PH-01

Antibiotic resistance and genomic characterization of *Enterobacteriaceae* involved in bovine in mastitis using Nanopore technology

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Objectives: Bovine mastitis is the most common and costly disease in dairy cattle. Among the causative pathogens, *Escherichia coli* has recently emerged as one of the most prevalent causative agents. Mastitis treatment is mainly based on the use of antibiotics, being one of the main causes for antibiotic use in farms. The use of antibiotics is considered a risk factor in the emergence of antibiotic resistant bacteria. Therefore, the objective of this study was to evaluate the presence of resistance determinants in mastitis pathogens from Spanish dairy farms.

Materials & Methods: More than 10.000 bacterial isolates were recovered over the past 10 years from clinical and subclinical mastitis. The causative agents and their antimicrobial resistance pattern was recorded. Further, mastitis30 isolates from both clinical and subclinical mastitis from different dairy farms in Spain collected during 2019. Bacterial identification was performed using MALDI-TOF (Matrix Assisted Laser Desorption/Ionization- Time of flight). Agar disk diffusion method was used to determine the susceptibility of all isolates. 15 *E. coli* isolates were tested for their antimicrobial susceptibility against fourteen antibiotics via Minimal Inhibitory Concentration (MIC), based on the criteria of the European Comittee on Antimicrobial Susceptibility Testing (EUCAST). All *E. coli* isolates were sequenced using Nanopore technology in order to determine its resistance genes.

Results: Analyzing the trends in antimicrobial resistance over the last 10 years, we can observe the resistance to ampicillin has been maintained in these 10 years. Ampicillin, amoxicillin clavulanic and trimethoprim/sulfamide are the antibiotics with the highest levels of resistance. We observed that the bacteria that is causing the highest number of clinical mastitis is *E.coli*, followed by *Streprococcus uberis*; on the other hand *Streptococcus uberis* and coagulase negative *S. aureus* are the ones that cause subclinical mastitits.

E. coli was detected in 15 samples, *Klebsiella oxytoca* was detected in 2 samples, *Klebsiella pneumonie* was detected in 2 samples. The highest antimicrobial resistance was observed for sulfonamides and tetracyclines due to the presence of the genes *sul1*, *sul2*, *sul3*, *tet*(A), *tet*(B), *tet*(M), bla_{TEM-1B}, bla_{TEM-1A}, bla_{CARB-2}. These genes are related with mobile elements that can favour the dissemination of resistance genes to other bacteria.

Conclusions: The main pathogens that cause mastitis are *Streptococcus uberis*, coagulase negative *Staphylococcus*, *Staphylococcus coagulase positive*, *E. coli* and *Klebsiella spp*. *Staphylococcus coagulase negative*, the bacteria with a higher incidence. The tendency of antimicrobial resistance, shown in the different *E. coli* isolates that cause clinical mastitis, have slightly grown towards penicilin, cephalosporins and fluoroquinolones resistance. This is worrying, as these antibiotics are of great importance for human health, according to WHO and the One Health approach. In the collected isolates during 2019 we can observe an extensive resistance to ampicillin with resistance to to 3rd generation cehalosporins. The genes encoding these resistances are also found in human isolates. The levels of resistance found underline the imortance of reducing the use of antibiotics to preserve their action as long as possible

Keywords: Mastitis, antibiotic resistance, Escherichia coli.

PH-02

Molecular diagnosis of *Coxiella burnetii* in bovine milk tank from small rural properties in Brazil

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Objectives: Therefore, the aim of this study was to investigate *Coxiella burnetii* in milk from cattle from small rural properties in the state of São Paulo, Brazil.

Material & Methods: Milk samples from bulk tank were collected from 102 family farms and sent to the laboratory for molecular testing. DNA extraction from milk samples was performed and subsequently the polymerase chain reaction (PCR) was performed using specific primers targeting the *C. burnetii* IS1111 transposase region. For the conventional PCR the set of primers Trans1/Trans2 were used and for the nested PCR, the primers N3+ and N4- were used. Then, electrophoresis was performed to estimate possible positive bands, and negative and positive controls were used.

Results: Of the one hundred and two samples collected, 16 (15.6%) were positive for the bacteria by molecular tests. Subsequently, these positive samples were sequenced and compared with sequences in Blastn. The samples demonstrated a similarity level of 98.4 to 100% with the samples in the database.

Conclusion: The presence of the bacterium in milk demonstrates the need for its active research into properties, as it can cause economic losses and infect humans. In addition, it demonstrates the circulation of *C. burnetii* in different municipalities, showing that the bacteria have the potential to

cause outbreaks and cases of Q fever. Therefore, it is necessary that surveillance be carried out continuously so rural producers can be made aware and to draw the attention of public health agencies to the adverse effects of infection by the agent and contamination of food, such as milk and its by-products, in addition to contamination environmental, since *Coxiella burnetii* is reported worldwide.

Keywords: Coxiellosis, Q Fever, Bulk tank, Bovine milk, Molecular diagnosis.

PH-03

Molecular investigation of *Toxoplasma gondii* in raw bovine milk from small rural properties in Brazil

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Objectives: This study aimed to investigate *Toxoplasma gondii* in 102 samples of bovine milk from expansion tanks, in small properties located in different cities of the Midwest region of São Paulo, Brazil.

Material & Methods: Samples from bulk tank milk were collected from 102 family farms. The collection of milk samples was approved by the Ethics Committee on the Use of Animals – CEUA – protocol 1367/2020, of the Botucatu Medical School - UNESP. The samples were submitted to DNA extraction using the commercial GFX Genomic Blood Kit (GE Healthcare) with modifications and subsequently the polymerase chain reaction (PCR) was performed using specific primers targeting the *Toxoplasma gondii* ITS1 region. For the PCR the set of primers NN1 and NN2 were used and for the nested PCR, the primers TgNP1 and TgNP2 were used. The amplicons were subjected to 2% agarose gel electrophoresis, with fragments amplified at 227 bp.

Results: Thirteen samples (12,74%) were positive for *T. gondii.*

Conclusion: The presence of *Toxoplasma gondii* DNA in milk demonstrates the need for investigated into properties, due it can cause economic losses and infect humans. In addition, it demonstrates that circulation of *T. gondii* in different municipalities, showing the potential to cause outbreaks and cases of Toxoplasmosis. Therefore, it is necessary that surveillance be carried out continuously and to draw the attention of public health agencies to the adverse effects of infection by the agent and contamination of food, such as milk and deriv-

atives, as well as contamination environmental, since *Toxoplasma gondii* is reported worldwide. Other diagnostic tests, like bioassay in experimental animals, would be opportune to validate the infection capacity of these samples.

Keywords: Toxoplasmosis, Bulk tank, Bovine milk, Molecular diagnosis, Zoonosis.

PH-04

Antimicrobial resistance profiles of *Escherichia coli* from dairy farms participating on an antimicrobial stewardship educational training program for farm employees

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Objective: The increase in antimicrobial resistant (AMR) bacteria is one of the biggest public health threats of our time. Although current AMR is monitored through reports such as the U.S. National Antimicrobial Resistance Monitoring System integrated report, there is a knowledge gap for on-farm AMR monitoring data. Our goal was to evaluate the antimicrobial resistance of *Escherichia coli* (EC) from pooled fecal samples before and after implementation of an on-farm animal health and diagnostic training program for farm workers in antimicrobial stewardship in adult dairy cattle.

Methods: Pooled fecal pat samples were collected from the hospital pen (cows treated with antimicrobials with a milk withhold period), the fresh pen (1 to 3 DIM) and the mid-lactation pens (90 to 150 DIM) in conventional dairies in CA (n=9) and OH (n=9). Fecal samples were collected as part of a larger study with a quasi-experimental design that assigned farms to training intervention group (TG; 9 per state) or control group (CG; 3 per state). For the TG, farm worker(s) identified as having the task of diagnosis and treatment of adult cows on the farm participated in training program on antimicrobial stewardship practices. Samples were collected at enrollment and three months after completing the intervention. For each pooled sample, EC was isolated. Standard culture, antimicrobial sensitivity testing using the broth microdilution approach, and categorization of isolates as susceptible, intermediate, or resistant was used. Logistic regression models were used to evaluate the association between EC antimicrobial resistance profiles and farm-level factors.

Results: A total of 504 EC isolates were tested for antimicrobial susceptibility. All isolates were susceptible to azithromycin and sulfisoxazole. The antimicrobial resistance more commonly found was against tetracycline (TET, 18.3%), streptomycin (STR, 16.3%), ceftriaxone (AXO, 10.7%) and ampicil-

lin (AMP, 11.1%). Resistance to ceftiofur (XNL) was only found in 1.98% of the isolates, one of the most commonly used drug on the study farms to treat sick cows, while 15.3% isolates were classified as multidrug resistant. Among the most commonly found MDR patterns were streptomycin-ceftriaxone-tetracycline (n=5), streptomycin-chloramphenicol-tetracycline (n=5), and streptomycin-ampicillin-chloramphenicol-tetracycline (n=4).

No significant effect on the proportion of AMR isolates was found associated to the intervention compared to the control farms. However, the univariate analysis showed that samples from OH had a higher proportion of AMR isolates to nalidixic acid (NAL) and TET, while samples from CA had a higher proportion of isolates resistant to XNL. There was also a higher proportion of EC isolates resistant to STR, XNL and TET from the enrollment visit compared to the visit after intervention was completed. Finally, samples from the fresh cow pen had significantly higher proportion of EC isolates resistant to cefoxitin (FOX), STR, AMP and NAL, while samples from the hospital and mid-lactation pens had higher proportion of isolates resistant to XNL and amoxicillin/clavulanic acid (AUG), respectively.

Conclusion: Differences on the antimicrobial resistance profiles of *Escherichia coli* from dairy farms included in this project were detected, and although, there was not a significant difference in the AMR profiles after the educational intervention, this supports the need for more research to get a better understanding of the resistome and its changes over time on dairy farms.

Keywords: Antimicrobial resistance, education, cattle.

PH-05

***ESBL* resistance genes in fecal *E. coli* of calves fed waste milk with antimicrobial residues**

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Objectives: Beta-lactamases are enzymes capable of hydrolysing β-lactam antimicrobials, conferring resistance to Gram-negative bacteria. Among β–lactamase enzymes, the extended-spectrum β–lactamase enzymes (ESBL) provide resistance to a wide variety of β-lactam antimicrobials including penicillin and 2nd, 3rd and 4th generation cephalosporins. Infections caused by Gram-negative bacteria harbouring these enzymes are challenging to treat and have increased in incidence in the human as well as in the dairy cattle population. Feeding waste milk to calves, a mixture of excess colostrum, transition milk and non-saleable milk from cows that are being treated with antimicrobials, has been observed to lead to increased antimicrobial resistance in faecal isolates of calves. This study aimed to evaluate the association between feeding waste milk to calves and the occurrence of antimicrobial multi-resistance by extended spectrum β-lactamase enzymes through determining ESBL production by E. coli isolates from 32 dairy farms.

Material & Methods: In each farm, fecal samples were collected from the rectum of five healthy calves in the first month of life and pooled into a single container. Five isolates from each sample were selected and confirmed to be *E. coli* by amplification of the 16S gene. ESBL production was determined phenotypically on 148 isolates from 31 farms by use of antimicrobial susceptibility testing to cefotaxime, ceftazidine both alone and after impregnation with clavulanate. Genotypic confirmation of ESBL production was performed by PCR for the genes blaCTX-M-1, -2, -8, -9 and blaCMY-2. A questionnaire was performed regarding potential risk factors for the emergence of antimicrobial resistance. A univariate analysis was performed to evaluate which risk factors should be included in a multivariable logistic regression. Statistical analysis was performed with the software R®, version 3.5.1, with p<0.05 considered statistically significant.

Results: Phenotypically 40 *E. coli* isolates from 15 farms (48.4%) were found, whereas genotypically 55 isolates from 20 farms (64.5%) were found. The questionnaire revealed that 28 out of 31 farms (90.3%) fed waste milk to calves. On all of the farms that had this practice, waste milk comprised milk from animals being treated with antimicrobials and milk originating from animals during the withdrawal period for antimicrobial treatment. For 17 of the farms, waste milk also included milk from animals with high somatic cell counts. On 7 of the 31 farms an antimicrobial was added to milk, either waste milk or milk replacer, in a preventative way. The questionnaire also revealed that the number of different intramammary tubes used as treatment options for mastitis in the past year varied from 1 to 7. The multivariate analysis identified feeding milk with preventative antimicrobial to calves as the main risk factor with this routine leading to a 2.5 times higher risk of calves in their first month of life shedding ESBL-producing *E. coli*. Another risk factor identified by our study was the use of a higher number of antimicrobial treatment options for the treatment of mastitis, which led to a 1.3 times higher risk of calves shedding ESBL-producing *E. coli* on those farms. In our study, feeding waste milk to calves was not significantly associated with an increased risk for the presence of *E. coli* producing ESBL in the faeces of calves in the first month of life, although farms that had this routine, had a 5.2 higher risk of ESBL-producing E. coli.

Conclusion: Feeding waste milk to calves was a very common practice on the participating farms, with it including milk from animals under antibiotic treatment, during the withdrawal period for antibiotic treatments and milk from animals with subclinical mastitis. Some farmers also added antibiotic to waste milk to prevent neonatal calf diarrhea, a procedure that increased significantly the likelihood of calves in their first month of life shedding ESBL-producing *E. coli* in their feces. The other risk factor that significantly contributed to this outcome was the use of a higher number of intramammary antibiotic options to treat mastitis.

Keywords: ESBL, waste milk, calves, antimicrobial resistance.

An observational cohort study on antimicrobial usage in dairy farms from Québec, Canada

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Quantification of antimicrobial use (AMU) is crucial to measure the impact of intervention programs targeting a more judicious use, to determine associations between usage and resistance, to compare populations and to promote benchmarking.

Objectives: The objective of the study was to describe quantitatively AMU in Québec dairy herds over one year as a whole, then by administration route (intramammary, injectable, oral, intrauterine), and by category based on importance in human medicine according to Health Canada (4 categories: Category I "Very high importance", II "High importance", III "Medium importance", and IV "Low importance"). A secondary objective was to evaluate the effect of herd size, production level, and disease level on AMU.

Material & Methods: Data were obtained from 101 dairy farms randomly selected in 3 regions of the Québec province of Canada (Montérégie, Estrie, Centre-du-Québec) by collecting and recording all empty drug packaging between spring 2017 and spring 2018 (garbage can audit method). The quantity of medicated feed sold during the same period was obtained directly from the feed mills. Antimicrobial usage was reported in number of defined course doses (DCDbovCA) per 100 cows-year (calculated as a whole, by administration route, and by category). The intramammary route was separated between antimicrobial agents (AMs) administered during lactation for treatment of mastitis and AMs administrated at the time of drying-off. The oral route was separated between AMs administered as individual treatments (boluses, oral solutions, etc.) and AMs administered in the feed. The participating dairy producers completed an in-person questionnaire between January and March 2018. The number of dairy cattle in the farm was collected by age group, as well as the amount of milk produced. Questions were asked regarding number of animals experiencing some common diseases during the last 12 months by age group. Effect of herd size, production level, and diseases level on AMU was evaluated by using negative binomial regression models. A predictive model was also built to predict how the level of infectious diseases on a farm could explain its AMU.

Results: The average size of the population was 66.5 cows per farm (range 20-150); 2/101 farms were organic. Overall, a median of 429 DCDbovCA /100 cows-year was used. The most frequent administration was through the intramammary route: median of 161 and 67 DCDbovCA /100 cows-year for lactating and dry cow formulations respectively. A median of 47 and 12 DCDbovCA /100 cows-year was observed for the injectable route and the feed route respectively. The intrauterine route and the oral individual route (other than in the feed) were infrequently used (median of 0 DCDbovCA /100 cows-year). Category II antimicrobials were the most frequently used (median 181 DCDbovCA /100 cows-year), followed by categories III, I, and IV antimicrobials (median of 79, 62, and 23 DCDbovCA /100 cows-year

respectively). Category I AMs were more frequently used as intramammary than injectable formulations. A positive and linear association was identified between herd size and global AMU. A positive and non-linear association was identified between diseases level and global AMU. Incidence of diseases only explained 31% of the global AMU, and 2.2% of Category I AMU.

Conclusion: This study provides a detailed description of the AMU over one year on Québec dairy farms. Oral and intrauterine formulations were infrequently collected and other methods of data collection should be considered to obtain a more complete picture of AMU for these formulations. The technique based on garbage can audit could be imperfect and incomplete for reporting on AMU in dairy farms; other methods such as using veterinary prescriptions (or invoices) or treatment records could be used in Québec to estimate the AMU. This study highlights that AMU is positively associated with herd size (mainly for Category IV AMs) and with level of diseases.

Keywords: Antimicrobial usage, dairy cattle, defined course dose, garbage can audit, quantification.

Salmonella eradication on 57 dairy cattle farms in Finland

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Objectives: Finland has a salmonella control program that covers cattle, pigs, and most of the poultry species. The aim of the control program is to maintain *Salmonella* prevalence of all serotypes below 1% in the food chain and thus reduce the risk of human salmonellosis. If *Salmonella* is detected on a farm or at the slaughterhouse sampling, it must be eradicated from the farm. On a dairy farm eradication includes e.g., sampling of animals and the environment, eliminating the possible infection source, and thorough cleaning and disinfection. For the present group insurances through dairies have been available for farms to partly cover the costs of eradication.

Our aim was to investigate *Salmonella* eradications on dairy farms, describe the principles of the eradication process and to clear out the most likely cause of the infection. Based on these findings, it would be easier to advice the prevention and guide the eradication of *Salmonella* on dairy herds.

Materials and methods: Our data included 57 dairy herds with *Salmonella* infection and an eradication that an experienced veterinary advisor (Dipl. ECBHM Olli Ruoho) had planned and guided during 2009–2019. These dairy herds had an average 76 milking cows (range 17-260). The most common serotypes detected were *S.* Typhimurium (n=30), *S.* Enteritidis (n=11) and *S.* Altona (n=4). We calculated the length of the eradication from the start and end date of the official restrictions of the farm prescribed by legislation. Based on environmental and animal sampling at the beginning of the eradication process, we defined the *Salmonella* infection of

the farm as non-existent, restricted, average, or vast.

Results: The eradication lasted 21-533 days (mean 114, median 90). It lasted longer (52-533 days, mean 156, median 120) on 31 farms (54%) where the infection was considered as average or vast at the beginning. Other typical reasons for a prolonged eradication were: if the source of the infection was undetected at the beginning, if salmonella positive animals were not culled in time, if cleaning and disinfection procedures were inadequate or lack of labour leading to exhaustion. On 46 farms (81%) the source of the infection was birds or rodents, and in most of these cases birds. Occasionally, the possible source was *Salmonella* positive animals, humans, or contaminated feed. However, the management of salmonella risk in commercial feeds is at a high level in Finland.

Conclusions: Eradicating *Salmonella* from a dairy farm is possible. However, proper planning is essential as well as working systematically throughout the eradication process. Furthermore, a competent supervision and guidance during the process are important as well as having enough skilled labour on the farm. Nevertheless, the focus should be on *Salmonella* prevention by improving the on-farm biosecurity like protecting the barn and especially feed storage from birds in a way that there will be no easy access to feed. This includes furnishing the barns with anti-bird nets, covering all the feed storage, maintaining the barn and farm area tidy that uneaten feed is taken away regularly, and enhancing the overall pest control. Moreover, the whole sector should discuss the means how to reduce the risk of spreading *Salmonella* infections from dairy farms to calf rearing units and thereby to beef production chain since all calves can not be tested against *Salmonella*.

Keywords: Salmonella eradication, dairy cattle, biosecurity.

PH-08

Fractionation of milk for trace analysis of contaminants and residues

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Objectives: Contamination of dairy products with detergents and disinfectants and their degradation products such as chlorate and perchlorate raise challenges for stakeholders in the dairy food chain, as these substances are a potential threat for food safety. The aim of this work was to develop a passive sample preparation for raw milk, apart from the well-known Quechers or Quppe methods, which does not require any additional extraction agents and enables a cost-effective just-in-time analysis of milk raw materials for chlorate and perchlorate.

Materials and Methods: Unprocessed raw milk was separated into the phases "water", "fat" and "protein", using a fractionation unit (FraMiTrACR C/PC®). The separation by FraMiTrACR C/PC® requires the use of a standard table top centrifuge. Different sample volumes were processed, both 10 mL raw milk using a fixed angle rotor at 30 minutes and 2,000 x g and 5 mL raw milk using a "swing out" rotor at 30 minutes and 4,000 x g. For both settings, half of the volume of feed added was recovered as the water phase. For the analysis of the water phase, an ion chromatography system was used, the 930 Compact IC Flex System with Dosin gradient technology for the determination of anions after sequential suppression and conductivity detection (IC-CD). For each determination, 0.25 mL of water phase was injected into the analyser. At the beginning of the development, the anion matrix in the water phase was collected from milk samples that were free of chlorate and perchlorate. To improve the detection limit, the characteristic anion matrix of the water phase has been subtracted when evaluating the results. The chlorate and perchlorate content was determined using spiked samples in standard series and also in native samples. In order to obtain comparability with previously used methods, the investigated raw milk samples were simultaneously analysed in a contract laboratory using the modified Quppe method and liquid chromatography with tandem mass spectrometry coupling (LC-MS/MS).

Results: In direct comparison to well-established sample preparation methods, such as Quechers and Quppe, there is a significant saving in working time and thus personell costs due to the passive sample preparation using a table top centrifuge. In addition, the complete elimination of extraction solvents or other additives leads to further cost savings in the laboratory process chain, simultaneously excluding the risk of unwanted contamination. In the water phase, detection limit of 0.003 mg/kg chlorate and perchlorate could be achieved by using IC-CD. In the analysis of the water phase by means of LC-MS/MS detection limit of down to 0.001 mg/kg chlorate and perchlorate could be achieved.

Conclusions: The application of the fractionation unit (FraMiTrACR C/PC) shows that it is possible to determine defined analytes directly from the water phase of the milk without further preparation. The advantage of this method is that milk samples are prepared in one step, passively and without further additives. This leads not only to a reduction in personnel costs by reducing the active working time of the laboratory staff, but also to a reduction in the costs of operating resources, stock-keeping and stock management of operating resources. Sample contamination is prevented as well, as the sample only needs to be filled into the fractionation unit (FraMiTrACR C/PC) and then closed. The risk of carry-over or contamination within the laboratory can thus be reduced to a minimum. With the method described, it will also be possible, depending on the analytical equipment available, to analyse raw milk just-in-time before processing. It should be emphasised that sample preparation and subsequent analysis using a comparatively low-cost method, IC-CD, led to comparable results to sample preparation using the modified Quppe method and analysis by LC-MS/MS. In the methods compared, the limit of quantification was 0.01 mg/kg chlorate and perchlorate and the detection limit was below 0.01 mg/kg chlorate and perchlorate. This also shows that the sample preparation method can be used universally for many other analytical methods. Basically, the fractionation unit (FraMiTrACR C/PC) opens up new possibilities for residue and contaminant determination in dairy products. More analytes will be investigated with this method in further studies.

PH-09

Knowledge, behaviours and attitudes of Scottish dairy farmers towards antimicrobial use

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Objectives: Scientific evidence demonstrates that antimicrobial usage (AMU) in animals may contribute to the emergence of antimicrobial resistance (AMR). In the UK, dairy farmers can administer antibiotics without the veterinarian being present and thus, they play a crucial role in the decision making around AMU. Further understanding of how antimicrobials are used in dairy production as well as stakeholder beliefs relating to their use is essential to help ensure responsible AMU. To the authors' knowledge, no work to date has been carried out exploring these aspects in the Scottish dairy sector. Therefore, the aims of this study were: to identify the factors influencing knowledge, AMU and attitudes towards AMR and to determine the barriers and motivators to uptake of best practice recommendations.

Materials and Methods: To address this study, a questionnaire was designed for administration via an online survey; the target population was Scottish dairy farmers (n=840). To inform the design of the questionnaire, a focus group and a workshop were held with representatives of the industry with the aim of eliciting free discussion around the topics of AMU and AMR. As a result of the COVID-19 pandemic, the focus group was held via Zoom, and participants (n=5) were a convenience sample of farmers. The workshop was part of an online agricultural event (Agriscot).

The questionnaire was structured in four main sections. Section One explored farmers' awareness and understanding of antibiotics and AMR. Section Two contained some questions to identify factors that may influence farmers' AMU, as well as the main barriers and drivers for responsible usage. In addition, some specific scenarios of dairy cows' diseases were posed to assess farmers' behaviour. Section Three investigated farmers' attitudes towards AMR and AMU best practices. Section Four covered demographic information of the participants and the farm.

A pilot study was performed using a preselected group of farmers (n=5) to test survey duration and suitability of the questions to the target population. The questionnaire was launched online and was open from May to September 2021. The survey URL was promoted via multiple ways (farming press, social media, veterinary practices, and milk buyers).

Data analysis was performed using R Core Team (2020). Responses were analysed using descriptive statistics. Two outcomes were explored in initial analyses – participant knowledge and AMU. These were derived scales, determined by answers provided in sections One and Two. Univariable logistic regression models were established to explore the association of knowledge and AMU with demographic characteristics, attitudes and other various factors such as frequency of contact with the veterinarian and implementation of AMU best practices. Significant explanatory variables from univariable analysis (P<0.2) were included in multivariable logistic regression models for the two outcomes.

Results: The survey was completed by 61 respondents (7.3 % of the total target population). Overall, farmers expressed good understanding of the phrase antimicrobial resistance (AMR) however, 31 % thought antibiotics were effective against viruses and 25 % against parasites. The vast majority (90%) had implemented practices to reduce AMU, such as written treatment protocols and selective dry cow therapy. Additionally, 70 % expected a further reduction in AMU in the future. Limited knowledge and poor facilities were reported as the main barriers. It was found that veterinarians are usually not consulted before treating animals with antibiotics, even if they are regarded as the most influencing and reliable source of information. The majority of farmers (89 %) thought it was important to reduce AMU on farm; however, only half of them were concerned about AMR in the dairy sector. Future work will look at associations between outcomes, such as farmer knowledge and AMU, and selected explanatory variables.

Conclusions: For dairy practitioners, understanding farmers behaviour and attitudes is essential to engage with them towards antimicrobial usage reduction. These preliminary results suggest farmers' need for more training and support for responsible AMU and veterinary practitioners have a key role on this. Farmers generally agreed on the importance to reduce AMU; however, they often do not perceive AMR as a current threat to their own farm. It is important for veterinarians to consider the different levels of attitude and awareness of farmers in order to implement tailored interventions.

Keywords: Antimicrobial resistance, dairy, farmer behaviour.

PH-10

Antimicrobials resistance in *Escherichia coli* and *Enterococcus fecalis* commensal bacteria in north-eastern Italian dairy farms

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Objectives: Antimicrobial resistance (AMR) is of public and animal health concern worldwide.

Aim of our work was to identify an AMR indicator to classify healthy dairy herd according to antimicrobial susceptibility (AST) in indicator bacteria *Escherichia coli* (EC) and *Enterococcus fecalis* (ENT) towards an harmonized list of antimicro-

bials as suggested (European Food Safety Agency (EFSA), 2019). The overall farm health condition or prevalence of animal diseases were not considered yet the animal category (whether milking cows (VL), dry cows (VA), heifers (M) and calves (V)) of the isolate origin was analyzed to identify within heard different source of AMR.

Materials and methods: Thirty-three dairy farms were enrolled in this study, caring that both small and larger farms located in foothills or ground level of the Veneto and Friuli-Venezia Giulia regions of Italy were selected. According to preliminary analysis (data not shown in this paper) in each herd the following sampling protocol was applied: individual fecal samples were collected VL, VA, M and V to isolate 20 *Escherichia coli* (EC) and 10 *Enterococcus fecalis* (ENT). Isolates were AST with broth microdilution (CLSI VET01, 2020) using a commercial kit EUVSEC3 and EUVENC (ThermoFisher, US) to determine the minimum inhibitory concentration (MIC). MICs were interpreted according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) epidemiological cut-off val-

ue (ECOFFs;www.eucast.org/mic_distributions_and_ecoffs/). Isolates displaying MICs above the ECOFF were classified as non-wild type. Antimicrobial resistance index (ARI) was calculated for each bacterial isolate by dividing the number of resistances by the number of antimicrobials tested to provide descriptive statistics of the AMR herd indicator as reported (Agnoletti, 2018).

Results: 620 EC isolates and 229 ENT were AST assessed. The percentage of non-wild strains for each animal categories for each active ingredient are summarized in table 1 and table 2 for EC and ENT, respectively. EC isolates showed high proportions of non-wild phenotype towards sulfamethoxazole and tetracycline. In calves EC non-wild phenotypes are largely diffuses when compared to the other animal categories. Among ENT, non-wild phenotypes were mainly observed for gentamicin (dry cows), erythromycin (calves) and tetracycline (calves).

The herd level indicator ARI ranged from 0 to 0.14 (mean 0.08) for *E. coli* towards 13 antimicrobials tested (presumptive

Tables.

Antimicrobial	A	L	M	V
Sulfamethoxazole	35/68(51.5)	131/254(51.6)	69/160(43.1)	103/138(74.6)
Trimethoprim	2/68(2.9)	5/254(2)	6/160(3.8)	29/138(21)
Ciprofloxacin	1/68(1.5)	2/254(0.8)	3/160(1.9)	28/138(20.3)
Tetracycline	3/68(4.4)	14/254(5.5)	15/160(9.4)	71/138(51.4)
Meropenem	0/68(0)	0/254(0)	0/160(0)	0/138(0)
Nalidixic Acid	1/68(1.5)	0/254(0)	3/160(1.9)	24/138(17.4)
Azithromycin	0/68(0)	0/254(0)	0/160(0)	0/138(0)
Cefotaxime	1/68(1.5)	4/254(1.6)	0/160(0)	8/138(5.8)
Chloramphenicol	2/68(2.9)	3/254(1.2)	1/160(0.6)	19/138(13.8)
Tigecycline	0/68(0)	0/254(0)	0/160(0)	0/138(0)
Ceftazidime	0/68(0)	0/254(0)	2/160(1.2)	2/138(1.4)
Colistin	0/68(0)	1/254(0.4)	0/160(0)	0/138(0)
Ampicillin	4/68(5.9)	8/254(3.1)	8/160(5)	53/138(38.4)
Gentamicin	0/68(0)	0/254(0)	1/160(0.6)	6/138(4.3)
Presumptive_ESBL	1/68(1)	4/254(2)	2/160(1)	8/138(6)

Table 1. Number of non-wild strains of E. coli divided for animal category.

Antimicrobial	A	L	M	V
Gentamicin	26/33(79)	40/73(55	8/16(50	60/107(56)
Chloramphenicol	0/33(0)	5/73(7)	0/16(0)	21/107(20)
Ampicillin	0/33(0)	1/73(1)	0/16(0)	1/107(1)
Vancomycin	0/33(0)	0/73(0)	0/16(0)	0/107(0)
Teicoplanin	0/33(0)	0/73(0)	0/16(0)	0/107(0)
Erythromycin	10/33(30)	15/73(21)	3/16(19)	38/107(36)
Quinuopristin_Dalfopristin	4/33(12)	9/73(12)	2/16(13)	9/107(8)
Tetracycline	14/33(42)	19/73(26)	2/16(13)	73/107(68)
Tigecycline	0/33(0)	0/73(0)	0/16(0)	0/107(0)
Linezolid	0/33(0)	2/73(3)	0/16(0)	0/107(0)
Daptomycin	3/33(9)	4/73(5)	0/16(0)	3/107(3)
Ciprofloxacin	0/33(0)	0/73(0)	0/16(0)	2/107(2)

Table 2. Number of non-wild strains of *E. fecalis* divided by animal category.

ESBL were defined as being not wild for at least one among cefotaxime and ceftazidime) and 0.04 to 0.29 (mean 0.13) for *E. faecalis* towards (12 antimicrobials tested).

Conclusions: Microbiologic resistance against molecules widely employed in Veterinary Medicine (sulphonamides, tetracyclines, aminoglycosides, macrolides, and phenicols) was detected in the sampled dairy herds. Yet with the exception of fluorchinolones, EC isolated showed low levels of microbiologic resistance against antimicrobials classified of highest critical importance for human therapy (hstCIA; WHO, 2020). However, enterococci displayed high levels of microbiologic resistance towards erythromycin and gentamycin. Compared to adult bovines, including heifers 6 months aged, isolates from calves were keener to microbiologic resistance, which may be attributable to inappropriate farmer behavior of feeding calves with waste milk from cows treated with antimicrobials. Calves must be considered when monitoring AMR to avoid underestimate data.

References:

Agnoletti F, et al. Longitudinal study on antimicrobial consumption and resistance in rabbit farming. International Journal of Antimicrobial Agents 2018.

Keywords: Antimicrobial resistance, dairy cows, calves.

Critically important antimicrobials are not needed for treating clinical mastitis in lactating dairy cows

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Objectives: There is ongoing debate regarding whether critically important antimicrobials (CIAs) should be used to treat infections in food-producing animals. Mastitis accounts for majority of worldwide antimicrobial usage (including CIAs) in dairy cattle. Objectives of this network meta-analysis (NMAs) were to evaluate whether critically important antimicrobials (CIAs) were necessary to treat non-severe clinical mastitis (CM) caused by the most commonly isolated bacterial pathogens worldwide. The literature reporting on bacteriological cure (BC) rates of antimicrobials used to treat lactating dairy cows with non-severe CM was analyzed using a set of networks.

Material and Methods: On March 30, 2019, the databases CAB Abstracts, Web of Science (all databases), MEDLINE, Scopus (Elsevier) and PubMed were screened for potentially relevant articles reporting on pathogen-specific BC rates of antimicrobials for treating non-severe CM in lactating dairy cows. Two reviewers reviewed all titles and abstracts independently. Prior to analyses, categories of antimicrobial treatment protocols, supportive therapy and pathogens were generated. Antimicrobial treatment protocols were grouped using categories defined a priori based on the WHO 5th revision of Critically Important Antimicrobials for Human Medicine and route of administration (systemic and intramammary). A frequentist approach using generalized linear models applied using graph theory was used to develop networks. Five NMAs were carried out according to most commonly reported bacteria causing CM worldwide (*Staphylococcus aureus*, *Escherichia coli*, non-*aureus* staphylococci, environmental streptococci and *Klebsiella* spp.). Potential sources of heterogeneity and inconsistency, including use of supportive therapy, were assessed using sensitivity analysis.

Results: Our search strategy yielded 9,173 records, from which 30 studies were included. Out of 30 studies, 25 were randomized controlled trials. *Staphylococcus aureus* was the most frequently studied pathogen (22 studies), whereas relatively few studies (n = 8) reported on BC of *Klebsiella* spp. There was no evidence supporting the need of CIAs for treating non-severe CM caused by most commonly reported bacteria worldwide; no protocol including the use of CIAs had superior BC rates of non-severe CM than protocols relying on non-CIAs. Additionally, there was no evidence to support use of antimicrobials for treating non-severe CM caused by *Klebsiella* spp. or *E. coli*, as the probability of BC was similar for treated versus untreated cows.

Conclusions: To our knowledge, this is the first systematic review comparing efficacy of CIAs and non-CIAs for treating non-severe CM caused by the 5 most commonly isolated bovine mastitis pathogens worldwide. Antimicrobials other than CIAs were equally effective to treat non-severe CM in dairy cows. In addition, antimicrobial treatments did not alter BC rates of non-severe CM caused by the Gram-negative bacteria *E. coli* and *Klebsiella* spp., although the relatively low number of studies reporting on BC rates of CM caused by *Klebsiella* spp. warrants further investigation. Due to the comparable efficacy of CIAs and non-CIAs and assuming all other variables impacting safety, choice and use of antimicrobials are equal, no adverse effects in terms of animal health and welfare should be expected by ceasing use of CIAs for treating non-severe CM in dairy cattle. Findings from this study will be important to inform public strategies aimed to promote antimicrobial stewardship in veterinary medicine.

Keywords: Antimicrobial resistance, antimicrobial use, critically important antimicrobials, dairy cows, mastitis.

Longitudinal study of fecal commensal Gram-negative bacteria resistant to critically important antimicrobials found in healthy lactating dairy cattle on three farms from Île-de-France

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Objectives: The specific objectives of the project were to evaluate on a convenient sample of three dairy cattle farms the baseline intestinal colonization with commensal gram-negative bacteria resistant to carbapenems and 3rd generation

cephalosporins (3GC) using specific selective media and normalized methods for evaluation of antibiotic resistance.

Materials and methods: Three peri-urban dairy farms were selected because they are included in a regular reproductive follow-up by Alfort Bovine Theriogenology Unit. The study lasted 1 complete year. Basically, 10 healthy producing cows were sampled in each herd on a monthly basis. The samples consisted in rectal swabs, easy to make and to proceed rapidly afterwards in the lab. In addition, the bulk tank filter was sampled monthly in 2 out of 3 herds. The selective used media aimed at selecting for Gram-negative bacteria resistant to 3rd generation cephalosporins and carbapenems. For that purpose, we used 2 commercial media (Carba and Oxa48) (ChromID®, Biomérieux) and one especially prepared media on the basis of scientific literature (MacConkey with 1 mg cefotaxime /L). Isolates were identified through a Maldi-Tof device, and resistance to critically important antibiotics was confirmed via disk diffusion antimicrobial susceptibility testing implemented on Mueller Hinton agar according to EUCAST recommendations.

Results: In farm A, the average monthly percentage of sampled cows harboring bacteria resistant to 3rd generation cephalosporins was over 15% (table 1). This percentage was highest in June at 70%. Among the 16 resistant isolates collected during the year, antimicrobial susceptibility revealed that none was resistant to carbapenem, but all were displaying an AmpC or an ESBL phenotype. AmpC producers all belonged to *Enterobacter* genus; one ESBL producer belonged to the *Pseudomonas* genus, and all others belonged to the *Escherichia* genus. All these ESBL producers were multi-drug resistant (MDR) bacteria according to the classification of Magiorakos *et al.*

In farm B, only one resistant bacterium was isolated on one cow in June; this was a *Pseudomonas aeruginosa* exhibiting an AmpC-producing phenotype. By contrast, the monthly sampling of bulk tank filters revealed the presence and persistence almost entirely throughout the year of AmpC-producing *Pseudomonas aeruginosa* in the milking machine (table 2). All of these isolates were resistant to imipenem and MDR.

In farm C, no bacterium resistant to 3rd generation cephalosporins or carbapenems was isolated in cattle during the year. As in farm B, the monthly analysis of bulk tank filters also revealed the presence and persistence throughout the year of AmpC-producing *Pseudomonas aeruginosa*. Only 20% of these isolates were resistant to imipenem, yet all of them were still considered MDR.

	Farm A	Farm B	Farm C
Total number of phenotypically resistant bacteria	75	21	36
Number of presumptive ESBL and/or AmpC producers (%)	16 (21.3%)	1 (4.8%)	0 (0%)
Number of presumptive ESBL producers (%)	14 (18.7%)	0 (0%)	0 (0%)
Number of presumptive AmpC producers (%)	2 (2.7%)	1 (4.8%)	0 (0%)
Number of MDR bacteria (%)	14 (18.7%)	1 (4.8%)	0 (0%)

Table 1: number of resistant bacteria harbored by sampled healthy dairy cattle in each farm during the year 2018.

	Farm B	Farm C
Total number of phenotypically resistant bacteria	83	117
Number of presumptive ESBL producers (%)	0 (0%)	0 (0%)
Number of presumptive AmpC producers (%)	12 (14.5%)	10 (8.5%)
Number of MDR bacteria (%)	12 (14.5%)	10 (8.5%)

Table 2: number of resistant bacteria isolated in bulk tank filters for farms B and C during the year 2018.

Conclusions: The intestinal colonization baseline of healthy dairy cattle by Gram-negative bacteria resistant to 3GC or carbapenems on a convenient sample seems to vary greatly between farms. By monitoring this resistance for a whole year on a monthly basis, we observe an increase of the proportion of resistant bacteria during summer. These data will be confronted to the antimicrobial consumption data as well as pasture and fertilization conditions in these farms.

The presence in the milking machine of AmpC-producing phenotype *Pseudomonas aeruginosa* in Farms B and C needs to be confirmed and investigated further by determining the support of this resistance. The milking industry would be greatly impacted in terms of public health image should this reservoir be confirmed.

PH-13

Monitoring antimicrobial consumption in the Dairy Sector in Portugal - system structure and results

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Objectives: Monitoring antimicrobial (AM) consumption in the dairy sector is relevant to assess the quantity, quality and reasons for antimicrobials usage.

The presentation describes the Portuguese system for antimicrobial consumption (PSAC) of the dairy sector and presents first results on antimicrobial consumption patterns from three years of farm activity.

The PSAC provides data for the ESVAC system, records and analyse the data to provide benchmark information to farmers and veterinarians, help in establishing the baseline for future target setting at farm level and serves also scientific purposes.

Material & Methods: The PSAC is a conjoint initiative from the Portuguese Veterinary Authority (DGAV), the Portuguese Dairy Farmers Association (ANABLE) and Faculty of Biomedical Sciences Abel Salazar- Porto University (ICBAS-UP).

Antimicrobial consumption (AMC) data used by PSAC is supplied by the BOVNIFOR ® system. This is a WEB base information system that harbours more than 70% of the dairy farms in Portugal: The Continent and Azores Islands. It is operated at national level by the dairy farmers' association AN-ABLE. Farms in PSAC adhere on a voluntary basis and are selected from the database of BOVINFOR ® in order to represent, stratified by region and by size, the Portuguese dairy sector. The objective is to reach 250 dairy farms.

BOVINFOR ® MEDICAMENTOS records all batches of AM purchased by the farms using the system; the registry at the farm, of AM and other medicines prescribed by the veterinarian is compulsory in Portugal. Beside AM consumption, PSAC also collects from BOVINFOR ® data from every animal present at the farm the last day every month, categorized as milking cow, dry cow or by age group otherwise – from calf to heifer; milk production for every milking cow and somatic cell count are also collected.

Data treatment involves the calculation of Defined Daily Doses for animals (DDDvet) and Defined Course Doses (DCDvet) after the ESVAC recommendation, 2016. The consumption of each AM substance per farm and the average weight of animals' is calculated for the entire year after ESVAC, 2016. In the analysis DDDvet or DCDvet are expressed by 100 cow/day or cows/year. Other metrics are also calculated, like DDDvet or DCDvet per ton of milk produced yearly. Analysis is performed for injectable, oral, intramammary and intrauterine formulations. The use of Critically Important (CIA) AM is analysed separately.

Standardized costing of AMC within the herds is performed attributing an average cost per product after yearly consultation of the market. Total quantity of products acquired per year provides basis for calculation.

Descriptive statiscical analysis of the data is performed as well as Principal Component Analysis to look for dominant consumption patterns.

Reporting is available on line, to users, using an interactive web-based dashboard. The dashboard allows for the farms have a benchmark with farms from the same region and size category. Standardized costing of AMC per farm, per cow in milk/year and per ton of milk is calculated and presented in dashboard.

Results: The structure and operation of the PSAC is presented and discussed. Advantages and limitations of treating data from AM purchasing rather than from consumption, as well as the methodology for cost calculation are discussed.

Data from more than 100 farms of AMC and heterogeneity among farms is analysed. The variation in DDDvet or DCDvet reaches fivefold. The profile of AM substances used is wide even when the same prescriber is involved: the farmer seems to play an important role deciding the AM substances used. The use of CIA is analysed and doesn't seem to play important role in higher production of better SCC.

The dashboard will be explored in the presentation and the way forward is discussed.

Conclusion: The PSAC approach involves the relevant stakeholders: veterinary authorities, farmers, farm veterinarians and academia which has so far proven to be a very positive solution.

Data analysis suggests high heterogeneity of AMC among herds offers ample opportunities for reduction and improvement . Analysis of the use CIA raise interesting hypothesis about the relevance of systematic use and deserves investigation in the future.

The use of interactive web-based dashboard is a popular solution for the farnmers and veterinarians involved.

Keywords: ESVAC, Dairy, Antimicrobial Consumption.

PH-14

Evaluation of the longitudinal effect of metaphylaxis treatment of preweaned dairy calves with enrofloxacin or tulathromycin on the susceptibility of antimicrobial resistant fecal *E. coli*

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Objective: The objective of this study was to longitudinally quantify *E. coli* resistant to ciprofloxacin and ceftriaxone in calves treated with enrofloxacin or tulathromycin for the control of bovine respiratory disease. Dairy.

Material and Methods: Calves 2 to 3 weeks old were randomly selected and enrolled in each study group: (1) receiving single label dose of enrofloxacin (ENR)(n=22) ; (2) receiving single label dose of tulathromycin (TUL)(n=23); or (3) serving as a control and not receiving an antimicrobial treatment (CTL)(n=20). Calves were housed in individual hutches and at approximately 60 days of age weaned and housed in group pens. Fecal samples were collected immediately before the administration of the antimicrobial treatment and at days 2, 4, 7, 14, 21, 28, 56, and 112 days after beginning treatment. Samples were used for qualification of *E. coli* using a selective hydrophobic grid membrane filter (HGMF) master grid. Kruskal-Wallis test was used to compare proportion of *E. coli* resistant to ciprofloxacin and proportion of *E. coli* resistant to ceftriaxone by treatment group over time.

Results: ENR had a significantly higher proportion of *E. coli* resistant to ciprofloxacin when compared to the CTL and TUL at time points 2, 4 and 7. At time point 28, a significantly higher proportion of *E. coli* resistant to ciprofloxacin was observed only when compared to CTL. TUL had a significantly higher proportion of *E. coli* resistant to ciprofloxacin when compared to the CTL at time points 2, 4 and 7. Lack of significant difference in shedding of ciprofloxacin resistant *E.coli* in ENR and TUL compared to CTL was because of lower total

CFU/g of feces shedding of ciprofloxacin resistant *E.coli* in these treatment group. None of the treatment groups resulted in a significantly higher proportion of *E. coli* isolates resistant to ceftriaxone.

Conclusion: Our study identified that treatment of calves at high risk of developing BDR with either enrofloxacin or tulathromycin resulted in a consistently higher proportion of ciprofloxacin resistant *E. coli* in fecal samples.

Keywords: ENrofloxacin; prohylaxis; dairy calves; BRD.

PH-15

Effect of heat and pH treatments on degradation of ceftiofur in whole milk

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Objectives: Waste milk (milk that contains drug residues and high somatic cell counts) feeding practices of preweaned dairy calves have been implicated as a potential source for disseminating antimicrobial resistant bacteria among animals and the environment. Two interventions that have shown potential for degrading antimicrobial drugs in milk are heat and pH treatment. The aim of this study was to evaluate the effect of heat and pH treatments on the degradation of ceftiofur and ceftiofur free acid equivalents in milk at concentrations previously found in waste milk on dairy farms by spiking saleable pasteurized whole milk with ceftiofur sodium.

Material and Methods: Three heat treatments of ceftiofur sodium spiked milk were evaluated for their ability to degrade ceftiofur: 63°C for 30 minutes (LTLT), 72°C for 15 seconds (HTST) and 92°C for 20 minutes (HTLT). Two pH treatments of ceftiofur sodium spiked milk were evaluated: pH 4.0 (LpH) and pH 10 (HpH). Control samples spiked with ceftiofur sodium were kept at room temperature and samples collected at corresponding times for heat and pH treatments. Four treatment replicates were performed for each treatment group. Ceftiofur was quantified in milk samples using liquid chromatography mass spectrometry (LC-MS/MS) and ceftiofur free acid equivalents (CFAE) were measured using high-performance liquid chromatography (HPLC).

Results: HTLT resulted in a degradation of 35.24% of the initial concentration of ceftiofur. Ceftiofur degradation did not differ between control and the remaining two heat treatment groups (LTLT and HTST). HpH resulted in degradation of the 95.72 and 96.28% of the initial concentration of ceftiofur and CFAE, respectively.No significant changes in the degradation of ceftiofur or CFAE were observed for control or LpH treatments.

Conclusion: In conclusion, our study results were that alkalinizing milk to pH 10 and heating milk to 92°C for 20 minutes degraded ceftiofur and CFAE in spiked simulated waste milk demonstrated promising potential as treatment options for degrading ceftiofur and CFAE in waste milk, and further research is needed to evaluate the viability for implementation

of these treatments in dairy farms.

Keywords: waste milk; drug residues; antimicrobial resistance.

PH-18

Antimicrobial sensitivities of mastitis pathogens amongst herds with different dry cow therapy usage history

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Objectives: Antimicrobial use in animal production faces increasing scrutiny due to concerns about potential selection for antimicrobial resistance. Intra-mammary dry cow therapy (DCT) is the largest single indication for antimicrobial use on dairy farms in New Zealand (Compton and McDougall 2014). DCT containing the beta lactams cephalonium, cloxacillin or cloxacillin/ampicillin in combination, are the most commonly-used in New Zealand. However, the relationship between the use of cephalonium or ampicillin/cloxacillin-based DCT and the development of antimicrobial resistance has not been examined in New Zealand. The objective of this study was to compare the distribution of Minimum Inhibitory Concentrations (MICs) for common mastitis pathogens from cows in herds exposed to cephalonium or cloxacillin-based DCT, relative to isolates from cows on organic farms where there has been no recent antimicrobial exposure.

Material and Methods: Herds were selected on the basis of not having used any antimicrobial for at least three years (organic; n=7 herds) or having used either cloxacillin/ampicillin dry cow therapy (cloxacillin/ampicillin; n=11 herds) or cephalonium DCT (cephalonium; n=8 herds) as the predominant DCT in the preceding three years.

In each of these herds, quarter-level milk samples (n=793) were collected from all cows (n=984) with an SCC >200,000 cells /mL at the most recent herd test. Cows that had been treated with antibiotics for mastitis in the 30 days prior to sampling were excluded. The MIC of coagulase-negative Staphylococci (CNS), *Staphylococcus aureus*, *Streptococcus uberis* and *Streptococcus dysgalactiae* isolates were determined using a broth microdilution plate which included 10 antimicrobials (Mastitis plate, Trek Diagnostics, CMV1AMAF).

The MIC values were categorised as sensitive or resistant using Clinical and Laboratory Standards Institute (CLSI) categories (where available) and the effect of herd DCT exposure on the MICs within each pathogen/antimicrobial combination was analysed using the nonparametric Kruskal Wallis rank test and by ordinal logistic regression.

Results: For CNS isolates (n=240), 13% and 32% were resistant to ampicillin and to penicillin, respectively. Less than 3% of the CNS isolates were resistant to the other 7 antimicrobials tested (including 0.8% resistant to cephalothin). Isolates from organic herds had a lower risk of being in a higher MIC category for ampicillin (OR=0.14) and penicillin (OR=0.18) than isolates from cephalonium herds. For 29% and 35% of

isolates were resistant to ampicillin or penicillin, respectively. Resistance was detected in 0.3% of *S. aureus* isolates to erythromycin and 1.2% of isolates were resistant to cloxacillin. Isolates from organic herds had lower MICs for tetracycline than those from cephalonium (OR<0.01) herds and had lower MICs for ampicillin and penicillin than for cloxacillin/ampicillin exposed herds (both P<0.05). The MICs for ampicillin and penicillin were lower (P<0.05) in cloxacillin exposed herds than cephalonium exposed herds but were not different between organic and cephalonium herds. For *S. dysgalactiae* (n=50), 2% of isolates were resistant to ceftiofur, 4% were resistant to erythromycin, 2% were resistant penicillin/novobiocin, 2% were resistant to pirlimycin, and 8% were resistant to tetracyclines. There were insufficient isolates of *S. dysgalactiae* to analyse the effect of DCT-exposure on MIC. Amongst *S. uberis* isolates, 1.1% were resistant to erythromycin, 1.6% were resistant to pirlimycin, and 0.5% were resistant to tetracycline. Organic herds had lower MIC's for ampicillin, ceftiofur, cephalothin, penicillin, pirlimycin, sulphadimethoxine, and tetracycline than isolates from cephalonium herds, and similarly organic herd isolates had lower MICs for ampicillin, ceftiofur, cephalothin, penicillin, pirlimycin, and tetracycline than those from cloxacillin/ampicillin herds.

Conclusions: Antimicrobial resistance was detected amongst some bovine mastitis pathogen/antimicrobial combinations. Higher MICs were observed for some pathogen/antimicrobial combinations amongst isolates drawn from herds with a history of exposure to either cloxacillin or cephalonium DCT, compared with isolates from organic herds. It should be noted that these differences in MIC distribution generally occurred at MIC values below clinical cutpoints as defined by CLSI, such that the antimicrobials are likely to remain clinically effective. Bimodal MIC distributions for some antimicrobials within some bacterial species were observed in organic herds suggesting that recent DCT usage is not the only factor affecting MICs. Given the observed findings, further work is required to determine if indeed exposure to DCT is in fact causal of elevated MICs, and whether reduction or removal of DCT from herds alters the MIC of mastitis pathogens from herds.

Keywords: Antimicrobial resistance, dry cow treatment, dairy cow.

PH-19

Antimicrobial resistance of *E. coli* in bulk tank milk from dairy farms in Germany – A 10 year perspective

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Objectives: The objective of this study was to compare current antimicrobial resistance (AMR) in *E. coli* from bulk tank milk in German dairy herds with AMR of *E. coli* from previous years collected in the same sampling framework. While the risk of consumers due to AMR in *E. coli* from bulk tank milk is typically low as most of the milk is heat treated before marketing and consumption, it gives a good indication of the overall resistance situation in the dairy herds and may mirror antimicrobial use.

Material and Methods: The study was carried out in the framework of a national monitoring program on zoonotic bacteria and antimicrobial resistance in the food chain in Germany. Randomly chosen dairy herds (n=471) were included in this study in 2009, 2010, 2014 and 2019. A new random selection was carried out in every year, i.e. different herds were tested over the years. In 2014 an additional sampling frame included organic dairy herds. No. of conventional herds was distributed across the country proportionate to the number of dairy cows in the respective federal state. Data for 2019 are still subject to further validation. Final data will be presented at the conference.

Escherichia coli were isolated from bulk tank milk by regional state laboratories using routine methods. Isolates were submitted to the National Reference Laboratory for Antimicrobial Resistance (NRL-AR) for antimicrobial susceptibility testing (AST) using the broth microdilution method according to CLSI guidelines. For the comparison, minimum inhibitory concentrations were evaluated based on epidemiological cut off values (ECOFF) provided by EUCAST and Commission Implementing Decision 2013/652/EU. Fourteen antimicrobials were included in the testing annually. However, due to a change in the panel of antimicrobials only ten antimicrobials (gentamicin, chloramphenicol, cefotaxime, ceftazidime, ciprofloxacin, nalidixic acid, ampicillin, sulfamethoxazole, trimethoprim and tetracycline) were available for comparison across all years, while some antimicrobials were only included in the first two years (kanamycin, streptomycin and florfenicol) and replaced by others (azithromycin, tigecycline and meropenem) in 2014. Colistin was tested in all years. However, concentrations tested in 2009 and 2010 did not cover the current ECOFF. Therefore results from those years could not be included in the analysis.

Results: Overall, antimicrobial resistance in the tested *E. coli* was low in all years, with more than 80 % of the isolates susceptible to all antimicrobials. The highest proportion (95.9 %) of fully susceptible isolates was observed in isolates from organic farms (n=74) that were tested in 2014. The lowest rate was seen in 2010 with 75.8 % fully susceptible isolates. Overall, highest resistance rates were observed to sulfamethoxazole (10.0 %), tetracycline (6.2 %) and ampicillin (5.9 %), while resistance to azithromycin, tigecycline and meropenem (tested only in 2014 and 2019) was absent. Resistance to third generation cephalosporins (cefotaxime and ceftazidime) was absent in 2009 and in isolates from organic farms that were additionally tested in 2014. Highest resistance rates to these substances were observed in 2019 (6.9 % and 5.7 %) while overall resistance rate was low (1.9 %). Resistance to fluoroquinolones (2.3 %) was highest in 2010 (5.3 %) and was only absent in isolates from organic farms in 2014. Resistance to colistin was observed in one isolate from a conventional farm in 2014, but absent in isolates from organic farms in 2014 and dairy farms in 2019.

Conclusions: Overall results indicate a constantly low rate of AMR in *E. coli* from bulk tank milk in Germany over the years. On the other hand, observed resistance patterns have been including antimicrobials of highest priority to human

medicine indicating room for further improvement.

Keywords: Antimicrobial Resistance, Bulk Tank Milk, Dairy, E. coli.

PH-20

Quantifying, benchmarking and rationalising medicines use in the UK beef industry

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Objectives: There is growing awareness of the threat of antimicrobial resistance (AMR) to animal, human and ecosystem health, therefore livestock industries must demonstrate a clear intent to reduce, replace and refine antimicrobial use (AMU). Individual farmers and veterinarians must also demonstrate responsible, evidence-based medicine use to maintain productivity, animal health and public health, and to ensure consumer confidence in animal welfare and food safety. A whole-system approach to livestock health is required, incorporating measuring and improving on-farm medicines use, which is facilitated by benchmarking between time periods, farms and industry sectors. Various medicines benchmarking metrics are already in use within livestock production (e.g. mg AMs/kg liveweight, defined daily dose (DDD), etc.), with the relevance, applicability and adoptability of each metric depending on the quality and availability of input data. However, within the UK livestock industry, beef producers are uniquely heterogenous, with farms spanning a wide range of cattle breeds, herd sizes, production systems and marketing strategies, which poses significant challenges for medicines benchmarking.

This study analysed on-farm medicine use on beef operations within and across UK beef sectors in order to develop medicines metrics and methodologies for on-farm data collection, recording and benchmarking; and to inform the development of a national electronic medicine data recording hub (eMH) for UK cattle.

Materials and methods: A multimodal approach with industry input at every stage was employed, using farmer questionnaires in conjunction with on-farm medicine and veterinary prescribing records to assess the quality and quantity of medicines data available on UK beef farms and to develop adoptable, appropriate and effective data collection methodologies. Focus groups involving farmers, veterinarians, suitably-qualified persons (SQPs) and beef industry stakeholders were convened, with participatory methodology used to gain insight into opportunities for and barriers to data collection.

Results: Although keeping detailed on-farm medicine records is a legal requirement, no standard approach exists for UK beef operations, with considerable variation in the quality, accessibility and extent of data recorded on farms. Medicines records obtained in this study were shown to be consistent with existing databases. However, they were often confounded by multiple beef operations on a single holding or the co-presence of sheep, pigs or dairy operations. Furthermore, many beef operators did not weigh their cattle and growing cattle moved between operations without medicine records being simultaneously transferred. It was therefore difficult to calculate accurate livestock weight-based metrics (e.g. mg AMs/kg cattle liveweight), dose-related metrics (e.g. DDD) or individual animal metrics (total AM use over the animal's lifetime/kg beef produced). Nevertheless, novel metrics that allowed cattle producers to benchmark and compare medicines use between cattle groups or timepoints (e.g. percent of cattle treated with a specific medicine) and allowed AMU to be quantified and compared were both achievable by, and applicable to, the UK beef industry, providing that the denominator could be accurately determined. Consequently, a standard beef cattle unit (SBCU) was developed, based on characteristic and representative cattle liveweights according to cattle breed (beef or dairy), age, sex and system (intensive or extensive). The most appropriate metrics for AMU benchmarking were determined to be:

- Total AMU (mg/SBCU)
- Total Highest Priority Critically Important Antimicrobial (HPCIA) (mg/SBCU)
- Total non-HPCIA (mg/SBCU)

The most-appropriate metric for benchmarking other medicines use (e.g. vaccines, parasiticides, steroids, etc.) was the percent of the at-risk population treated with the medicine per annum. These novel metrics have since informed national AMU standards for the beef sector and the cattle eMH.

Conclusion: Establishing and adopting on-farm medicines use metrics will encourage farmers and veterinarians to improve animal health and responsible medicines use. However, it is also recognised that there is value in augmenting the core benchmarking metrics recommended in this study with more accurate farm-specific information as well as in moving in the future to recording lifetime health and medicine administration information. In future, electronic cattle identification, data collection apps and the development of an integrated data hub that can include birth dates, movements, live/slaughter weights, laboratory tests, herd health status, medicine administration and treatment outcome would add value for the industry.

Keywords: AMR, Medicine, Beef, Cattle, Metric.

PH-21

An outbreak of Salmonella Typhimurium RDNC in a dairy herd

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Objectives: An advisory visit and a subsequent zoonosis sampling exercise were conducted on a dairy herd following the isolation of Salmonella Typhimurium RDNC in the adult herd. The aim of the advisory visit was to establish a cause for acute milk drop in 80% of the adult herd. When confirmed as being due to Salmonellosis a second visit was carried out to epidemiologically assess the site and establish the likely route of entry for the bacteria into the herd. This visit was also an opportunity to reinforce the zoonotic risk posed by the organism.

Materials and Methods: An acute episode of milk drop was reported over one weekend in a 120 cow dairy herd with 80% of the herd affected. The bulk tank volume dropped by over 50% and the private veterinary surgeon involved requested an advisory vist by an Animal and Plant Health Agency Veterinary Investigation Officer. At the time of this initial visit the cows were observed to be dull, with vague signs of abdominal discomfort and inappetance. Pyrexia had been reported in a small proportion and supportive treatment had been adminstered. Walking the grazing area of the cows, casts of intesinal mucosa were observed with frank blood staining also.

Blood and faecal samples were submitted for assessemt with the primary differential being alimentary tract disease.

Following isolation of Salmonella Typhimurium RDNC from all faecal samples, an advisory zoonosis visit was conducted. This resulted in a detailed overview of the entire farm, its management and a substantial amount of environmental sampling.

Results: The blood results were unremarkable.

The faecal samples collected all yielded Salmonella Typhimurium RDNC.

The environmental samples collected demonstrated widespread contamination of the adult cow and feed areas, with Salmonella Typhimurium RDNC

Whole genome sequencing confirmed the isolate to be one of concern in an ongoing human health issue under investigation by Public Health England.

Conclusion: This was an unusual presentation of Salmonellosis in a group of adult cattle, given the morbidity and acute onset. At no point was disease observed or suspected in other age groups in the herd and it was considered that this was a measure of the robust biosecurity already in place. Though additional means to enhance this were suggested. The cows recovered within a few weeks with moderate losses. This comprised of one abortion, one cow death and three cases of tail tip necrosis. No further losses were reported some three motnhs later and a full return to expected yields was reported. The strain invoved in this case had been the subject of an information leaflet published by APHA in Novemner 2018 following a significant issue of mortlality in sheep flocks and associated human health incidents. The latter had been linked to the consumption of lamb and mutton. One of the flocks involved in this case was near to this dairy farm. At the advisory visit it was established that the outbreak coincided with the opening up of a maize silage clamp, which had been heavily contaminated by wild birds. Sampling of the feed area recovered the Salmonella of interest and it was proosed that the most likely route of entry onto the farm had been wild bird movements from another infected premisies. The provison of the maize silage via a TMR had then enabled large scale innoculation of the adult herd in one event, leading to the uniformity of the clinical signs.

Salmonella Dublin is routinely reported as the predomnant serotype of concern in the bovine currently. As veterinary surgeons we must always be aware that other strains can be just as significant or even more so on an individual farm basis. New and emerging threats such as this with, established human health significance; are timely reminders that we should never become complacent in the control of this bacteria.

Keywords: Zoonosis, Salmonella Typhimurium RDNC, Dairy.

PH-22

There's more work to do: New Zealand Dairy Farmers' understanding of One Health, Antimicrobial Resistance, and the Restricted Veterinary Medicines process

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Objectives: Veterinarians play a key role in managing the threat of antimicrobial resistance in animal and human populations, not only by adopting antimicrobial stewardship in their treatment and prescribing decisions, but also through education of stakeholders. The Restricted Veterinary Medicines (RVM) procedure under the Agricultural Compounds and Veterinary Medicines (ACVM) Act 1997 is one method that veterinarians in New Zealand use to monitor antimicrobial use on dairy farms and provides an opportunity for veterinarians to introduce farmers to the One Health model.

The aim of this study was to generate a pilot set of data on the opinions and understanding of New Zealand Dairy Farmers with regards to 'One Health', 'Antimicrobial Resistance (AMR)' and the 'Restricted Veterinary Medicines' operating plan. Furthermore, it aimed to allow a greater understanding of how these topics impact dairy farmers' lives and to lay the groundwork for further research in this globally important area.

Materials and methods: A questionnaire including 55 questions (40 open questions and 15 closed questions) was distributed to a convenience sample of dairy farm clients of the Massey Farm Services Clinic. The questionnaire was then completed via in-person interviews with the principal researcher. The study period was from September 2021 to November 2021. Results were entered into a database and processed with Microsoft Excel.

Results: 15 out of 16 farms participated in the survey (response rate of 93.75%). The average herd size of this study was 181 cows. Only one farm reported being aware of the term 'One Health' and none of the interviewees could define the discipline. In comparison, 73% of interviewed farms were aware of antimicrobial resistance and could attempt to define it. 53% of clients interviewed were unaware of the existence of the New Zealand Veterinary Association's (NZVA) 'Antimicrobial Traffic Light system' and only 33% considered following the traffic light system when choosing antibiotic treatment. 53% of respondents did not use antibiotics as first line treatment

without seeking veterinary advice, 80% of which said they had decreased their antibiotic use over the past 5 years.

Conclusions: The World Health organization has categorized AMR as one of the top threats facing public health and created the global action plan on AMR in 2015. The NZVA antimicrobial traffic light system dividing antimicrobials into three groups (green, orange, and red) was launched in 2016, encouraging preferential use of green light antibiotics which are less important to human health compared to orange or red rated antibiotics. New Zealand Veterinarians have a key role to play within this plan and by following the NZVA traffic light system and using the RVM process as tools to combat AMR, the New Zealand veterinary industry is in a strong position to keep AMR at the low levels we currently see in large animal veterinary practice. However, ensuring all relevant parties understand the terms that are used to achieve One Health goals is crucial for success and for ensuring buy-in from farmers. This study identified a lack of understanding of the term 'One Health' in farmers which is concerning. Furthermore, in 2021, a 'traffic light system' for the COVID-19 response was launched in New Zealand which could cause confusion of terms, especially when using search engines for example.

Overall, farmers interviewed in this study found the RVM of use to their farm; providing opportunity for clinical veterinarians to communicate the key themes of One Health and Antimicrobial Resistance to receptive clientele. This study has laid the groundwork for future research into farmer opinions of AMR and One Health and allow all those who work in this field to collaborate on this important global health threat.

Keywords: Antimicrobial resistance, One Health, farmer survey, antimicrobial traffic light system.