bonardi, 2017). These serotypes are among the top 10 serotypes found in human cases in France.

In our study, the disinfection protocols (with QAC based disinfectants) used on these farms in farrowing rooms were effective to reduce by 1,000 to 10,000-fold the level of total *E. coli* and antibiotic resistant *E. coli*. The antibiotic resistance profiles for *E. coli* strains were similar to those reported by the French National Reference Laboratory for Antimicrobial resistance and, EFSA and ECDC (2019). Even though experimental trials carried out under in vitro conditions (Soumet et al., 2016) indicate that repeated exposure to biocides may select to bacteria with antibiotic resistance, such a selection was not observed in this first field study involving a limited number of samples. Further field studies are needed to better assess these preliminary findings.

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## Elevated platforms in broiler barns– any risk for climate stability?

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### Introduction

Elevated platforms offer an option for structuring broiler barns. Even though the use of an elevated platform is beneficial to animal behaviour, it is still unclear whether there are any negative effects on the housing climate below the platform. Therefore, within the Model and Demonstration Project for animal welfare “MaVeTi”, climate conditions in barns provided with an elevated platform were evaluated.

### Animals, material and method

On a commercial farm in a barn with about 33,000 broilers (Ross 308, Aviagen Group, Huntsville, USA), an elevated platform with perforated plastic floor, manure belt and drinking line was installed over the entire length of the house (platform: 90 m long, 120 cm wide, 70 cm high). Continuous measurements of relevant climate parameters were carried out below the platform and in the middle of the barn (control). Sensors (DOL Sensor technic, Dol-sensors A/S; Aarhus, Denmark) for recording temperature (C°; Sensor DOL-114) and relative humidity (%; Sensor DOL-114), carbon dioxide (CO<sub>2</sub>; Sensor DOL 19E) and ammonia (NH<sub>3</sub>; Sensor DOL 53) were installed below the elevated platform (n = 1) and in the middle of the barn (n = 3) to monitor the indoor climate continuously. Over three fattening periods (March-August 2021), data were recorded continuously at animal's height for 24 hours (stored every 30 minutes) from fattening day one to fattening day 42.

### Results

With an average temperature of 27.32 C°, relative humidity of 60.27 %, CO<sub>2</sub> content of 1,974.17 ppm on average, and NH<sub>3</sub> of 4.11 ppm below the platform, the determined climate parameters were comparable or slightly lower to the mean values recorded in the control area in the middle of the barn (27.67 C°, 65.70 %, 2,097.98 ppm, 5.01 ppm). However, on the three warmest days of the record with outdoor temperatures over 30 °C (16.06.2021-18.06.2021; fattening day 37-39), an increased temperature below the platform by up to 2 °C was recorded late afternoon and evening.

### Conclusions

In conclusion, the measurements showed no permanent increase of pollutant gas or temperature below the elevated platform. On the contrary, the air below the platform was slightly drier and ammonia levels tended to be lower than in the middle of the barn.

## Founding

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## Microbiological and physico-chemical investigations of drinking water sources in cattle farms in Lower Saxony, Germany.

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### Introduction

Drinking water must be provided in sufficient quantity and quality to ensure the health and performance of livestock. Biological and physico-chemical safety in relation to the water's origin and hygienic distribution are essential factors for its suitability as drinking water. Polluted surface water, contaminated pipe systems and cisterns, or the entry of surface water into wells used for agricultural purposes can cause microbiological contamination and lead to health hazards in animals. Moreover, mineral and heavy metal contamination can affect both the palatability of drinking water and animal health [5,7]. The availability of drinking water sources is often related to the geographical location of farms. In addition to the use of the public drinking water network, farmers use primarily on-farm wells or they collect rainwater that supply water for the animals [1,4].

With respect to cattle grazing, surface waters such as drainage ditches, ponds, streams or rivers often serve as direct water sources or from which troughs are fed by pumps [1,7]. Since drinking water is an important component of animal care in agriculture in order to ensure the welfare of farm animals, the Federal Ministry of Food and Agriculture (FMFA), Germany, has published guidelines for the quality of drinking water, which are used for evaluating the hygienic quality of drinking water for food producing animals in accordance with the current regulatory framework [5,7].

The purpose of this preliminary study was to investigate the hygiene quality of drinking water on farms with cattle husbandry and to identify possible differences in bacterial contamination of different water sources provided to cattle as drinking water.

### Material & Methods

Drinking water samples from 1) wells; 2) water troughs fed from well water; 3) ditches and 4) water troughs fed from ditch water were collected on four farms (farm A, B, C, D) with cattle husbandry in the region of Friesland, Lower Saxony, Germany, between September and November 2020. The sampling of drinking water was carried out twice on each farm. All water samples were collected in sterile laboratory bottles, with the first fill discarded and the second fill serving as the test sample. The samples were transported in a precooled cool box under permanent cooling conditions - maintaining a storage temperature between 5°C and 8°C - to the laboratory within 24 h after collection. The temperature of each transport was monitored with a data logger (Testo 174 H, Testo SE & Co. KGaA, Germany).

The analysis of physico-chemical parameters (pH, conductivity and salinity) was performed directly on site using a mobile measuring device (PCE-PHD 1, PCE Deutschland GmbH, Germany). The semiquantitative measurement of nitrite and nitrate was conducted with colorimetric test strips with a measuring range of 0,5-10 mg/L for nitrite and 10-500 mg/L for nitrate (Merck KGaA, Germany).

Biological drinking water quality was determined with regard to cultivable aerobic, mesophilic total viable count (TVC) and total coliform bacteria (TC) as CFU ml<sup>-1</sup> on selective nutrient media. Tenfold serial dilutions of each water sample were made in sterile sodium chloride solution (0.9 % w/v). Aliquots of 0.1 ml or 0.5 ml of stock samples and serial dilutions were spread-plated in triplicates on suitable nutrient media by using the spread plate technique. TVC was enumerated on R2A nutrient media (Carl Roth GmbH, Germany) incubated at both 20°C and 36°C for 24 h to 5 d. TC was enumerated on Chromocult® coliform nutrient media (CCA, Merck KGaA, Germany) incubated at 36°C for 24 to 48 h with pink-colored colonies indicating TC and purple-colored colonies indicating *Escherichia coli*.

Simultaneously, all water samples were qualitatively tested for the presence of TC and *E. coli* using the Colitag™ test kit (Neogen Europe Ltd., United Kingdom) to ensure the detection of very low concentrations of the forementioned bacteria in drinking water. A water sample of 100 ml was mixed with the powdery Colitag™ reagent and incubated at 36 °C for 24 h. After incubation, a yellow color of the enriched culture indicated the presence of coliform bacteria and a blue fluorescence under UV light (365 nm) the presence of *E. coli*. Positive valued Colitag™ enrichments for the species *E. coli* was used for subsequent cultivation of *E. coli* that was performed by spreading an aliquot of 0.1 ml of 1:1000 diluted Colitag™ enrichment on CCA. After incubating the plates for 24 h at 36 °C, purple-colored colonies were considered positive for *E. coli*. Isolated purple-colonies were periodically transferred to fresh Standard I nutrient agar (St I, Merck KGaA, Germany) in order to obtain pure cultures. Subsequent confirmation of *E. coli* was performed biochemically by the API® 20 E bacterial identification test strip (Biomérieux Deutschland GmbH, Germany) for identification and differentiation of members of the family *Enterobacteriaceae*. With regard to API® 20 E test the identification of *E. coli* species was detected in 13 isolates. Colitag™ and API® 20 E tests were carried out according to the manufacturer's instructions.

All 13 isolates which were positively identified for *E. coli* were screened for antibiotic susceptibility by the standard agar disk diffusion technique following the guidelines of EUCAST (European Committee on Antimicrobial Susceptibility Testing) [2]. Commercial antibiotic disks used for the test were from Thermo Scientific™ Oxoid™ (Germany): Ampicillin (AMP, 10 µg), Cefoxitin (FOX, 30 µg), Ceftriaxone (CRO, 30 µg), Ciprofloxacin (CIP, 5 µg), Gentamicin (CN, 10 µg), Trimethoprim (W, 5 µg). All tests were carried out in triplicates on Mueller Hinton agar inoculated with fresh culture of isolated *E. coli* adjusted to 0.5 McFarland turbidity standard with sterile sodium chloride solution (0.9 % w/v). Antibiotic disks were placed on each plate using an antimicrobial susceptibility disk dispenser (Thermo Scientific™ Oxoid™, Germany) and incubated at 36°C for 24 h. The size of inhibition zone diameters were interpreted according to EUCAST inhibition zone diameter distributions for *Enterobacterales* [3].

## Results

Tables 1 and 2 summarize the results of the investigated drinking water samples with respect to physico-chemical and bacterial parameters reported as means and compared with the guideline values for the evaluation of the hygienic quality of drinking water [5].

The physico-chemical parameters of drinking water samples were within the guideline values for suitability as drinking water with respect to salinity, nitrate and nitrite on all four farms. A slight increase in conductivity was shown only in the ditch water fed trough sample tested on farm A (3140 µS/cm). Additionally, water samples on farm D showed pH shifts in well water (pH 6.1), trough water

fed from ditch water (pH 6.3) and trough water fed from well water (pH 5.8) which are lower than the guideline values (Table 1).

In three farms, with the exception of all well water samples, the guideline value for the hygienic quality of drinking water was exceeded in terms of TVC after incubation at 36°C ( $<1 \times 10^3$  CFU ml<sup>-1</sup>) and 20°C ( $<1 \times 10^4$  CFU ml<sup>-1</sup>). The highest values of TVC incubated at 36°C was found to be  $5.6 \times 10^4$  CFU ml<sup>-1</sup> for ditch water and  $6.8 \times 10^4$  CFU ml<sup>-1</sup> for trough water fed from the appropriate ditch on farm D. The highest value of TVC incubated at 20°C was found to be  $5.4 \times 10^6$  CFU ml<sup>-1</sup> for trough water fed from well water on farm B. The well water of the same farm has shown a lower bacterial load of  $2.5 \times 10^3$  CFU ml<sup>-1</sup>. With respect to the determination of TC,  $8.3 \times 10^1$  CFU ml<sup>-1</sup> were quantified in the well water on farm C, while no coliforms were detected in the well water samples from the other three farms A, B and D. However, all ditch water and trough water samples showed moderate TC contamination (Table 2). The species *E. coli* was detected qualitatively in two water sources including ditch water and troughs fed from ditch water on all farms using Colitag™. *E. coli* isolated from ditch water from farm A and farm B showed cefoxitin resistance.

**Table 1.** Physico-chemical and chemical parameters in drinking water from different water sources

	Unit	Water source	Farms				Guideline values <sup>a</sup>
Parameter			Farm A	Farm B	Farm C	Farm D	
Physico-chemical parameters							
pH		Ditch	7.0	7.6	6.9	7.2	6,5 – 9,5
		Well	n.a. <sup>g</sup>	8.5	6.9	6.1	
		Trough <sup>b</sup>	7.0	7.8	7.1	6.3	
		Trough <sup>c</sup>	7.2	8.3	7.0	5.8	
Conductivity	(µS/cm)	Ditch	274	1209	296	268	<3000
		Well	n.a. <sup>g</sup>	291	542	302	
		Trough <sup>b</sup>	3140	1227	308	290	
		Trough <sup>c</sup>	291	291	552	310	
Salinity	(g/L)	Ditch	0.1	0.6	0.2	0.2	<2,5
		Well	n.a. <sup>g</sup>	0.2	0.3	0.2	
		Trough <sup>b</sup>	0.1	0.6	0.2	0.2	
		Trough <sup>c</sup>	0.2	0.2	0.3	0.2	
Chemical parameters							
Nitrate (NO <sub>3</sub> <sup>-</sup> )	(mg/L)	Ditch	n.d. <sup>f</sup>	n.d. <sup>f</sup>	10	10	<300 <sup>d</sup> <200 <sup>e</sup>
		Well	n.a. <sup>g</sup>	n.d. <sup>f</sup>	10	10	
		Trough <sup>b</sup>	n.d. <sup>f</sup>	n.d. <sup>f</sup>	10	n.d. <sup>f</sup>	
		Trough <sup>c</sup>	n.d. <sup>f</sup>	n.d. <sup>f</sup>	10	25	
Nitrite (NO <sub>2</sub> <sup>-</sup> )	(mg/L)	Ditch	n.d. <sup>f</sup>	n.d. <sup>f</sup>	0.5	n.d. <sup>f</sup>	<30
		Well	n.a. <sup>g</sup>	n.d. <sup>f</sup>	n.d. <sup>f</sup>	n.d. <sup>f</sup>	
		Trough <sup>b</sup>	n.d. <sup>f</sup>	0.5	0.5	n.d. <sup>f</sup>	
		Trough <sup>c</sup>	n.d. <sup>f</sup>	n.d. <sup>f</sup>	n.d. <sup>f</sup>	0.5	

<sup>a</sup> Guideline values for the suitability of drinking water, FMFA guidance framework 25/05/2007

<sup>b</sup> Trough fed from ditch water

<sup>c</sup> Trough fed from well water

<sup>d</sup> Ruminant animals

<sup>e</sup> Calves

<sup>f</sup> n.d.: not detectable

<sup>g</sup> n.a.: not available

**Table 2.** Bacterial enumeration in drinking water from different water sources

Parameter	Water Source	Farms				Guideline values <sup>d</sup>
		Farm A	Farm B	Farm C	Farm D	
TVC, 20°C (cfu ml <sup>-1</sup> ) <sup>a</sup>	Ditch	3.8 x 10 <sup>3</sup>	1.4 x 10 <sup>4</sup>	1.3 x 10 <sup>5</sup>	1.3 x 10 <sup>6</sup>	<1x10 <sup>4</sup>
	Well	1.1 x 10 <sup>3</sup>	2.5 x 10 <sup>3</sup>	7.4 x 10 <sup>2</sup>	1.9 x 10 <sup>2</sup>	
	Trough <sup>b</sup>	7.0 x 10 <sup>3</sup>	3.6 x 10 <sup>4</sup>	8.2 x 10 <sup>4</sup>	8.5 x 10 <sup>5</sup>	
	Trough <sup>c</sup>	3.2 x 10 <sup>4</sup>	5.4 x 10 <sup>6</sup>	2.8 x 10 <sup>4</sup>	5.2 x 10 <sup>3</sup>	
TVC, 36°C (cfu ml <sup>-1</sup> ) <sup>a</sup>	Ditch	5.9 x 10 <sup>3</sup>	1.7 x 10 <sup>3</sup>	1.3 x 10 <sup>4</sup>	5.6 x 10 <sup>4</sup>	< x10 <sup>3</sup>
	Well	1.3 x 10 <sup>2</sup>	8.0 x 10 <sup>2</sup>	8.4 x 10 <sup>2</sup>	1.5 x 10 <sup>2</sup>	
	Trough <sup>b</sup>	2.8 x 10 <sup>3</sup>	5.8 x 10 <sup>3</sup>	1.4 x 10 <sup>4</sup>	6.8 x 10 <sup>4</sup>	
	Trough <sup>c</sup>	4.7 x 10 <sup>3</sup>	2.3 x 10 <sup>4</sup>	9.6 x 10 <sup>3</sup>	1.4 x 10 <sup>3</sup>	
TC, 36°C (cfu ml <sup>-1</sup> ) <sup>a</sup>	Ditch	4.2 x 10 <sup>2</sup>	2.5 x 10 <sup>2</sup>	5.2 x 10 <sup>2</sup>	1.2 x 10 <sup>3</sup>	No guideline value
	Well	n.d. <sup>e</sup>	n.d. <sup>e</sup>	8.3 x 10 <sup>1</sup>	n.d. <sup>e</sup>	
	Trough <sup>b</sup>	4.1 x 10 <sup>2</sup>	2.5 x 10 <sup>2</sup>	7.3 x 10 <sup>2</sup>	1.3 x 10 <sup>3</sup>	
	Trough <sup>c</sup>	4.4 x 10 <sup>1</sup>	3.0 x 10 <sup>2</sup>	7.4 x 10 <sup>1</sup>	7.7 x 10 <sup>1</sup>	

<sup>a</sup> Mean values calculated from two independent measurements in colony forming units per milliliter

<sup>b</sup> Trough fed from ditch water

<sup>c</sup> Trough fed from well water

<sup>d</sup> Guideline values for the suitability of drinking water, FMFA guidance framework 25/05/2007

<sup>e</sup> n.d.: not detectable

## Discussion

Physico-chemical parameters such as pH, conductivity or salinity, for example, are generally used to assess the quality of the drinking water as well as the hygiene status with regard to microbiological contamination.

Electrolytes that may have entered the water through urine or feces may result in increased conductivity [7] that was measured in the water of troughs fed from ditch water on farm A.

PH values outside the range of 6.5 - 9.5 can affect the uptake of drinking water in livestock. A low pH value in the well water from farm D may have been caused by humic acid transfer to groundwater [7]. Well water was found to be the least contaminated in terms of TVC and TC findings. However, well water-fed troughs had elevated total bacterial and coliform counts, suggesting that this bacterial contamination may be due to bacterial input from the environment. Ditch water and water troughs that made ditch water available to animals generally had higher total bacterial and coliform counts than well water, suggesting that the use of such drinking water may cause an increased bacterial exposure in cattle. Possible sources of contamination could be the cattle themselves due to the entry of feed remains from the mouth cavity and fecal materials. Furthermore, the deposition of dust, feed or animal bedding material into troughs may play also a considerable role. In addition, the water

source from which the troughs are fed may already contain initial high levels of microbiological contamination [6]. *E. coli* indicates fecal contamination and poorly disinfected troughs may harbor other pathogens that can multiply in trough water and sediment [1]. The presence of ceftiofur-resistant *E. coli* indicates that water might be a notable pathway to transmit antibiotic resistance to animals. The actual significance of this observation on animal performance and health cannot be conclusively determined from the studies conducted here.

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## The mould contamination of an indoor riding arena floor as a function of the particle size of the floor material

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### Introduction

The so-called Equine asthma syndrome can be caused by the inhalation of airborne mould spores and dust. Apart from contaminated feed and bedding material, the contamination of riding arena floors with moulds could be an additional trigger for respiratory diseases in horses due to released particles from these floors. The smaller the floor particles that are brought into an airborne state, the greater their residence time in the air and thus their respiratory availability. Consequently, it can be assumed that small and inhalable particles loaded with fungal spores can penetrate into deeper regions of the lung of horses and could therefore have a detrimental effect on the respiratory tract. The aim of the investigation is to determine the mould burden in different sized particles of the floor material and its mycological diversity on the basis of sieve fractions of the arena floor material.

### Material & Methods

By means of an automated sieve fraction analysis with mesh sizes between 1000 µm and 20 µm, samples of a riding arena floor were analyzed during autumn and winter in respect to particle size and mould concentration. For this purpose, the particulates from the sieve fractions were cultivated on selective nutrient media after laboratory processing and the findings were recorded as colony forming units (CFU) per gram of the floor material. In addition, typical morphological characteristics of the mould colonies and hyphae were used to determine the mould genus under a microscope. The subsoil of a riding arena was investigated, whose soil can be used for various types of equestrian sports due to its physical properties.

### Results

In the smallest sieve fraction with a mesh size of 20 µm, high mean mould concentrations of  $2.08 \times 10^7$  CFU/g were found. Comparatively low concentrations of  $2.45 \times 10^2$  CFU/g were detected in the sieve fraction of 200 µm. *Aspergillus spp.*, *Fusarium spp.*, *Penicillium spp.* and *Mucor spp.* were identified as the most common mould genera.

### Discussion

It has been shown that smaller floor-related particles can harbour considerably more moulds than bigger particles. Since it can be assumed that such small particles are frequently released into the air of riding arenas, increased mould-associated exposures of horses may occur during activities in the indoor riding arena. Especially with regard to genera such as *Aspergillus spp.* and their allergic-inflammatory potential, appropriate measures should be taken (e.g., floor material with more coarse particles, more frequent floor renewal) to establish a preventive effect on the respiratory health of horses.



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## Ambient temperature interaction on serum cortisol levels in growing pigs

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### Introduction

Indoor thermal environment is critical for pig health, animal productivity and welfare<sup>(1)</sup>. The increased frequency of hot days due to climate change can potentially impair the environmental and economic performance of pig-fattening farms <sup>(2)</sup>. Ambient temperatures above the thermoneutral zone for growing pigs can be a source of extensive financial loss in production systems by affecting the performance and welfare of pigs <sup>(3)</sup>. Due to the global warming, heat stress is becoming an increasingly important issue for the production animals and their breeders. A one of the methods to quantify heat stress is to measure the cortisol level in body fluids.

**The aim** of the current study was to determine of the ambient temperature interactions on serum cortisol levels in growing pigs.

### Material & Methods

All the research was conducted in compliance with the Law of Veterinary Medicine of the Republic of Lithuania (new wording from 1 July 2011: No. XI-1189, 30 November 2010: No. 148-7563 (20.10.2012)) and the Law on Animal Welfare and Protection of the Republic of Lithuania (No. XI-2271, 03.10.2012, Official Gazette 2012). Care and usage requirements of animals used for scientific and educational purposes were approved by the State Food and Veterinary Service by official letter (No. B1-866, 31.10.2012).

One industrial pig farm were selected for study. The study was conducted since July 2018 till May 2019. A total of 56 healthy growing pigs ( $41 \pm 1,8$  kg) were randomly selected in the study (28 gilts (12 Landrace breed and 16 mixed-breed) and 28 castrate males mixed-breed).

Pig's blood samples was collected 4 times from *v.jugularis* before feeding for the determination of the serum cortisol level. For quantitative measurement of cortisol level in serum was used Cortisol ELISA Kit.

The average of ambient temperature in the farm during the study varied respectively  $18,3 \pm 1,2^{\circ}\text{C}$  (in November)  $13,7 \pm 1,7^{\circ}\text{C}$  (in January);  $15,3 \pm 1,4^{\circ}\text{C}$  (in May) and  $27 \pm 1,9^{\circ}\text{C}$  (in July). Temperature has been measured with FHT 100 (Humidity and Temperature Meter) at four locations.

## Results

Mean serum cortisol levels were highest in summer and lowest in autumn, mean values respectively  $22.13 \pm 1.14$  ng/ml and  $14.48 \pm 0.98$  ng/ml ( $p < 0.05$ ).

At the lowest ambient temperature ( $13.7^{\circ}\text{C}$ ) mean serum cortisol levels were  $16.32 \pm 0.95$  ng/ml and during spring mean serum cortisol levels –  $15.39 \pm 0.64$  ng/ml.

Serum cortisol levels differ between of gilts and castrated males or landrace and mixed-breeds. During all seasons the serum cortisol levels in gilts were 10.79% ( $p < 0.05$ ) smaller than in castrates males. A comparison of the cortisol levels in gilts and castrated males serum when ambient temperature 27 degrees showed the higher 2.23 ng/ml concentration of cortisol in serum of castrated males than in gilts and 1.28 ng/ml in Landrace breed compared with mixed breed.

## Discussion and Conclusion

The comfort temperature of fattening pigs is  $16-27^{\circ}\text{C}$ , with optimal temperature  $21^{\circ}\text{C}$ . The current study shows that the growing cortisol level in serum is predisposed by ambient temperature of  $27^{\circ}\text{C}$  in contrast to low temperatures have no significant effect on changes in cortisol level. Despite changes in ambient temperature, higher serum levels of cortisol were found in castrate males than in gilts and respectively in landrace breed gilts than in mixed breed gilts.

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## Biotransformed rapeseed meal in the feeding of laying hens

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### Introduction

Post-extraction rapeseed meal is the feed material which presents high economic potential in poultry nutrition. Still, non-processed rapeseed meal shows a high level of anti-nutritional factors, which makes its use not entirely effective (Khajali and Slominski, 2012). The solution to improve the value of rapeseed meal can be obtained by its biotransformation by solid-state fermentation with *Bacillus subtilis* (Konkol et al., 2019). The aim was to assess the possibility of using biotransformed rapeseed meal (BRM) in laying hens nutrition.

### Material & Methods

The research was carried out in the experimental henhouse of the Wrocław University of Environmental and Life Sciences (Poland). 108 Lohmann Brown line hens were kept in a cage system. Hens were divided into three experimental groups consisting of 12 replicates (3 birds per replicate). CN-group (negative control) has not received post-extraction rapeseed meal in the basal diet. CP-group (positive control) received the addition of 3% post-extraction rapeseed meal but not biotransformed. The 67-Group was fed a diet containing 3% post-extraction BMR with *B. subtilis* 67, the strain isolated from *Eisenia fetida* worms and used for biotransformation of rapeseed meal. The birds were fed for 90 days. According to the line manufacturer's recommendations, the birds were kept under controlled microclimatic conditions. Production parameters such as egg production (%), weight of eggs (g), feed intake (g) and feed conversion ratio (FCR) were determined. The results were statistically analyzed using Statistica ver. 13.1. The differences between the groups were assessed using the one-way ANOVA. The differences were statistically significant when  $P < 0.05$ .

### Results

After 30 days of the experiment, the CP-Group was characterized by a significantly lower feed intake ( $P < 0.05$ ) compared to the 67-Group, but it not significantly affect FCR. There were also no statistically significant differences in egg production and egg weight. After 60 days of the experiment, the weight of eggs in 67-Group was significantly higher ( $P < 0.01$ ) compared to the weight of eggs in the CP-Group. Feed intake in the CP-Group was significantly lower ( $P < 0.05$ ) compared to the CN-Group. There were no differences between the groups in egg production and FCR. After 90 days of the experiment, the feed intake was significantly higher ( $P < 0.05$ ) in the CP-Group compared to the CN-Group, which resulted in a significant difference in FCR ( $P < 0.05$ ) between these groups.

## Discussion

The BRM has no negative effect on the production parameters of laying hens. This could be since the content of anti-nutritional substances in the rapeseed meal after the biotransformation process was significantly lower. Moreover, *B. subtilis* 67 produces NSP enzymes which may improve the utilization of feed nutrients.

## Acknowledgement & Funding

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## Evaluation of the suitability of a full fat or defatted Mealworm (*Tenebrio molitor*, L.) larvae meal as ingredient for broiler chicken diets

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### Introduction

Poultry meat is popular and widely available, and a steady increase in its production and consumption has been observed around the world (OECD, 2020). Human population growth, accompanied by global climate change and a decline in the area under food crops, has promoted a search for alternative, effective protein sources for livestock diets. Attention has been paid to insects, which are a natural source of protein and other nutrients for omnivorous species of poultry in the wild (Józefiak et al. 2016). According to some authors (Gariglio et al., 2019), the use of insect products in poultry diets could reduce the negative environmental impacts of poultry production. *Tenebrio molitor* has been widely used as an ingredient of animal diets since its larvae are a rich source of fat and protein.

The aim of this study was to compare selected growth performance effects and carcass quality characteristics in broiler chickens fed diets with different full fat (TMF) or defatted (TMD) *Tenebrio molitor* (TM) larvae meal - processed animal proteins (PAP) content.

### Material & Methods

The experiment was run on a total of 420 1-day-old female Ross 308 broilers. At 1-day of age, the chickens were randomly assigned to 7 dietary treatments (6 replications per treatment, 10 birds per pen). The diets were formulated by including, on as-fed basis, increasing levels of full-fat or defatted TM larvae meal (5%, 10% and 15%; TMF<sub>5</sub>, TMF<sub>10</sub>, TMF<sub>15</sub>, TMD<sub>5</sub>, TMD<sub>10</sub> and TMD<sub>15</sub>, respectively). The birds were raised to 35 d of age and fed *ad libitum* (Starter 1-10 d, Grower 10-21 d, Finisher 21-35 d). At 35 d of age, two birds per pen were selected in each feeding group based on the average final BW ( $\pm 15\%$ ), and slaughtered. Carcasses were eviscerated, chilled at 4°C for around 24 h, and were

divided into the following cuts: breast muscles, legs and the remaining portion the carcass. The legs were subjected to detailed dissection (muscles+ bones+skin). The results are presented as means and the standard error of the mean ( $\pm$  SEM) The significance of differences in mean values between age groups was determined by Duncan's test. Significance was set at  $P \leq 0.05$ .

## Results

The final body weight (BW) of chickens fed diets containing TM-PAP was similar to BW in the control group, with the exception of the TMF<sub>5</sub> group ( $P \leq 0.05$ , respectively: 2139.4 g TMO, 2165.6 g TMF<sub>5</sub>, 1916.9 g TMF<sub>10</sub>, 2038.1 TMF<sub>15</sub>, 2034.4 TMD<sub>5</sub>, 2119.4 TMD<sub>10</sub>, 2123.8 TMD<sub>15</sub>). There was no effect of the diet on the weight of the pectoral muscles and the muscles of the legs.

The replacement of soybean protein to full-fat (5-10 %) or defatted (5-15 %) TM-PAP in the chicken's diet did not deteriorate the final BW and the basic characteristics of the slaughter value.

## Acknowledgement & Funding

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## ***Acinetobacter* spp. as a source of potentially zoonotic CRESS DNA in livestock environments**

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### **Introduction**

Circular Rep-encoding single-strand (CRESS) DNA viruses and molecules similar to them have been detected in various foods including milk products. They are discussed to be involved in the development of different human cancer forms and neurodegenerative diseases. To prevent their entry into the food chain, possible environmental sources are of particular interest (1). For Bovine Meat and Milk Factors (BMMF) - one group of such CRESS DNA molecules – environmental bacteria might act as reservoirs (2). Sequence entries in Genbank are particularly similar to plasmids of *Acinetobacter* spp.

### **Material/Methods**

Milk samples (n = 40) were taken when the milk was delivered from various farms to dairy companies in Schleswig-Holstein. Presence of *Acinetobacter* spp. and BMMF was proven by different microbiological and molecularbiological methods. Bacteria were differentiated by use of selective agar and confirmed via Maldi-TOF-MS analysis. After rolling circle amplification (RCA) extracts of cultured *Acinetobacter* spp. and the original milk samples were analysed by PCR followed by sequencing to detect the *rep*-gene of BMMF.

### **Results**

Molecular co-occurrence of *Acinetobacter*- and BMMF-DNA was observed in all 40 milk samples, and *Acinetobacter* spp. could be cultured from 22 samples. MALDI-TOF-analysis identified *A. guilloniae* as a probably involved species. The comparison of *rep*-sequences from bacterial extracts and milk extracts with Genbank entries rendered high similarity to *Acinetobacter* spp. as well as to sequences designated as BMMF.

### **Discussion/Conclusion**

The results showed partly different and partly homologous findings for the *rep* gene sequences in milk samples and pure cultures of the bacteria. These findings mark *Acinetobacter* spp. as one of several environmental reservoirs of BMMFs. Further research must clarify whether hygienic measures targeting bacteria might be suitable to decrease the occurrence of CRESS-DNA in milk.



## Biotransformed rapeseed meal in the feeding of broiler chickens

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### Introduction

Post-extraction rapeseed meal is the feed material which presents high economic potential in livestock nutrition. It is a second, after soya, protein raw material used in the world. Still, non-processed rapeseed meal shows a high level of anti-nutritional compounds, which makes its use not entirely effective (Khajali and Slominski, 2012). The solution to improve the value of this feed material can be obtained by its biotransformation by solid-state fermentation with *B. subtilis* (Konkol et al., 2019). The aim was to assess the possibility of using biotransformed rapeseed meal (BRM) in broiler nutrition.

### Material & Methods

The research was carried out in the experimental henhouse of the Wrocław University of Environmental and Life Sciences (Poland). Ross 308 male broilers were kept in a deep litter system in 24 boxes. Chickens were divided into three experimental groups consisting of 8 replicates (30 birds per replicate). CN-group (control) has not received post-extraction rapeseed meal in the basal feed. CP-group received the addition of 3% post-extraction rapeseed meal but not biotransformed. The 67-Group was fed a diet containing 3% post-extraction BMR with *B. subtilis* 67, the strain isolated from *Eisenia fetida* worms and used for biotransformation of rapeseed meal. The birds were fed for 35 days. According to the line manufacturer's recommendations, the birds were kept under controlled microclimatic conditions. Production parameters such as bodyweight(g), daily weight gain (g), feed intake (g), feed conversion ratio (FCR) and European Production Efficiency Factor (EPEF) were determined. At the end of the study, the breast and thigh muscles were collected from 8 random chickens per group and used to analyse selected carcass traits. The results were statistically analysed using Statistica ver. 13.1. The differences between the groups were assessed using the one-way ANOVA. P-value <0.05.

### Results

The use of BRM in the feeding of broiler chickens has a positive effect on their production parameters, especially bodyweight, daily gains and EPEF which does not affect the slaughter efficiency and carcass parameters. At the end of the experiment, the birds from 67-Group had higher ( $P < 0.05$ ) body weight than CN-Group and CP-Group. Birds from 67-Group showed higher EPEF than birds in the rest of the

groups. The highest carcass weight was observed in chickens from 67-Group. They were also characterized by the most significant mass of breast and thigh muscles. Moreover, they presented the highest ratio of breasts and thighs to carcass and breasts to live weight, but these differences were not statistically significant.

## Discussion

The BRM had a positive effect on the production parameters of broiler chickens. This could be since the content of anti-nutritional substances in the rapeseed meal after the biotransformation process was significantly lower. In addition, the feed mixtures containing the BRM showed lower amounts of fiber, which can improve the digestibility of the feed nutrients.

## Acknowledgement & Funding

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## Type of diluent on sperm quality of refrigerated sheep semen

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### Introduction

The conservation of semen allows the use of genetic resources in the long term, in such a way that the refrigeration of sheep semen allows to preserve for a longer time the fertilizing capacity of the spermatozoa, after obtaining the ejaculate. The pregnancy rate when using refrigerated semen varies between 45 and 65%, while when using fresh semen, it is around 84%; which is because spermatozoa experience damage at the level of the plasma membrane, with inevitable reduction of motility and probably also at the level of the acrosome during its conservation. These facts stimulate to continue working on the conservation of semen in refrigeration of this species. The objective of this work was to assess the effect of conservation in cooling of sheep semen on sperm quality (motility, viability and acrosomal integrity).

### Material and methods

Four sheep of the 2-year-old English Suffolk breed were used; of which 24 ejaculates were collected by means of artificial vagina, obtaining 3 ejaculates per week, during 4 weeks. Progressive motility, viability, acrosomal integrity (NAR) and pH were assessed. 3 diluents were added in a ratio of 1:3. The diluents used were: diluent 1 (Rangel, 1985); diluent 2 (egg yolk-Tris-fructose for ram semen -Salamon, 1990) and diluent 3 triladyl base; the semen was kept in refrigeration at 4°C for 24 hours and the afore mentioned variables were assessed at 0, 2, 4 and 24 hours. The results were analyzed using descriptive statistics, showing averages for each variable.

### Results

The results were analyzed using descriptive statistics, showing averages for each variable. It was found that the pH did not vary during the 24 hours in the three diluents. Motility and viability were improved in the triladyl base diluent for the duration of the experiment. However, acrosomal integrity was better in the first two diluents.

### Conclusions

In a study carried out by Milckzewski (2000) it was indicated that motility using tris-egg yolk as a diluent was less than 50% at 4 hours of refrigeration; found that using the diluents tris-egg yolk and tris-antibiotics, motility at 4 hours was 50 and 60%, respectively. Vázquez and Núñez (2000) reported that

the decrease in viability occurs between 6 and 10 hours in refrigeration; however, in the present work, it was observed that the decrease in viability occurs after 4 hours. Watson (2000) indicated that there is a favorable effect on the integrity of the acrosomes in the presence of egg yolk, contrary to the results obtained in this work, where it can be observed that the proportions in the egg yolk were variable, with greater concentration in diluent one, which presented the least acrosomal damage. The diluent based on triladyl, can present an alternative for the conservation of sheep semen in refrigeration.

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## Georeferenced detection of ambient particulate matter (PM<sub>10</sub>, PM<sub>2.5</sub>) with a mobile DIY aerosol measurement device – an initial idea and first attempts

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### Introduction

Airborne particulate matter (PM) can contribute to morbidity and premature mortality by damaging cardiovascular and respiratory systems (LELIEVELD *et al.*, 2019). But aerosol concentrations in ambient air dynamically differ according to varying local and regional sources and emission quantities. Additionally, atmospheric long-range transportation phenomena of PM interfere with local caused aerosol releases. Consequently, this can locally change the PM situation in the atmosphere over time (BOVALLIUS *et al.*, 1978; KELLOGG and GRIFFIN, 2006; KINDAP *et al.*, 2006; LIANOU *et al.*, 2011). It can be hypothesized, that these circumstances make it more difficult to find causative relationships between varying ambient aerosols and health hazards in a spatiotemporal context at any time.

It is desirable to improve the spatial resolution of PM concentrations in and outside rural areas with livestock operations in particular, because they are considerable PM emitters (SEEDORF, 2004) and are suspected to cause public health problems due to released bioaerosols (DOUGLAS *et al.*, 2018; RUIZ-GIL *et al.*, 2020). Unfortunately, in regions with a high livestock density official measurement stations can be relatively rare (see [https://www.umwelt.niedersachsen.de/startseite/themen/luftqualitat/lufthygienische\\_uberwachung\\_niedersachsen/lufthygienische-ueberwachung-niedersachsen-9107.html](https://www.umwelt.niedersachsen.de/startseite/themen/luftqualitat/lufthygienische_uberwachung_niedersachsen/lufthygienische-ueberwachung-niedersachsen-9107.html); accessed 28/07/2022). Unnecessary to emphasize that the availability of a mobile measurement concept is therefore very beneficial, because investigations can be conducted everywhere on demand without great organizational, logistical or extraordinary technical requirements.

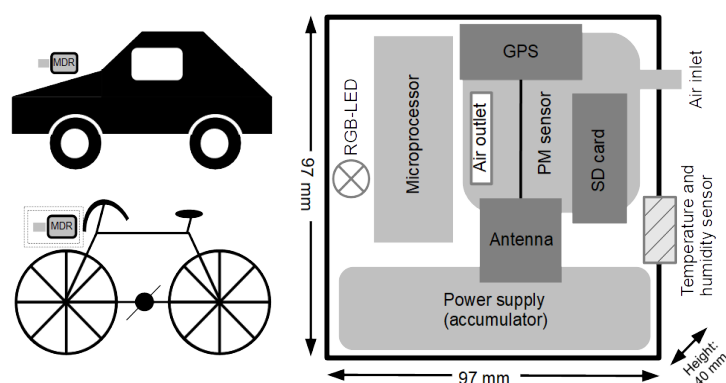
From this point of view it is demonstrated how and whether a mobile Do-it-yourself (DIY) apparatus is generally suitable to measure ambient PM concentrations in a spatial context. Furthermore, potential methodological shortcomings are selectively discussed.

### Material & Methods

A DIY device, called *MobileDustRecorder<sub>GPS</sub>* (MDR), was assembled from a low cost dust sensor (Nova SDS011) together with a temperature and humidity sensor in conjunction with a GPS (Global Positioning System) module and a programmable microprocessor, which controls and operates the electronic parts by an internal power supply. A RGB-LED indicate the successful recording of geocoordinates at the beginning of the measurements and serves as indicator for different categorized PM concentrations, which determine the colour of the active LED. The assembled device is finally integrated into a protective plastic based cable junction box. A car and a bicycle served as transport vehicle for the MDR (Fig. 1).

Extra fastening devices for a safety mobile usage serve as basis for the transportation of the MDR. A wire basket at the handlebars was used to move the MDR with the bicycle. During the measurement campaigns with the car the MDR was placed on the engine hood via magnets and strings. A 5 second interval was used as recording frequency for all measurements. In summary, five measurement trips have been performed with the bicycle and the car each in spring and early summer 2022. The travellings relate to 4322 and 1633 complete data sets (date, time, geocoordinates, speed, PM<sub>2.5</sub>, PM<sub>10</sub>, temperature, humidity), respectively. The tours were mainly located in an urban area and in the countryside with a transition into the peripheral site of the urban area.

Apart from some calculations with the R Statistical Software (R CORE TEAM, 2021) the collected outdoor data were additionally uploaded into a geographical information system (GIS) named QGIS (QGIS DEVELOPMENT TEAM, 2022) for further visual inspection and preliminary spatial data analysis (i.e., Inverse Distance Weighting Interpolation). The created maps are therefore rudimentary and will not show typical details such as scale bars, compass roses or fully explaining legends.



**Figure 1.** The basic structure of the MDR (right side). Dark grey coloured modules are mounted above the PM sensor. The accumulator is connected to the microprocessor from where all other modules are supplied. To keep the figure clear power supply wires and wires for receiving and transmitting signals are not shown. The black frame represents the device housing with its dimension in mm. The right side shows the relative position of the MDR on the engine hood of the car and within the wire basket in front of the handlebars of the bicycle.

## Results

One of the most obvious differences between cars and bicycles are the reachable speeds. Therefore, it is not surprising that the maximum speed for the car was nearly 88 km/h in contrast to 40 km/h in the case of the bicycle (**Tab. 1**). However, the mean values over all car and bicycle tours differ not very much (30.8 km/h vs. 23.4 km/h), because the route through an urban area with traffic lights, for instance, required several stops (0.0 km/h). Such interruptions during the travelling were also necessary from time to time during cycling, but less often along the countryside route.

The mean values of PM<sub>2.5</sub> and PM<sub>10</sub> are < 10 µg/m<sup>3</sup>. Interestingly, the bicycle-related investigations have shown the highest PM<sub>10</sub> yields indicated by a maximum value of 211.5 µg/m<sup>3</sup> that was caused by a dust cloud initiated by oncoming cars on an unpaved part of the road. The averaged ambient

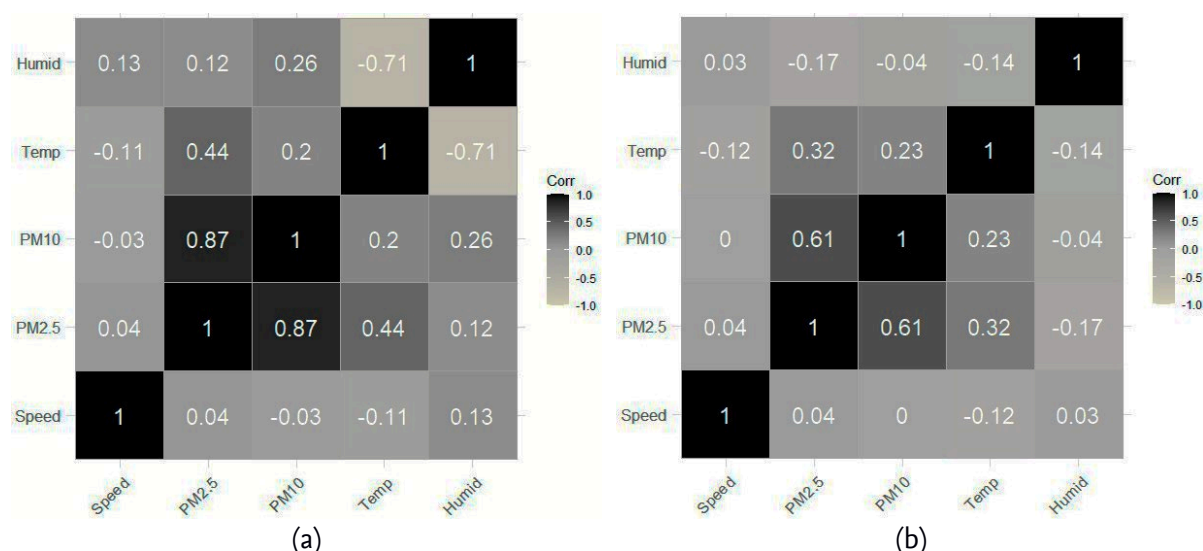
temperatures ranged between 23.5 °C and 25.2 °C. Such summer-like temperatures have corresponded to 48.7 % and 39.1 % relative humidity (Tab. 1).

Potential interactions among the recorded parameters were checked by a correlation matrix (Pearson correlation) using car- and bicycle-related data in respect to speed, PM<sub>2.5</sub>, PM<sub>10</sub>, temperature and humidity as combinable inputs (Fig. 2). Relative strong correlations were calculated between PM<sub>2.5</sub> and PM<sub>10</sub> ( $r = 0.87$  and  $0.61$ , respectively). Relationships between PM<sub>2.5</sub> and temperature were less strong with  $r = 0.44$  (car) and  $r = 0.32$  (bicycle). The correlations coefficients for the combination between PM<sub>10</sub> and temperature were  $r = 0.20$  (car) and  $r = 0.23$  (bicycle). The correlations for humidity were generally less clear, although the met environmental conditions during the car tours have caused a correlation coefficient of  $r = -0.71$  in conjunction with temperature. The importance of speed in context to all other selected parameters is also unclear due to low and heterogeneous correlation coefficients. This impression is additionally confirmed by p-levels of 0.13 and greater in case of measured particulate matter during the car tours. Pairing bicycle-related speed with PM<sub>10</sub> gave  $p = 0.86$ , but for PM<sub>2.5</sub> a p-level of 0.01 was calculated. The p-levels were all significant within the correlation calculations of PM<sub>2.5</sub>, PM<sub>10</sub>, temperature and humidity with the most less significance of  $p = 0.02$  when comparing PM<sub>10</sub> and temperature for the bicycle route.

**Table 1.** Summary of selected statistical data of the bicycle (n=5) and car tours (n=5) in terms of vehicle speed, particulate matter, temperature and relative humidity

Vehicle type	Statistical data type	Speed (km/h)	PM <sub>2.5</sub> (µg/m <sup>3</sup> )	PM <sub>10</sub> (µg/m <sup>3</sup> )	Temperature (°C)	rel. Humidity (%)
Car	Min.	0.0	0.4	0.5	16.6	35.6
	Mean	30.8	2.8	4.2	23.5	48.7
	Std.dev.	20.8	2.5	2.7	3.7	8.6
	Max.	87.8	21.8	23.4	29.8	64.4
Bicycle	Min.	0.0	1.0	1.3	20.0	27.8
	Mean	23.4	4.4	9.1	25.2	39.1
	Std.dev.	6.4	2.4	9.3	4.2	3.9
	Max.	40.2	25.4	211.5	34.5	50.9

Std.dev.: Standard deviation

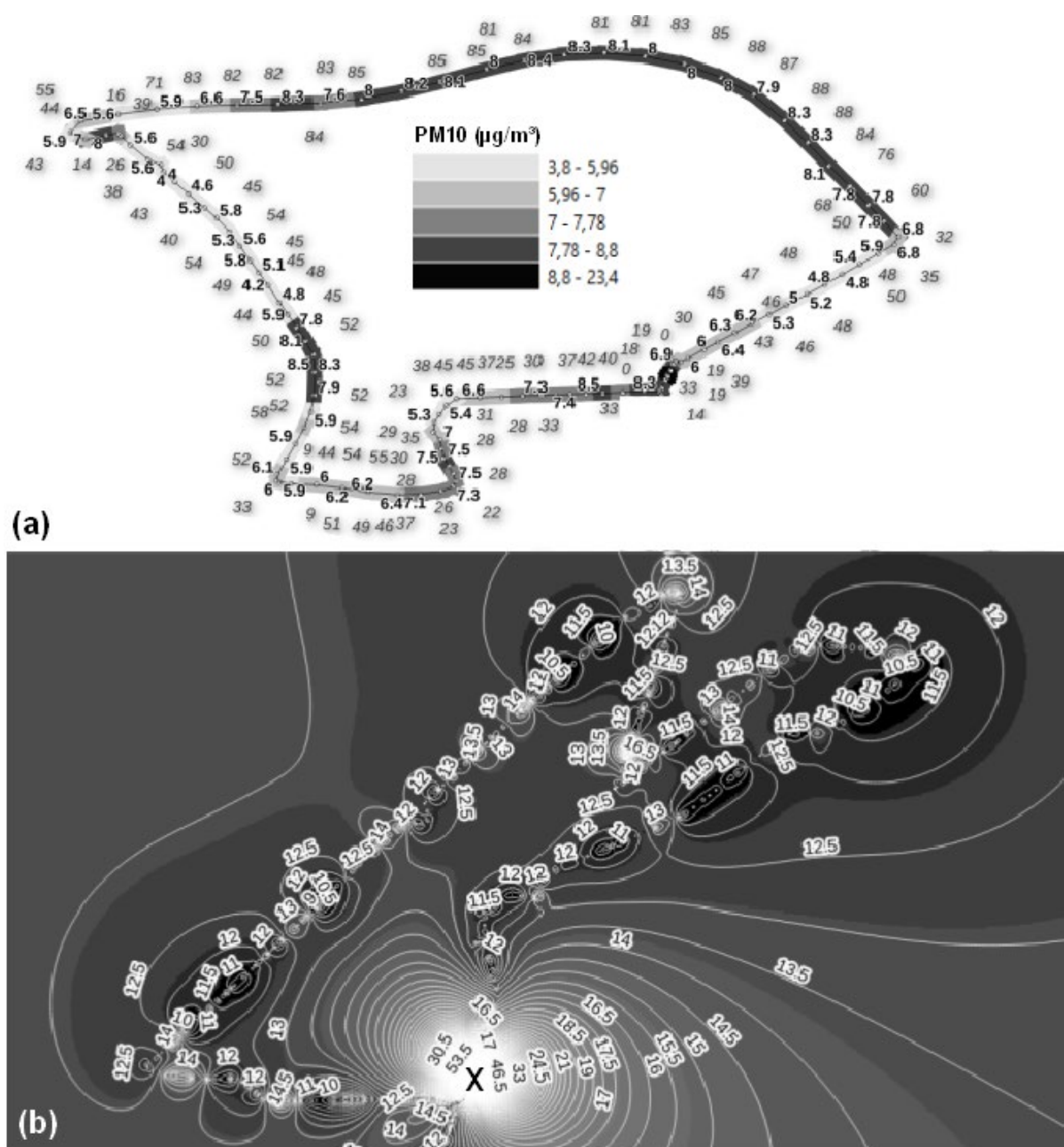


**Figure 2.** Correlation matrix of selected combined parameters for all (a) car and (b) bicycle routes. The shown values present correlation coefficients.

Recorded data sets for one selected car and bicycle tour were finally used to show exemplary the spatial resolved PM<sub>10</sub> concentrations along the routes and the theoretical PM<sub>10</sub> situations in the peripheral environment (**Fig. 3**). The PM<sub>10</sub> concentrations ranged between 3.8 and 23.4  $\mu\text{g}/\text{m}^3$  during the journey with the car along a route of approx. 14 km at a speed from 0 to 88 km/h. Pre-defined PM<sub>10</sub> classes indicate route sections with different concentration categories in **Fig. 3a**.

An alternative possibility to illustrate the PM<sub>10</sub> situation in the ambient atmosphere was chosen for the bicycle tour in **Figure 3b** with approx. 30 km in length. By means of a spatial interpolation in the GIS program theoretical PM<sub>10</sub> concentrations were calculated beyond the bicycle route to make PM<sub>10</sub> values available within a defined area where direct measurements did not take place. In this way, estimates for PM concentrations (or for other airborne factors) in the surrounding area can be made; for example, 100 m away from the driving route. From this point of view the influence of a real PM<sub>10</sub> concentration on the vicinity is assessable (see the X in **Fig. 3b**, for example).





**Figure 3.** Georeferenced PM<sub>10</sub> concentrations of (a) a car travelling event in an urban area and (b) along a bicycle route with displayed contour lines from a spatial interpolation procedure in GIS. In (a) the grey shaded concentration categories are related to  $\mu\text{g}/\text{m}^3$  in conjunction with the less big, but bold numbers. The marginal placed numbers in italic font with a light grey shadow present the speed of the car. Apart from the PM<sub>10</sub>-labelled contour lines in (b) the different shades of grey in the map are indirect indicators of the calculated PM<sub>10</sub> concentrations beyond the bicycle route. The X indicate a measured PM<sub>10</sub> concentration of  $211.5 \mu\text{g}/\text{m}^3$ .

## Discussion and conclusions

The applied low-cost measurement technique is generally suitable to detect PM concentrations outdoors with a high time resolution during mobile investigations, although some technical improvements are desirable. Apart from necessary suitable calibration protocols the sampling of

aerosols in moving air needs attention to be sure that no significant PM sampling variations occur among the different PM sizes due to a non-isokinetic and non-isoaxial sampling procedure (MITCHELL, 1995; VDI 2066 – Part 1, 2021). This is mainly important when using motor vehicles like cars, but here the MDR had only a simple air inlet that does not fulfil the desired operational demands. It would be a challenge to find a practical solution to take varying wind speeds and directions as well as different car velocities into account in relation to an appropriate architecture of the air inlet without getting an instrument to bulky or not easily to handle.

The mentioned shortcomings were probably less relevant during the bicycle tours, because the MDR was mounted within the wired basket of the bicycle. However, the upper part of the basket was not closed with a wire net. Nevertheless, such wired baskets might be a solution to act as a wind breaking device and to smooth the incoming air. If so, relative calm air can be expected in the basket similar to the magnitude of air movements indoors and therefore more representative aerosol measurements should be possible. This conclusion is comparable with the situation within livestock buildings where comprehensive air flow-related technical adaptations for aerosol sampling devices are not usual (WATHES 1995).

Spatial interpolation methods offer a couple of uncertainties in terms of reliable calculated predicted values within the geocoordinate system under consideration. It is not only the density of available measurement data but also the so-called distance coefficient as adjustable variable within the interpolation method. This coefficient indicates the magnitude of influence of a data point over distance. From this point of view different distance coefficients manipulate principally the calculated virtual values. Therefore, this argument – certainly among many other criteria – needs attention in further investigations. The application of alternative spatial interpolation methods must be also checked. It is also worth mentioning that statistical analysis such simple correlations needs a review in future (e.g., assessing the role of recorded and identified confounders).

The presented measurement values here only represent the potential of gathering big amounts of data with devices such as the MDR. When adequate adaptations in technology and performance succeed, prospective measurement campaigns would be able to create data pools for reliable results. This would give environmental monitoring an additional boost.

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## Environmental and operational factors influencing the total bacterial count in filtration systems of fresh- and saltwater aquaria

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### Introduction

To ensure fish welfare in aquaria, bacteria which metabolize toxic compounds like ammonia and nitrite are essential. These bacteria are found in communities with other bacteria, viruses and protozoa, forming the so-called microbiota. The microbiome is subject to constant changes in terms of amount and composition due to various factors. The aim of this study is to validate the total bacterial count (TBC) as an indirect indicator of changes in the bacterial community due to certain events during the establishing period of new zoo aquaria.

### Material & Methods

Water samples and swab samples were taken out of the filtration system from an outdoor freshwater aquarium and from indoor marine and freshwater aquaria. Samples were collected at regular intervals for one year to cover all seasons. A bacterial cultivation was carried out on caso (freshwater)/marine salt agar (seawater) at 25°C and sheep blood agar/salt agar at 37°C. Grown colonies were counted after 48h and TBC was calculated for each sample using the colony forming units (CFU) per mL.

### Results

Several deviations from the standard TBC were observed in the several occasions. Generally, TBC in the outdoor aquarium was higher by 2 folds than the indoor one. Stagnation of water movement due to a short-term power failure resulted in a slight increase in the TBC by log 10. A decrease in the CFU by log 10 was noticed after the replacement of older filter components as well as during a strong increase in the salt concentration in the seawater. No changes were observed with minor environmental influences, such as the introduction of single new fish to the tank.

### Discussion

Few factors have influenced the TBC of the water in the filtration system but generally without or minor effects on the fish welfare. The TBC is limited to the bacteria that can be cultivated on agar under standard lab conditions and only allows a conclusion to be drawn about changes in the amount of those bacteria. Screening the same samples by next generation sequencing will give more insight on the actual bacterial composition in the aquarium filtration system.

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We thank the Freundeskreis Tiermedizin der Veterinärmedizinischen Fakultät Leipzig e.V. for general support and Mario Reinhardt, Robert Küchler, Evelyn Brumme and Dana Rüster for expert technical assistance.

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# Tenacity of Feline Coronavirus on surfaces and in aerosols including the role of the particle size

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## Introduction

The emergence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) resulted in a large global outbreak. Three main transmission paths are discussed: Droplet, aerosol and contact transmission. Carriers exhale virus-containing particles of varying size and quantity. Large droplets get directly onto the mucous membranes of susceptible hosts, settle on inanimate surfaces or evaporate to form droplet nuclei before reaching the ground. Droplet nuclei and small aerosol particles remain airborne for several hours in enclosed environments. Therefore, we aimed to determine the stability of Feline Coronavirus (FCoV) in aerosols and on surfaces under various environmental conditions, including the influence of particle size, to better understand the relevance of the different transmission paths.

## Material & Methods

FCoV was used as a surrogate for SARS-CoV-2. The viral aerosol was generated by an ultrasonic nebulizer and homogeneously distributed in an airtight walk-in aerosol chamber. We first investigated the stability of aerosolized FCoV at different RH (30, 40, 50, 60 and 70%). Air samples were taken using two Coriolis  $\mu$  cyclone air samplers at different heights. In a further experimental series, we collected the aerosolized FCoV particle size-selectively via a six-stage Andersen cascade impactor (ACI), ranging from 0.6 to  $>7 \mu\text{m}$ . Additionally, the particle size distribution of the aerosol was measured using a laser based particle counter. Finally, we investigated the stability of FCoV on stainless-steel germ carriers with either sheep blood or yeast extract as organic soiling. Germ carriers were stored at 4°C and 20°C until no infectious virus was detectable. The tissue culture infectious dose 50 (TCID<sub>50</sub>) of all samples was calculated. ACI air samples were further analyzed using Real-time quantitative PCR (RT-qPCR).

## Results

Aerosolized FCoV showed maximum stability at 40% RH, significantly lower stability was observed at 50% RH with recovery rates ranging from 11.08% (40%) to 2.51% (50%). Using the ACI, viral RNA was detected in all particle fractions above 1.1  $\mu\text{m}$ , at concentrations from  $2.23 \times 10^5$  to  $3.18 \times 10^7$  copies/ $\text{m}^3$ . Infectious FCoV was detected in all particle fractions above 1.1  $\mu\text{m}$  in concentrations between  $1.48 \times 10^2$  and  $7.28 \times 10^4$  TCID<sub>50</sub>/ $\text{m}^3$ . On stainless-steel surfaces, FCoV showed maximum stability at 4°C using yeast extract as organic soiling, with a 5-log TCID<sub>50</sub> reduction after an average of 167 days. In contrast, at 4°C, we observed a 5-log TCID<sub>50</sub> reduction after 19 days. Remarkably, with sheep blood as organic soiling, FCoV showed comparable stability at 4°C and 20°C with a 5-log TCID<sub>50</sub> reduction after 54 or 58 days, respectively.

## Discussion

Our results indicate that FCoV may remain infectious for a prolonged period under natural environmental conditions, both in air and on surfaces. Moreover, we detected FCoV in a wide range of particle sizes. However, the viability of the virus is dependent on particle size.

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## Map of the area



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- 7 Mensa Herrenhaus Düppel
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