Department of Production Animal Medicine
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Antimicrobial use, meat inspection findings, and salivary biomarkers as porcine health-related indicators

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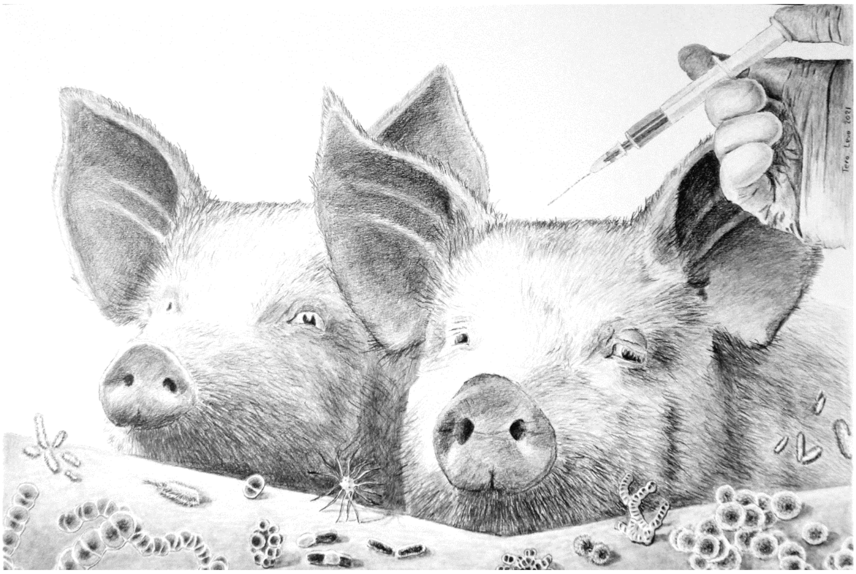

ACADEMIC DISSERTATION

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Without trying to climb, you will never know if a new wonderful world will open up from the hill. This work is dedicated to my husband, my child and to my parents.

ABSTRACT

Ill health in pigs may be difficult to notice. However, infectious and inflammatory diseases do occur in pigs, and are often related to production conditions. As pigs are typically group-housed, infectious diseases can easily spread, and antimicrobials are commonly used to treat these diseases. The use of antimicrobials increases the development of antimicrobial-resistant bacterial strains, and threatens the efficacy of currently available antimicrobials. Therefore, it is necessary to lower the need to use antimicrobials in pigs. This could be achieved by investigating methods to improve porcine health, as well as methods that allow the detection of diseased pigs as early and as easily as possible.

This dissertation is built upon three separate studies (I–III). In Study I, antimicrobial use (AMU) on nine commercial Finnish piglet-producing and fifteen finishing farms was investigated. Antimicrobial treatments of all age groups present on these farms during one year were obtained from the health classification register for pig farms in Finland (Sikava). We also gathered data on yearly meat inspection (MI) findings for sows and finisher pigs from Sikava and compared the prevalence of MI findings with overall AMU (as mg/PCU) and the treatment incidence (TI) of different disease categories in sows and finishing pigs. Furthermore, antimicrobial susceptibility testing of the indicator *Escherichia coli* (*E. coli*) bacteria isolated from pooled fecal samples, collected at the pen level from one unit on each farm, was conducted. Greater overall AMU (as mg/PCU) on piglet-producing farms compared to that on finishing farms, and the highest TI for piglets compared with sows and finishing pigs was expected. Positive relationships between AMU and MI findings on both farm types was hypothesized. Since more antimicrobials were expected to be used on piglet-producing farms, the prevalence of antimicrobial-resistant *E. coli* indicator bacteria was assumed to be higher on piglet-producing farms than on finishing farms.

As expected, large between-farm variation was observed regarding all the parameters studied, and overall AMU (as mg/PCU) was higher on piglet-producing farms compared to finishing farms. When quantified as TI and in percentage terms, suckling piglets were most frequently treated with antimicrobials, followed by sows, weaned piglets, and finishing pigs. The surprisingly high AMU in sows warrants further investigation. Altogether, seven antimicrobial groups were used on the study farms, and penicillin was by far the most used antimicrobial, regardless of the age group. Antimicrobials belonging to the World Health Organization list of critically important antimicrobials for human medicine (CIA) were also used, mainly consisting of amoxicillin and lesser amounts of fluoroquinolones, tylosin, and long-acting tulathromycin. The majority of pigs were individually treated with injectable antimicrobials, whereas oral group treatments were administered only

occasionally. Pleuritis was the most common MI finding recorded at slaughter, but was more prevalent in finishing pigs than in sows. Notably, the majority of the relationships between AMU and the prevalence of MI findings were negative, indicating that the greater the AMU, the fewer the MI findings. The occurrence of AMR on both farm types was similar. In general, *E. coli* indicator bacteria were susceptible to most of the fourteen tested antimicrobials, and primarily showed phenotypic resistance against tetracycline, sulfamethoxazole, trimethoprim, and ampicillin. The result is in accordance with national monitoring results.

Novel methods for pig health assessment include the determination of inflammatory and immune biomarkers from porcine saliva samples. Saliva sampling is less stressful for pigs compared to blood sampling, is easy to learn, and is applicable to a large number of animals, making it cost-effective. In pigs, among the proposed salivary biomarkers are acute phase protein haptoglobin (Hp) and adenosine deaminase (ADA), which is a marker of the activation of cell-mediated adaptive immunity. The quantification of Hp and ADA in saliva is more sensitive than their quantification in serum.

Study II was an experimental study conducted on one experimental farm. The study objective was to validate whether salivary Hp and ADA are appropriate inflammatory biomarkers using a lipopolysaccharide (LPS) model. Sixteen healthy female growing pigs were randomly allocated to two groups that intravenously received either saline only or saline accompanied by LPS. Repeated saliva samples were collected from these pigs prior to injections, and four times during a 72-hour period after injections. As expected, the concentrations of Hp and ADA increased as a result of LPS administration, being indicative of an acute phase reaction (APR). The dynamics of both biomarkers followed a similar course, with a significant peak measured at four hours post-injection and then a rapid return close to the baseline in the LPS group between four and 24 hours post-injection. Moreover, there was a significant correlation between Hp and ADA responses at four hours post-injection in the LPS group. Study II confirmed that the studied biomarkers are indicative of systemic acute phase immune activation under experimental conditions in growing pigs.

Some evidence suggests that salivary Hp and ADA concentrations in pigs are dependent on the production stage as well as the gender. However, longitudinal studies conducted under field conditions are scarce. Furthermore, the combination of several biomarkers may improve the diagnostic sensitivity. The objective in Study III, was therefore to investigate the dynamics of salivary Hp, ADA, and a biomarker of adaptive humoral immunity, immunoglobulin G (IgG), during a complete production cycle of commercial finishing pigs. Four Finnish piglet-producing farms and their respective four finishing farms were included in the study. Individual study pigs, including females and males, were selected at birth (n = 163) and followed until slaughter (n = 83). Saliva samples were collected four times, including the suckling, beginning and end of growing, and finishing stages.

Finally, individual MI findings were obtained from the slaughterhouse, and the associations of biomarker concentrations in finishing pigs and MI findings were examined. Salivary concentrations of all the studied biomarkers differed significantly between production stages, always being highest in suckling piglets compared to later production stages. Some gender differences were found, with males more often having a numerically higher salivary biomarker concentration compared to females. Positive and significant relationships between the studied salivary biomarkers were found in other production stages apart from suckling piglets. The results indicate, and further confirm previous findings, that salivary biomarkers could be utilized for analytical purposes in pig disease diagnostics. No association was found between biomarker concentrations and MI findings, which could be related to the time that had elapsed between saliva sampling and slaughter, as well as to most of the reported MI findings being chronic.

In summary, between-farm variation in AMU is considerable, but on the majority of the participating farms, non-CIA antimicrobials were predominantly used, and antimicrobial treatments were used to treat individual pigs instead of groups. The good phenotypic bacterial resistance situation is probably the result of the rational AMU, which Finland has been working on for decades. Farm-level AMU that takes into account not only the amount but also treatment indications could be utilized in pig health care in the future. However, in order to evaluate the need to use antimicrobials in pigs, porcine health must be comprehensively monitored. Salivary biomarkers appear to be suitable for porcine health monitoring in farm circumstances, and especially in the detection of acute illness. However, they are not applicable for the selection of individual pigs to be treated or not due to their non-specificity. Moreover, from a practical point of view, it is not advisable to sample suckling piglets, and more knowledge about the normal variation in salivary biomarker concentrations is needed before their practical use in older pigs.

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The three studies on which this dissertation is built were carried out on Finnish pig farms (II, III) and at the Norwegian University of Life Sciences (NMBU) (I). I am grateful for the previous cooperation with the personnel of Finnish A-Producers Ltd involved in the Antibiotic-Free Pork project, especially those who actively recruited the farms and assisted with data collection.

I am grateful for all the grants that made it possible to carry out this research, from the Ministry of Agriculture and Forestry (Makera), the Finnish Veterinary Foundation (ETTS), the Finnish Foundation of Veterinary Research (SELS), and the Doctoral Programme in Clinical Veterinary Medicine (DPCVM). Moreover, a grant received from the European Cooperation in Science and Technology (COST) enabled fruitful collaboration with NMBU.

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Helsinki, 31.10.2023

Virpi Piirainen

LIST OF ABBREVIATIONS

ADA	Adenosine deaminase
AMU	Antimicrobial use
AMR	Antimicrobial resistance
APP	Acute phase protein
APR	Acute phase reaction
AS	Antimicrobial active substance
CIA	Critically important antimicrobial for human medicine
DDD	Defined daily dose
Hp	Haptoglobin
Ig	Immunoglobulin
IgG	Immunoglobulin G
LPS	Lipopolysaccharide
MI	Meat inspection
PCU	Population correction unit
Sikava	The health classification register for pig farms in Finland
TI	Treatment incidence

CONTENTS

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following publications, which are referred to in the text with their Roman numerals (I–III). The original publications are reprinted with the permission of the publishers.

I Sali, V., Nykäsenoja, S., Heikinheimo, A., Hälli, O., Tirkkonen, T. & Heinonen, M. (2021). Antimicrobial use and susceptibility of indicator *Escherichia coli* in Finnish integrated pork production. Front. Microbiol. 12:754894. doi: 10.3389/fmicb.2021.754894.

II Sali, V., Veit, C., Valros, A., Junnikkala, S., Heinonen, M. & Nordgreen, J. (2021). Dynamics of salivary adenosine deaminase, haptoglobin, and cortisol in lipopolysaccharide-challenged growing pigs. Front. Vet. Sci. 8:698628. doi: 10.3389/fvets.2021.698628.

III Piirainen, V., Gutiérrez, A. M., Heinonen, M., König, E., Valros, A. & Junnikkala, S. (2023). Concentrations of porcine salivary and serum haptoglobin, adenosine deaminase, and immunoglobulin G through four production stages, and associations with meat inspection findings. Porc. Health Manag. (submitted)

1 INTRODUCTION

In pigs, infectious and inflammatory conditions are common, but detecting signs of disease in pigs can be challenging, especially among group-housed pigs (Miller et al., 2019). The occurrence of many pig diseases is dependent on the production stage and influenced by the prevailing pathogen load in the environment, as well as the housing and management factors (Zimmerman et al., 2019). Antimicrobials are commonly used in pigs (EMA, 2022), and their use related to the development and occurrence of antimicrobial resistance (AMR) (Chantziaras et al., 2014; Ceccarelli et al., 2020; ECDC, EFSA & EMA, 2021), which threatens the efficacy of currently available antimicrobials (WHO, 2012) not only in animals but also in humans. Better understanding of antimicrobial use (AMU), including the collection of both qualitative and quantitative data, will help in developing strategies to reduce the need for AMU in animals and thus slow down the development of AMR in bacteria (WHO, 2015; WHO, FAO & OIE, 2019).

Pork is the most common meat produced (Luke, 2023a) and consumed (Lihatiedotus, 2021) in Finland. To date, Finnish pig farming has been characterized by the good national situation concerning infectious diseases (Finnish Food Authority, 2022c), the low consumption of antimicrobials (EMA, 2022), and the low occurrence of AMR among indicator and pathogenic bacteria (Finnish Food Authority, 2022a). Finland has been developing the health classification register for pig farms, called Sikava, for more than two decades (Sikava, 2021). Sikava is a health monitoring tool, which contains information on, for example, medicine use, farm health care plans, as well as on meat inspection (MI) findings at slaughter (Sikava, 2021). Joining Sikava is subject to certain health requirements for pigs (Sikava, 2021), and currently it covers more than 95% of Finnish pig farms.

The number of Finnish pig farms has been decreasing for several years, with a parallel increase in farm size (Luke, 2023b). Along with this development, the proper identification of pigs having compromised health and possibly in need of medical treatment is important in order to prevent disease transmission, and to choose the appropriate treatment that could reduce unnecessary AMU (Zimmerman et al., 2019). Both qualitative and quantitative data on AMU at the farm level could be used as an indicator of the prevailing diseases (Jensen et al., 2012), but AMU alone it is not a sufficient indicator of whether antimicrobials have been used prudently. Other potential tools for porcine health assessment include MI findings (Stärk et al., 2014; Valros et al. 2020; Hernandez et al., 2023) and the measurement of salivary inflammatory and immune biomarkers (Gutiérrez et al., 2009a; Gutiérrez et al., 2012a; Sánchez et al., 2021; Cerón et al., 2022).

Serum acute phase proteins (APPs) are sensitive and non-specific biomarkers that have been utilized in clinical veterinary practice since the mid-1990s (Pradeep, M., 2014). However, many biomarkers are measurable in pig saliva (Cerón et al., 2022), which has even better sensitivity compared to serum in detecting some diseases in pigs (Sánchez et al., 2021). A combination of APPs and their combination with other biomarkers such as adenosine deaminase (ADA) can improve diagnostic sensitivity (Sánchez et al., 2021). Saliva sampling is considered as less stressful to pigs compared to blood sampling and is cost-effective when implemented at the farm level (Kaufman & Lamster, 2002; Goecke et al., 2020). Several inflammatory and immune biomarkers are dependent on the age (Curtis & Bourne, 1971; Piñeiro et al., 2009b; Sánchez et al., 2019; Ortín-Bustillo et al., 2022), and potentially on the sex (Gutiérrez et al., 2018; Sánchez et al., 2019; Ortín-Bustillo et al., 2022) of a pig. In pigs, the potential biomarkers measured in saliva include haptoglobin (Hp) (Gutiérrez et al., 2009c; Sánchez et al., 2021) ADA (Gutiérrez et al., 2017) and immunoglobulins (Igs) (Escribano et al., 2012).

Experimental studies conducted in well-controlled settings can be utilized in the validation of analytical methods. Lipopolysaccharide (LPS) is an antigenic structure of the cell wall of Gram-negative bacteria that can be used to induce inflammation experimentally (Seemann et al., 2017).

Thus, LPS can be used to examine the activation of the immune system (Seemann et al., 2017), and particularly the acute phase reaction (APR). Furthermore, knowledge derived from experimental studies can be extrapolated to field conditions. To improve porcine health management in the future, as well as to reduce the need for AMU and combat emerging AMR, it is important to learn how to use analytical methods in combination with other health indicators at the farm level.

2 REVIEW OF THE LITERATURE

In the following sections, options for assessing the health of pigs that could be more extensively used in future health care work are discussed. In addition, general features of pig husbandry and the development of the porcine immune system are presented.

2.1 INTRODUCTION TO PORK PRODUCTION

Pork is the most common meat produced (Luke, 2023a) and consumed (Lihatiedotus, 2021) in Finland, although at the European level, Finland is a small pork-producing country with a production volume of around 0.8% (European Commission, 2023). In 2021, there was 1. 1 million living pigs in Finland (Eurostat, 2023), and the volume of pork produced was 176 000 tons (Luke, 2023d). In Europe (European Union, 2020), including Finland (Luke, 2023b; Figure 1), the number of pig farms has generally been decreasing, accompanied by an increase in farm size.

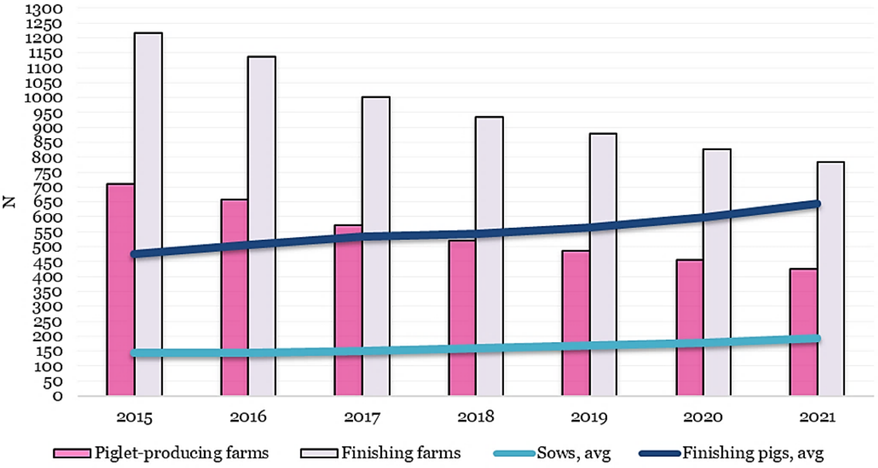

Figure 1 Changes in the number (N) of pig farms (columns) and average (avg) farm size (lines) in Finland between 2015 and 2021. On piglet-producing farms, finishing pigs can also be kept. Reproduced from the database of Natural Resources Institute Finland (Luke, 2023b).

In 2021, there were 864 pig farms in Finland, and the average size of piglet-producing farms was 190 sows, while that of finishing pig farms was 643 finishers (Luke, 2023b). Geographically, most pig farms are located in two major areas in southwestern and western Finland, where nearly half of all pork is produced (Luke, 2023a; 2023c). The Finnish pork industry is led by three slaughterhouses, where more than 99% of all pigs are slaughtered (Finnish Food Authority, 2022a). The slaughterhouses have collaborated to establish and finance a health classification register for pig farms in Finland called Sikava, which has replaced the slaughterhouses' own health registers since 2003 (Sikava, 2021).

Sikava has three levels which are based on the farm health status (Sikava, 2021). All farms start at the basic level, and to reach the national level, the herd health veterinarian must sign a document saying that the herd is clinically free from atrophic rhinitis, enzootic pneumonia, sarcoptic mange and swine dysentery (Sikava, 2021). In addition, *Salmonella* freedom (Sikava, 2021) needs to be shown with feces samples (ETT, 2021). After joining Sikava, the freedom of other diseases except from *Salmonella* is checked by visually inspecting the symptoms of these diseases. Freedom of *Salmonella* is proven by *Salmonella* –samples taken according to the salmonella sampling program (ETT, 2021). Besides, biosecurity status of farms at the national level must be evaluated once a year (Sikava, 2021). At the special level, farms must fulfil certain health and biosecurity criteria, and those farms are primarily breeding farms (Sikava, 2021). The farms that have registered in Sikava need to belong to at least national level in order to be able to sell pigs to the slaughterhouse company. Thus, slaughterhouses have a considerable impact on how pigs are kept.

Joining Sikava requires a health care agreement with a veterinarian, who makes a health plan and follows the disease status on farms through regular visits (Sikava, 2021). The health plan includes planning and providing advice on medicine use for pigs, and accordingly, farmers must record their medicine use bookkeeping in the electronic Sikava system (Sikava, 2021). Besides medicine use data, Sikava contains information on farm biosecurity, meat inspection findings, health care plans, documentation of regular health care visits, laboratory results, and welfare index. All Sikava information can be utilized in herd health monitoring (Sikava, 2021). Designated farm veterinarians and slaughterhouse personnel have access to the Sikava data of their customer farms, which allows systematic monitoring of the health situation on the pig farms (Sikava, 2021). In case the farm does not follow Sikava rules, its level can be lowered (Sikava, 2021). Currently, around 95% of Finnish pig farms have joined Sikava, accounting for about 97% of pork production (Sikava, 2021).

The national legislation provides a framework for pig management and housing, including space requirements and the provision of continuous enrichment material to pigs (Decree of the Ministry of Agriculture and Forestry 629/2012). On most farms, pigs have more space than required by the legislation, allowing them to be reared without docking their tails, which is prohibited by law (Animal Welfare Act 247/1996 and Animal Welfare Decree 396/1996). The majority of sows farrow in crates, and pregnant sows are generally group-housed for around two-thirds of the gestation period (Finnish Animal Welfare Centre (EHK), 2021). Most sows are routinely vaccinated against parvovirus and *Erysipelothrix rhusiopathiae*, and commonly vaccinated against *Escherichia coli* (*E. coli*) to protect the newborn piglets against colibacillosis (Finnish Food Authority, 2022b). Cross-fostering of piglets is a common practice. Male piglets are routinely surgically castrated within the first week of their life (Animal Welfare Decree 396/1996), and the castration pain is alleviated with systemic non-steroidal anti-inflammatory drugs on the majority of farms. Piglets cannot be weaned before 28 days of age without a special reason (Decree of the Ministry of Agriculture and Forestry, 629/2012). Piglet vaccination against porcine circovirus 2 before weaning is highly recommended (Animal Health ETT, 2018; Pohjola & Jääskeläinen, 2019).

Finland's successful strategy to preserve a good animal health situation and to reduce the need for antimicrobial treatments in animals has for decades been based on the implementation of eradication programs and control measures for infectious animal diseases, and the development of health programs for food-producing animals (Finnish Food Authority, 2022a). Moreover, the Finnish Food Authority complies annual summary reports of Animal Diseases in Finland (Finnish Food Authority, 2022c), and runs the mandatory surveillance program for veterinary antimicrobial consumption and the occurrence of resistance in bacteria isolated from food-producing animals (Finnish Food Authority, 2022a), in accordance with European Commission Directive 2003/99/EC (European Commission, 2003). Finland has an official disease-free status regarding Aujeszky disease, brucellosis, and classical swine fever, and additional guarantees regarding *Salmonella* (Finnish Food Authority, 2022c). Transmissible gastroenteritis has been detected in Finland last time in 1980, and belongs to the surveillance programme of animal diseases in Finland (Finnish Food Authority, 2022c). Moreover, African swine fever and porcine reproductive and respiratory syndrome (PRRS) have never been diagnosed in Finland (Finnish Food Authority, 2022c).

2.2 PORCINE IMMUNITY

The following sections outline general aspects of porcine immune development and innate and adaptive immunity, and introduce ways to examine the functioning of the immune system.

2.2.1 DEVELOPMENT OF IMMUNITY

The porcine immune system structurally develops until around five weeks of age (Sinkora & Butler, 2009). Hematopoietic activity in the bone marrow starts between 60 and 90 days of gestation, resulting in a rapid increase in the number of T and B lymphocytes in the blood circulation. Immune system development continues structurally and functionally to post-natal life and is influenced by antigen exposure from the environment and the ingestion of immune components from sow colostrum and milk (Sinkora & Butler, 2009). The development of the immune system gradually continues until puberty (Zimmerman et al., 2019).

2.2.2 INNATE IMMUNITY

The activation of innate immunity is immediate and non-specific (Murata et al., 2004; Sauce, L., 2018). It is characterized by two interrelated mechanisms, the first being the recruitment and activation of cellular components (neutrophils, macrophages, NK cells, and dendritic cells), which is accompanied by the release of signaling molecules (e.g., cytokines, chemokines, complement proteins, and antimicrobial peptides) (Sinkora & Butler, 2009). Infection (Murata et al., 2004; Gómez-Laguna et al., 2010), trauma (Murata et al., 2004), and a variety of endo- and exogeneous factors, including stress (Murata et al., 2004; Piñeiro et al., 2007a; Piñeiro et al., 2007b), activate sensory cells such as macrophages (Murata et al., 2004). This results in the release of pro-inflammatory cytokines, which trigger an acute phase reaction (APR) (Murata et al., 2004; Nordgreen et al., 2018). Consequently, the production of acute phase proteins (APPs) increases (Schrödl et al., 2016; Nordgreen et al., 2018), which predominantly occurs in the liver (Schrödl et al., 2016). The amount of APPs produced is related to the severity of the trigger during the threatening occasions, (Murata et al., 2004), but are continuously produced at least in the liver, which is considered as basal protective secretion (Schrödl et al., 2016). Some authors have also suggested local protection provided by APP production in extrahepatic tissues (Murata et al., 2004; Gómez-Laguna et al., 2010; Schrödl et al., 2016), which has been demonstrated in pigs (Skovgaard et al., 2009; Gutiérrez et al., 2012b). Systemic and local APP production may act together during inflammation, and extrahepatic production is considered particularly important in the case of focal injuries (Schrödl et al., 2016).

2.2.3 ADAPTIVE IMMUNITY

Adaptive immunity is functionally characterized by its memory, and the important counterparts for the adaptive immune system function are T lymphocytes and antibodies produced by B lymphocytes (Zimmerman et al., 2019). There is a temporal difference in the functioning of innate and adaptive immunity, although they overlap in some respects (Zimmerman et al., 2019). The former is activated within hours (Sauce, L., 2018; Zimmerman et al., 2019) or even minutes (Sauce, L., 2018) up to some days, and the latter within days to a few weeks (Sauce, L., 2018; Zimmerman et al., 2019).

Newborn piglets are considered to be immunocompetent, especially regarding innate immunity, and while they are at some level capable of cell-mediated adaptive responses, the adaptive humoral immunity is almost undeveloped due to the epitheliochorial sow placenta (Sinkora & Butler, 2009; Sauce, L., 2018). The placenta is impermeable to maternal immunoglobulins (Igs) and large molecules such as lymphocytes, which are instead secreted in the colostrum (Sauce, L., 2018). Thus, colostrum ingestion is crucial for piglet survival (Devillers et al., 2011; Decaluwé et al., 2014; Ferrari et al., 2014; Hasan et al., 2019) and health (Devillers et al., 2011; Ferrari et al., 2014; Hasan et al., 2019).

The absorption of Igs through the piglet intestine to the blood circulation takes place within 24–36 hours after birth (Sauce, L., 2018), and the Ig concentration of colostrum is highest within six hours after the birth of the first piglet (Decaluwe et al., 2014; Hasan et al., 2016). Furthermore, the lymphocytes in colostrum can only be absorbed if they originate from the colostrum of the piglet's biological dam (Tuboly et al., 1988). Depending on the amount of maternal immunoglobulins obtained from sow colostrum and milk, neonatal piglet is capable of some Ig production during the first weeks of their lives, and become gradually fully capable of endogenous Ig production at around four to five weeks of age (Zimmerman et al., 2019). Until then, IgG and immunoglobulin A (IgA) in sow milk offer the main protection to the piglet systemically and locally, respectively (Sauce, L., 2018).

2.2.4 EXAMINING IMMUNE SYSTEM FUNCTION

Lipopolysaccharide (LPS) is an antigenic structure of the cell wall in Gram-negative bacteria (Raez CR & Whitfield C., 2001). The activation of innate immunity could be assessed with LPS, as it induces a positive APR (Escribano et al., 2014; Seemann et al., 2017; Nordgreen et al., 2018; Veit et al., 2021), thus being applicable in systemic inflammation models (Seemann et al., 2017). In previous pig studies, variable doses of LPS (Dritz et al., 1996 (i.m.); Nordgreen et al., 2018 (i.v.); Veit et al., 2021 (i.v.), and including different *E. coli* serotypes (Dritz et al., 1996; Nordgreen et al., 2018), have been used in pig studies to examine APR. However, in systemic inflammatory models small doses of LPS have been shown to be sufficient to induce APR (Nordgreen et al., 2018), and sickness behaviour in pigs, including decreased general activity (Veit et al., 2021) and feed intake (Dritz et al., 1996; Veit et al., 2021) and changes in the lying behaviour (Veit et al., 2021).

In pigs, C-reactive protein (CRP), haptoglobin (Hp), Pig-MAP (Heegaard et al., 2011; Saco & Bassols, 2022) and serum amyloid A (SAA) (Saco & Bassols, 2022) are important positive APPs (Heegaard et al., 2011), meaning that their plasma concentration increases during an APR (Murata et al., 2004; Heegaard et al., 2011; Schrödl et al., 2016). Acute phase proteins differ in their biological functions, CRP being primarily involved in phagocytosis of bacteria, and Hp having a bacteriostatic effect as it binds free iron available for bacteria (Di Filippo et al., 2020). Pig-MAP is a pig-specific APP (Gonzalez-Ramón et al., 1995; Alava et al., 1997), with unknown exact function, but it probably has anti-inflammatory properties (Choi-Miura et al., 2000). Serum amyloid A has both chemotactic effect and suggested to be involved in chronic inflammatory diseases (Di Filippo et al., 2020). Thus, SAA has been investigated in inflammatory pig studies less than CRP, Hp or Pig-MAP.

Contrary to APPs, Igs are essential biomarkers of the development and functioning of adaptive humoral immunity (Salmon et al., 2009). IgG is a biomarker of systemic immune activation (Šinkora & Butler, 2009) and plays a central role in protecting pigs against many important infectious agents (Salmon et al., 2009). In pigs, both IgG and IgA in saliva have been validated as biomarkers for evaluating the humoral immune status in pigs (Escribano et al., 2012). Moreover, LPS could induce a local APR and humoral immune response, which is evaluable by measuring the Ig concentration in porcine saliva (Escribano et al., 2014).

One of the promising immune biomarkers, with an increasing number of studies conducted in pigs (Gutiérrez et al., 2017, Contreras-Aguilar et al., 2020; Sánchez et al., 2021), is adenosine deaminase (ADA). This is an enzyme encoded by the ADA gene and is functionally involved in the elimination of the lymphotoxic molecule deoxyadenosine, which is produced by the breakdown of DNA (National Library of Medicine, 2013). ADA catalyzes the reaction in which deoxyadenosine is converted to a non-harmful molecule, deoxyinosine. (National Library of Medicine, 2013; Bradford et al., 2017). The highest levels of ADA are expressed in lymphocytes (National Library of Medicine, 2013; Bradford et al., 2017). In human patients, the ADA activity in pleural exudate has been demonstrated to correlate with T-lymphocyte populations but not with B-lymphocyte populations, which suggests the possibility of a role in cell-mediated adaptive immunity (Baganha et al., 1990). The ADA molecular pathway is highly conserved in mammals (Bradford et al., 2017), and data from humans has therefore been extrapolated to animal studies, including pigs. In pigs, three isoenzymes of ADA have been characterized, namely ADA0, ADA1, and ADA2 (Widar J. & Ansay M., 1975), and the latter two have been investigated further in pig studies (Gutiérrez et al., 2011; Gutiérrez et al., 2017; Contreras-Aguilar et al., 2019).

2.3 PIG HEALTH AND RELATED INDICATORS

The monitoring of health and disease, and related production indicators such as pig growth and meat inspection (MI) findings are useful tools for successful health management in pig farming. In pigs, the clinical signs of the disease may be mild and indirect (Miller et al., 2019), which challenges the early detection of disease, especially in group-housed pigs. Furthermore, the occurrence of sub-clinical diseases allows the spread and persistence of diseases within and between pig farms. Thus, investment in health monitoring and disease preventive measures (Dewulf J. & Van Immerseeel F., 2018; Raasch et al., 2020) in a risk-based manner and considering all stages of production is of importance (Dewulf J. & Van Immerseeel F., 2018). Knowledge of the prevailing diseases is needed to develop disease preventive measures, as well as effective treatment strategies, which can lead to a lower need to use antimicrobials in pigs (see, for example, Fertner et al., 2015; Raasch et al., 2020). If uncontrolled, diseases may lead to an increase in antimicrobial use (AMU), which exacerbates both antimicrobial resistance (AMR) development and spread (WHO, 2012), although the relationships between AMU and AMR are multifactorial (Prestinaci et al., 2015). Factors to be considered are not only the antimicrobial compounds used but also the treatment indications (Jensen et al., 2012), accuracy of dosing, treatment frequency, and duration of antimicrobial treatment courses (Timmerman et al., 2006), as well as the history of AMU (Andersson & Hughes, 2011). In the following chapters, the potential to use biomarkers, AMU, and MI findings in pig health assessment is reviewed.

2.3.1 TARGETED HEALTH MANAGEMENT

The identification of animals in need of extra care and those in need of medical treatment forms the starting point for targeted health management. In general, pregnant sows (Hansen, P., 2011; Mor et al., 2017), and newborn piglets are immunologically the most sensitive groups (Šinkora & Butler, 2009). Pig mortality is highest during the suckling period (Calderón Díaz et al., 2017). With a greater litter size, there is an increased risk of lower piglet birth weight (Calderón Díaz et al., 2017), lower intake of colostrum immunoglobulins (Cabrera et al., 2012), and increased risk of likelihood of becoming sick as well as increased risk of pig mortality in the long term (Cabrera et al., 2012; Calderón Díaz et al., 2017). The moving and re-grouping of pigs is very stressful for the animals. Stress weakens the immune system of pigs, so in a grouping situation, the risk of spreading infectious diseases is present. Investment in the conditions of sows during gestation and of sows and piglets during lactation is beneficial for both sow longevity and, in the long term, for piglet well-being and health.

Analytical saliva samples could be used in the screening of pathogens (Goecke et al., 2020; Henao Diaz et al., 2020), in detecting sub-clinical disease (Gutierrez et al., 2012a; Cerón, J., 2019), and, accompanied with the veterinary clinical examination, in disease diagnosis, as well as the monitoring of recovery (Cerón, J., 2019). This will lead to more efficient disease detection and also allow early intervention. Saliva sampling offers a minimally invasive and less stressful alternative to blood sampling (Kaufman & Lamster, 2002). Additionally, saliva collection is quick, suitable for repeatable measurements, and applicable at both the group level (Goecke et al., 2020) and individual level (Gutiérrez et al., 2009a). Saliva collection is easy to learn (Kaufman & Lamster, 2002), allowing it to be implemented by farm employees and also making it cost-effective. The salivary concentration of many biomarkers is usually lower than in serum (Gutiérrez et al., 2009a; Cerón et al., 2022), but currently validated sensitive assays are available for porcine Hp (Gutiérrez et al., 2009b), ADA (Gutiérrez et al., 2017), and IgG (Escribano et al. 2012).

2.3.2 APPLICATION OF BIOMARKERS

According to the European Medicines Agency (2021), *a biomarker is a biological molecule found in blood, other body fluids, or tissues that can be used to follow body processes and diseases in humans and animals*.

Since the 1990s, APPs have been applied in veterinary diagnostics (Schrödl et al., 2016). As a biomarker, APPs are sensitive but non-specific (Schrödl et al., 2016), and the APR is usually more prominent in the case of a non-infectious challenge than an infectious one (Murata et al., 2004; Heegaard et al., 2011, Shrödl et al., 2016; Cerón, J., 2019). Furthermore, viruses and bacteria can trigger APRs differing in speed (Heegaard et al., 2011) and magnitude (Heegaard et al., 2011; Shrödl et al., 2016). Viruses alone have been reported to result in less prominent and delayed APR in comparison to bacteria (Heegaard et al. 2011). Moreover, pig studies under field conditions have demonstrated that in the case of natural infections, the APR is stronger in animals having clinical signs (Petersen et al. 2002; Saco & Bassols, 2022) and infected with multiple pathogens than in sub-clinically infected animals or those with infections caused by a single pathogen (Saco & Bassols, 2022). APP concentrations in both serum and saliva have been shown to correlate positively with the severity of clinical signs (Gómez-Laguna et al., 2010). However, being very sensitive, APPs respond to an inflammatory stimulus before clinical signs appear, which supports the use of APPs as a marker of subclinical conditions (Eckersall, P., 2000; Gutiérrez et al., 2012; Pomorska-Mól et al., 2014; Cerón et al., 2022). Moreover, measurement of APPs at the slaughterhouse would be useful to evaluate the health status of pigs at farm level (Van den Berg et al., 2007; Saco et al., 2011; Pomorska-Mól et al., 2014; Gutiérrez et al., 2015a; 2015b)

A combination of APPs (Heegaard et al., 2011) or the combination of APPs with other health biomarkers (Sánchez et al., 2021) has been suggested to improve the health evaluation of pigs. In pig studies, elevated salivary ADA concentrations have been reported in the case of lameness (Gutiérrez et al., 2017; Tecles et al., 2018; Contreras-Aguilar et al., 2020), respiratory disease (Gutiérrez et al., 2017), gastrointestinal disease (Gutiérrez et al., 2017), and growth retardation (Gutiérrez et al., 2017), being significantly higher compared to clinically healthy pigs (Gutiérrez et al., 2017; Sánchez et al., 2021). As Igs are biomarkers of the activation of humoral adaptive immunity (Šinkora & Butler, 2009), especially when the antigen is infectious (Salmon et al., 2009), it could be expected that the combination of IgG and ADA would provide better information on the health status of pigs, but no scientific literature is available on this topic. However, there is no current evidence that the interpretation of analytical saliva samples requires consideration of at least the production stage (Gutiérrez et al., 2012; Sánchez et al., 2019; Ortín-Bustillo et al., 2022) and gender (Sánchez et al., 2019; Ortín-Bustillo et al., 2022) of pigs.

2.3.3 USE OF SLAUGHTERHOUSE DATA

In the EU, meat inspection must be according to the regulation (EU) 2019/627 (European Commission, 2019), and is one of the cornerstones of food safety system as it has been developed to ensure public health (Edwards et al., 1997). Besides, MI-findings could be used in animal health monitoring (Stärk et al., 2014; Valros et al., 2020; Hernandez et al., 2023). Available studies have focused on visual inspection of pigs in order to evaluate pig health and welfare prior to slaughter (Teixeira et al., 2020). As different types of lesions occur in pigs, some of them are clearer to detect while others are more open to interpretation (Teixeira et al., 2020; Comin et al., 2023). Differences between *ante-mortem* and *post-mortem* findings have been reported as sub-clinical and chronic conditions such as pleuritis and pericarditis are not apparent in living animals (Andoni et al., 2023). On the other hand, mild or subclinical conditions may remain unnoticed at slaughter (Stärk et al., 2014). Thus, health-related indicators such as stress biomarkers and APPs could be used to evaluate pig health on farm and predict pork quality (Čobanović et al., 2020).

Pleurisy is a common finding in slaughtered finishing pigs in Finland (Hälli et al., 2020; Finnish Food Authority, 2023) and elsewhere (Meyns et al., 2011; Alban et al., 2015; Andoni et al., 2023). Chronic pleurisy is not clinically apparent but results in production losses due to reduced growth (Zimmerman et al., 2019) and rejections at meat inspection (Zimmerman et al., 2019; Valros et al., 2020). Under experimental conditions, presence and severity of clinical signs of respiratory disease has been shown to be positively correlated with the severity of lung lesions in finishing pigs infected swine influenza virus (Pomorska-Mól et al., 2014).

Valros et al. (2020) reported, that tail biting lesions of various severity, and scored at slaughter, were positively associated with the increased risk of occurrence of arthritis, pericarditis, pleuritis, pneumonia, and skin infection. Previously, a Finnish study of Heinonen et al. (2010) has shown that besides resulting in an inflammatory response, tail biting causes abscess formation elsewhere in the body. In a study of Valros et al. (2020), both whole and partial carcass condemnations were recorded at higher levels in pigs with tail lesions compared to those having intact tails (Valros et al., 2020). Comparison of AMU data with MI findings could be used to comprehensively assess and monitor health status of pig farms, (Nienhaus et al., 2020; Grosse-Kleimann et al., 2021), and assist in developing strategies to reduce AMU on farms.

2.4 ANTIMICROBIAL USE

The following chapters review some methods of veterinary AMU assessment, the characteristics of AMU in pigs, as well as AMU and AMR surveillance programs that have been implemented.

2.4.1 MEASUREMENT OF ANTIMICROBIAL USE

Antimicrobial use data can be collected for qualitative and quantitative purposes, and at several levels (Collineau et al., 2017a). Several methods to measure AMU are available, each with strengths and weaknesses (Collineau et al., 2017a; Kasabova et al., 2019).

Technical units of variable precision have been developed for AMU quantification (EMA, 2013; Collineau et al., 2017a), many of them being based on assumptions and estimations (Collineau et al., 2017a; Kasabova et al., 2019) that might be related to pig husbandry in the given country (for example, see Raash et al., 2018). Different calculation methods yield different results (Timmerman et al., 2006; Jensen et al., 2012; Kasabova et al., 2019), which further complicates comparisons between studies. Moreover, it is important to be able to choose the appropriate method for AMU quantification, which should be based on the objective of data collection (Collineaeu et al., 2017a).

The outcome indicator of AMU relies on the determination of the population at risk of being treated with antimicrobials, which could be based on the animal biomass or the number of individual animals (Collineau et al., 2017a). A population correction unit (PCU) has been developed to harmonize AMU quantification at the population level, and is used to measure antimicrobial consumption within a given population that could be potentially treated with antimicrobials (EMA, 2022). Calculations based on PCU are reported as milligrams of antimicrobial active substance per PCU (mg/PCU) (EMA, 2022), and the calculation is thus relative to animal biomass (see Figure 2 on the Materials and methods section). The population at risk of being treated with antimicrobials is, at the national level, based on the reported animal numbers in a certain weight category representative of different stages of pig production (EMA, 2013). At the farm level, a similar approach could be used when the number of animals within a certain weight group or production type is known (EMA, 2013; Yun et al., 2021) or estimated (Kasabova et al., 2019).

The defined daily dose (DDD), when applied in veterinary medicine, means *the assumed average maintenance dose per day per kilogram of animal of a given species for a drug used for its main indication* (EMA, 2013). The maintenance dose (mg/kg) is usually extrapolated from the Summary of Product Characteristics (SPC) in the given country (EMA, 2013). The DDD is used to calculate treatment incidence (TI), which is another technical unit of AMU quantification (Timmerman et al., 2006; Collineau et al., 2017a). It indicates the number of animals out of a theoretical group of 1000 animals treated daily with a defined dose of antimicrobials (Timmerman et al., 2006; Collineau et al., 2017a). More detailed data on the use of antimicrobials are needed to calculate TI, including the total amount of antimicrobials administered, the defined period at risk of receiving treatment, the number of animals being treated, and their respective weights (Timmerman et al., 2006). In comparison to mg/PCU, TI allows a more accurate assessment of the use of antimicrobials on the farm.

2.4.2 ANTIMICROBIAL USE IN PIGS

The majority of antimicrobials of food-producing animals in Finland and other European countries are consumed in the pig and cattle sectors (EMA, 2022). In general, AMU in pigs varies between European countries (Chantziaras et al., 2014; Postma et al., 2015; DeBriyne et al., 2016; Sjölund et al., 2016; Raasch et al., 2018; 2020; Lekagul et al., 2019; Ceccarelli et al., 2020; EMA, 2022), over time (Jensen et al., 2012; EMA, 2022; Finnish Food Authority, 2022a; EMA, 2022), between farms (Timmerman et al., 2006; Callens et al., 2012; Sjölund et al., 2016; Stygar et al., 2020; Yun et al., 2021), and between production stages (Sjölund et al., 2016; Yun et al., 2021; Lekagul et al., 2019; Raasch et al., 2020). Usually more antimicrobials are used for suckling and weaned piglets compared to adult pigs (Jensen et al., 2012; Sjölund et al., 2016; Raasch et al., 2018; Raasch et al., 2020; Yun et al., 2021).

Oral administration of antimicrobials as a group treatment is very common in Europe (Postma et al., 2015; Lekagul et al., 2019; EMA, 2022), although it is less common in the Nordic countries (EMA, 2022), including Finland (EMA, 2022; Yun et al., 2021). The oral administration of antimicrobials via feed or water is common, especially in the group medication of weaned piglets (Jensen et al., 2012; Lekagul et al., 2019), whereas parenteral drug administration is preferred in suckling piglets (Timmerman et al., 2006; Jensen et al., 2012) and sows (Jensen et al., 2012). Orally administrable antimicrobials are more often underdosed and parenterally administrable antimicrobials overdosed (Timmerman et al., 2006). The correct dosing of antimicrobials in pigs is challenging, because the actual body weight is rarely measured prior to antimicrobial administration (see for example, Timmerman et al., 2006).

Antimicrobial therapy that is chosen based on the clinical presentation alone is referred to as empiric antimicrobial therapy (Leekha et al., 2011), and is common in pigs. Prudent AMU, however, includes the definition of the bacteria causing the disease together with susceptibility testing (Leekha et al., 2011). Prophylactic AMU refers to when antimicrobials are administered to a group of animals, or an individual animal, without any clinical signs of disease, being completely a preventive treatment (Giguére, Prescott & Dowling, 2013). Antimicrobial metaphylaxis occurs when some diseased animals are detected in a group, and both diseased and clinically healthy animals are treated (Giguére, Prescott & Dowling, 2013).

Irrespective of the production stage, the most common antimicrobial groups used in pigs include penicillin and other beta-lactam antimicrobials (Jensen et al., 2012; De Briyne et al., 2014; Sjölund et al., 2016; Yun et al., 2021). The use of tetracyclines is also common (De Briyne et al., 2014), especially in weaned piglets (Jensen et al., 2012; Sjölund et al., 2016) and finishing pigs (Jensen et al., 2012), whereas trimethoprim in combination with sulfonamides is mostly used for sows (Jensen et al., 2012). The antimicrobial choice for the same indication also differs across production stages (Jensen et al., 2012), which could be related to the available pharmaceutical products (e.g., oral and injectable formulas) and their price.

Gastrointestinal and respiratory diseases are the most common reasons for antimicrobial prescription in pigs in Europe (De Briyne et al., 2014). Gastrointestinal diseases in weaned piglets were reported to be the main indication for antimicrobial treatment in Danish pig farms nationally (Jensen et al., 2012). In Finland, tail biting has been reported to be amongst the most common indications for antimicrobial treatment, followed by musculoskeletal and respiratory diseases (Heinonen et al., 2001; Stygar et al., 2020). Elsewhere in the Europe, respiratory diseases are among the most common indications for antimicrobial treatment in finishing pigs (Callens et al., 2012 [Belgium]; Jensen et al., 2012 [Denmark]). Fewer reports are available in the scientific literature on antimicrobial use in sows compared to growing pigs. In one available report, AMU in piglets has been accompanied with that in sows, and according to this report, diseases related to the locomotory system, as well as neurological and skin disorders, were the most common indications for antimicrobial treatment in the farrowing unit (Jensen et al., 2012). However, lower antimicrobial use has been reported for sows compared to other production stages (Raasch et al., 2018; 2020; Yun et al., 2021).

2.4.3 SURVEILLANCE OF ANTIMICROBIAL USE AND RESISTANCE

The antibiotic era in veterinary medicine has been developing since penicillin was discovered in 1928 (Giguére, Prescott & Dowling, 2013). The problem of AMR emerged in the mid-1950s, when the first penicillin-resistant human infections became clinically significant (Giguére, Prescott & Dowling, 2013). For several years, AMR has been stated as one of the most important threats to human health, and subsequently also to veterinary medicine (WHO, 2012; 2015; WHO, FAO & OIE, 2019).

Amongst the first actions to intervene in the problem of AMR, the use of antimicrobial feed additives was banned in the EU in 2006 (European Commission, 2005), being influenced by a British initiative called the Swann report, published in 1969 (Swann report, 1969). Finland was among the first countries in Europe to voluntarily ban the use of antimicrobials as growth promoters in the 1990s (EELA, 2004). The use of virginiamycin ended in 1990, bacitracin in 1992, flavomycin in 1996, and avoparcin in 1996 (EELA, 2004). Moreover, the feed additives carbadox and olaquindox were banned in the EU in 1999 (European Commission, 1998)

Global actors, such as the World Health Organization (WHO) (WHO, 2015) and World Organization for Animal Health (WOAH, former OIE) (WOAH, 2023), have been working internationally to develop strategies for the prudent AMU, and to monitor and campaign against AMR. In Finland, the first national recommendations on prudent veterinary AMU were published in 1996, and they have been updated three times, with the latest guidelines being published in 2016 (Finnish Food Authority, 2016). Understanding of the trends and drivers of veterinary AMU will assist in the implementation of strategies to slow the development of AMR. The Finnish Food Safety Authority launched the FINRES-vet program in 2002, which includes the annual monitoring of veterinary AMU and consumption, as well as AMR occurrence among indicator and pathogenic bacteria (EELA, 2004).

Despite the fact that the consumption of veterinary antimicrobials in Europe has declined, and in particular the consumption of antimicrobials classified as critically important (CIA) for human medicine (EMA, 2022), with increasing understanding it is clear how much work remains to be done. Thus, close cooperation is needed between animal owners, veterinarians, and other stakeholders, as well as between policy makers (WHO, FAO & OIE, 2019).

3 OBJECTIVES AND HYPOTHESES

In this thesis, potential indicators for pig health monitoring at the farm level are assessed, including data on AMU and treatment indications, MI findings, as well as inflammatory and immune biomarkers in saliva. In order to achieve the overall aim of the thesis, three separate studies (I–III) were conducted. The target groups to utilize the results of these studies in the future were primarily veterinarians in their health care work and farmers as a part of their herd monitoring.

The aim of Study I, was to describe AMU and the prevalence of MI findings over one year, and to investigate the occurrence of phenotypic resistance in the fecal indicator *Eschericia coli* (*E. coli*) bacteria on commercial Finnish piglet-producing and finishing farms. Another objective was to compare AMU and MI findings in sows and finishing pigs for the same year on these farms (I). Greater AMU for young pigs compared to sows and finishing pigs was hypothesized. A positive relationship between overall AMU and MI findings was expected, and that antimicrobial treatment indications would reflect these findings (I). It was also hypothesized that the occurrence of AMR on the study farms would be low (I), as reported nationally and compared with other European countries.

Study II was a validation study, in which two proposed biomarkers, Hp and ADA, were measured in the saliva of growing pigs, followed by a LPS challenge. The interests regarding this experimental model of acute inflammation were related to the dynamics of both biomarkers, as well as the relationship between Hp and ADA responses (II). It was predicted that porcine salivary Hp and ADA concentrations increase in response to LPS, and that a positive relationship exists between Hp and ADA responses (II).

A few studies have been conducted on porcine salivary biomarker dynamics under field conditions and including a longitudinal approach. Therefore, three selected biomarkers, Hp, ADA, and IgG, were investigated in pig saliva in four production stages during a complete production cycle of finishing pigs under commercial conditions (III). The effect of the production stage and gender was examined for all three biomarkers, and the relationships between all the biomarkers at each production stage were investigated (III). As the biomarkers may be indicative of chronic and/or sub-clinical conditions, the associations of their concentrations and MI findings in finishing pigs, which were used as an indicator of the health status of individual pigs, were further assessed. (III). It was predicted that all the salivary biomarkers differ according to both gender and production stage of pigs (III). Another hypothesis was that pigs with inflammatory and/or infectious MI findings would have higher biomarker concentrations compared to those with no such findings (III).

4 MATERIAL AND METHODS

The methods used in Studies I–III are overviewed in this section and described in detail in the original publications, which are reproduced at the end of this thesis.

4.1 ETHICAL APPROVAL

Studies I and III were approved by the Regional State Administrative Agency for Southern Finland (ESAVI/16950/2018), and written consent was obtained from the farm owners at the beginning of these studies. Study II was approved by the Norwegian Animal Research Authority (FOTS id 15232). All the experiments were in accordance with EU legislation on the protection of animals for scientific purposes (Directive 2010/63/EU).

4.2 DATA SETS USED (I–III)

All data were collected between April 2018 and July 2019. For studies I and III, a Finnish slaughterhouse company recruited the farms, and farms participating in Study III were a subset from Study I. Study II took place at the Livestock Production Research Center of the Norwegian University of Life Sciences (NMBU), campus Ås. A general description of each study is presented in Table 1.

Table 1. Descriptive information on Studies I–III.

	Study I	Study II	Study III
Study type	Field study, register-based study	Blinded randomized clinical trial	Field study
Production stages included	Suckling piglets, weaned piglets, sows and finishing pigs Boars, young breeding pigs (gilts)	Weaned piglets	Suckling piglets, weaned piglets and finishing pigs
Type and number of farms	24 commercial farms: 9 piglet-producing (P1–P9) and 15 finishing farms (F1–F15) [1]	One experimental farm	8 commercial farms: 4 piglet-producing and 4 finishing farms, forming 4 farm pairs (Farms 1–4) [1]
Number of sows and finishing pigs [2]	Median (min–max) number of sows on P1–P9: 896 (248–3422) Median (min–max) number of finishing pigs on F1–F15: 13 320 (3431–47255)	-	Farm 1: 248 sows, 25238 finishing pigs Farm 2: 450 sows, 8164 finishing pigs Farm 3: 458 sows, 3431 finishing pigs Farm 4: 1122 sows, 12046 finishing pigs
Number and type of study pigs	No individual data collected	Sixteen female pigs	Eighty-three pigs: 35 females and 48 males
Sampling	Pen-level fecal samples (n = 10 per farm), total n = 240 samples	Individual repeated saliva samples	Individual repeated saliva samples
Other data	Medicine use during one year, including amount, treatment indications and administration route from Sikava [3] Meat inspection findings during one year from Sikava [3] Monthly number of pigs present on each farm from the Finnish Swine Registry System	Weight	Weight Individual meat inspection findings from the slaughterhouse database

[1] Production of the Finnish farms was integrated, which in this context means that pigs were reared on the piglet-producing farms until the end of growing period and then sold to their respective finishing farms at an average target weight of 30 kg and ten weeks of age. [2] Sow numbers represent the average number of sows kept during one year on the study farms and the number of finishing pigs represents the total number of finishing pigs reported to the Finnish Swine Registry System. [3] The health classification register for pig farms in Finland.

4.3 STUDY I

4.3.1 COLLECTION AND PROCESSING OF SIKAVA DATA (I)

The raw data from the Sikava register, including all treatment records, were collected during one year from the nine piglet-producing farms (P1–P9) and fifteen finishing farms (F1–F15) (Figure 2), and pre-processed in Microsoft Excel. Pigs at risk of antimicrobial exposure were obtained from the Finnish Swine Registry (Figure 2), in which farmers are obliged to report the monthly number of different animal groups present on the farm, including suckling piglets, weaned piglets, sows, finishing pigs, boars, and young breeders corresponding to gilts. Sows at risk were defined by summarizing the monthly sow numbers reported in the registry in 2018 and calculating the average number of sows.

Based on the summary of product characteristics, antimicrobial product names in Sikava were converted to active antimicrobial substances (ASs), and further combined into seven groups (Figure 2; Table 2). The antimicrobials were further categorized as CIA (WHO, 2019) and other antimicrobial groups as non-CIA (Table 2).

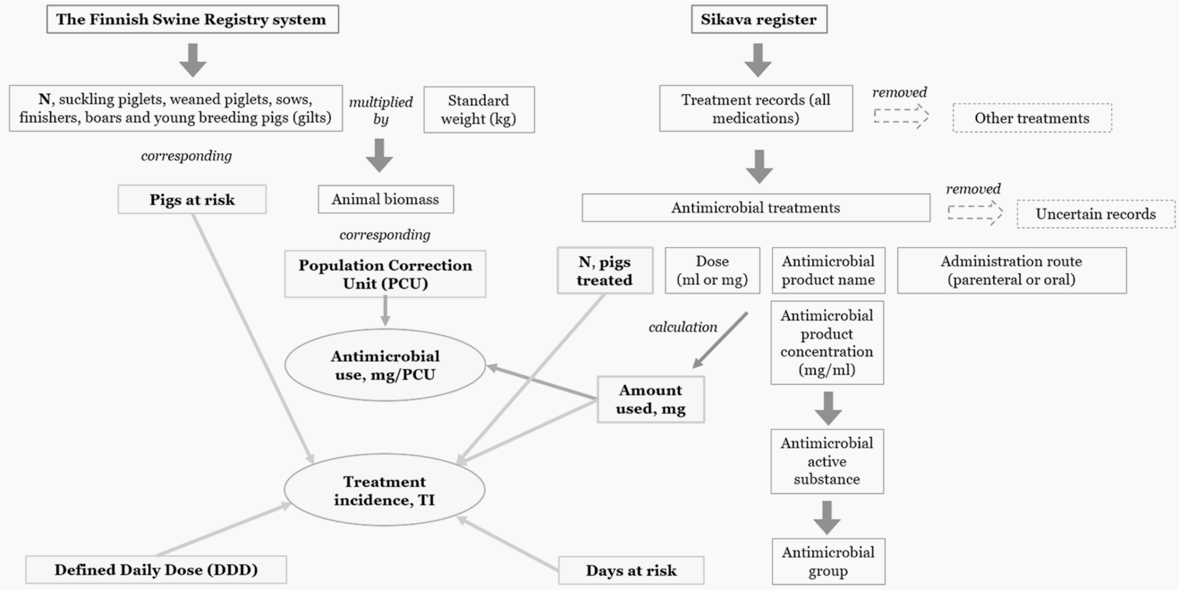

Figure 2 Illustration of data collection and processing for antimicrobial use calculations at the farm level with respect to animal biomass (mg/PCU) and at the individual pig level as treatment incidence. Standard weights used for calculations were 4 kg for suckling piglets, 12 kg for weaned piglets, 50 kg for finishers, and 220 kg for sows (EMA, 2013). The corresponding days at risk were 28, 42, 130, and 365 days, and were defined as time periods when a pig could receive an antimicrobial treatment in each production stage. Defined daily dose values were obtained from the publication of Postma et al. (2015).

Table 2. Summary of antimicrobials used on nine commercial Finnish piglet-producing and fifteen finishing farms during one year.

Antimicrobial active substance1)	Antimicrobial group
Penicillin	Penicillin
Amoxicillin	Beta-lactams other than penicillin
Sulfadiazine/-doxine in combination with trimethoprim	Sulfa-trimethoprim
Danofloxacin, enrofloxacin, marbofloxacin	Fluoroquinolones
Chlortetracycline, oxytetracycline	Tetracyclines
Tylosin, tulathromycin (LA) 2)	Macrolides
Lincomycin	Lincosamides

1) According to the Summary of Product Characteristics, 2) Long acting. Red-shaded antimicrobial active substances are listed as critically important antimicrobials for human medicine, according to WHO (2019).

Treatment indications were obtained from Sikava (Sikava, 2021). The indications were grouped according to the registry grouping as respiratory, gastrointestinal, skin, locomotory, reproductive and general diseases, and other diseases (including fever without other symptoms, anorexia, cachexia, supportive treatment of a piglet, tail biting, and a health care visit). Moreover, in some occasional cases, sow treatment had been indicated for piglets, and these were grouped separately (*Clostridium perfringens* type A diarrhea, hypothermia, and supportive treatment of a piglet).

Summaries of the MI findings from slaughtered sows and finishing pigs were obtained during one year from Sikava. According to the legislation (Decree of the Ministry of Agriculture and Forestry 6/EEO/2012), the following conditions are recorded at slaughter by the official veterinarians: pleuritis, pneumonia, pericarditis, abscesses, arthritis, tail biting, liver milk spots indicative of an *Ascaris suum* infection, and mycobacteria. Moreover, shoulder sores are recorded for sows (Decree of the Ministry of Agriculture and Forestry 6/EEO/2012).

4.3.2 MEASUREMENT OF ANTIMICROBIAL USE (I)

Figure 2 summarizes the processing of data and calculation of AMU as mg/PCU (EMA, 2013), and as TI (Timmerman et al., 2006; see also Formula 1).

$$\text{TI}\left(\frac{\text{amount of antimicrobial active substance (mg)}}{\text{DDD}\left(\frac{\text{mg}}{\text{kg}}\right)\text{ x days at risk (d) x number of pigs treated x standard weight (kg)}}\right)\text{x 1000 pigs at risk}$$

Formula 1. Treatment incidence according to Timmerman et al. (2006).

Furthermore, the following measures were computed:

- Total AMU (mg/PCU), comprising all age groups present on farms in the year 2021 according to the Finnish Swine Registry System;
- The percentage of treated pigs out of the total number of pigs present, separately for suckling piglets, weaned piglets, sows, and finishing pigs;
- TI, separately for suckling piglets, weaned piglets, sows, and finishing pigs;

- TI, separately for CIA and non-CIA antimicrobial classes;
- TI, separately for the indication groups.

4.3.3 ANTIMICROBIAL SUSCEPTIBILITY TESTING AND RESISTANCE OCCURRENCE (I)

The antimicrobial susceptibility of the indicator *E.coli* bacteria, isolated from pen-level pooled feces samples, was tested. We chose one room from each farm for sampling: on P1-P9 one room housing on average 10-week-old pigs weighing around 30 kg, and on F1-F15 one room housing finishing pigs with the average age of 6 months and weighing around 110 kg. . The fecal samples were collected in each selected room from approximately every second pen and altogether from ten pens on each farm. Within each pen, three feces piles were swabbed with one sterile cotton swab, which was then inserted into a culture medium tube (M40 Transystem Amies Agar Gel, Copan Diagnostics, Brescia, Italy). The phenotypic resistance of indicator *E. coli* was tested with broth microdilution (SensititreTM panels, TREK diagnostic systems, Cleveland, OH, United States) by determining minimal inhibitory concentration (MIC) values for the following fourteen antimicrobials: ampicillin, azithromycin, cefotaxime, ceftazidime, chloramphenicol, ciprofloxacin, colistin, gentamicin, meropenem, nalidixic acid, sulfamethoxazole, tetracycline, tigecycline, and trimethoprim. The bacteria were categorized as either susceptible (wild type) or resistant (non-wild type) based on epidemiological cut-off (ECOFF) values for indicator *E. coli* available on 25.1.2019 (EUCAST, 2021). Because no ECOFF value for azithromycin was available, a value suggested by the European Food Safety Authority (EFSA) was used (EFSA, 2020).

A single sample was determined as resistant if at least one indicator *E. coli* isolate out of ten isolates studied was resistant to any of the tested fourteen antimicrobials. A sample was determined as multi-resistant (MR) if resistance against three or more antimicrobials was observed (Schwarz et al., 2010). The following calculations were computed:

- The overall occurrence of resistance was determined as the proportion of resistant samples, and was calculated by dividing the number of resistant samples by the total number (n = 10) of studied samples per farm.
- The resistance against the fourteen studied antimicrobials was calculated. On each farm, we counted how many of the studied antimicrobials the indicator *E.coli* were resistant against, and this was divided by the total number of antimicrobials (n = 14) included in the susceptibility testing panel.
- The proportion of resistant and MR isolates from the total sample was determined (n = 240).

4.4 STUDY II

The description of methods presented below are derived from a subset of pigs (n= 16) included in the original publication (II). Pigs with intramuscular ketoprofen treatment (II) were not included in the thesis as the topic was not according to the aims of this thesis.

4.4.1 MANAGEMENT AND HOUSING (II)

The pigs were group-housed under the experimental conditions in one room in pens of four experimental and two companion male pigs. The pigs were moved there two weeks before the experiment was initiated, which was considered as an acclimatization period. The room temperature was set to 20 °C and water sprinklers were suspended over each pen. Lights were on sixteen hours per day between 6 am and 10 pm, and for the remaining eight hours, the room was dimmed with night lights. The pen size was 7.7 m^2, of which one half consisted of a solid lying area with bedding provided twice daily and the other half consisted of a slatted floor. The stocking density of the pens was set as 1.3 m^2 per pig, and the pigs had limited tactile contact with other pigs in the adjacent pen. Each pen was equipped with three nipple drinkers and a feeding trough, with an animal-to-feeding-place ratio of 3:1, and pelleted dry feed was offered *ad libitum*.

4.4.2 LIPOPOLYSACCHARIDE-INDUCED SYSTEMIC INFLAMMATION MODEL (II)

At the beginning of the study, the pigs were 68–85 (median 83) days old. The weight of the pigs was recorded one day before the experiment started, and the pigs weighed between 17 and 51 (median 42) kg. Two experimental groups were formed, to which study pigs within each pen were randomly allocated to receive one of the following combinations of two substances: saline–saline (SS, n = 9) or saline–LPS (SL, n = 7). The individual LPS dose was calculated based on the body weight of the pig according to Nordgreen et al. (2018) and Munsterhjelm et al. (2019). Saline was administered intramuscularly around one hour (61 ± 16 min) prior to intravenous administration of either saline or LPS through a permanent ear vein catheter that was kept in place until the end of the study. The pigs were closely observed in the hours after injections in order to detect a possible overreaction to LPS. Five individual saliva samples were collected from each pig by allowing the pig to chew a cotton sponge until it was moist. The first sampling occurred before any substance was administered, which was considered as the baseline (T0). Other samples were taken at 4, 24, 48, and 72 hours (T4-T72) after the second injection.

4.5 STUDY III

4.5.1 MANAGEMENT AND HOUSING (III)

The study pigs originated from four piglet-producing farms, from which the pigs were sold to their respective finishing farms at an average age of ten weeks and at an approximate weight of 30 kg. The study farms followed their normal management and housing throughout the study.

Pregnant sows were group-housed in a separate gestation unit until they were moved to the farrowing unit three (Farm 2) or five (Farms 1, 3, and 4) days prior to the expected farrowing day. In the farrowing unit, sows were kept in pens of a standard size (4.6–4.8 m^2) with a partially slatted floor and with no bedding. The pens were equipped with a farrowing crate, a feeding trough, and a drinking nipple for the sow. The sows were fed with a standard barley-based liquid meal. For piglets, a nest provided with a heat lamp was available in a corner or on one side of the farrowing pen.

Sow farrowing on the study farms was supervised by the farm employees during working hours, but not during the night. On all farms, piglets were cross-fostered within a few days after birth according to the farm management practices. Male piglets were castrated according to Finnish legislation before seven days of age (Animal Welfare Decree 396/1996). The piglets were weaned at an average age of 27, 28, 25, and 30 days on Farms 1–4, respectively, and moved to growing units that were located on the same piglet-producing farm. Prior to weaning, piglets were vaccinated against circovirus type 2 and sows against erysipelas and parvovirus.

Pens in the growing units and finishing farms had partly slatted floors, with a space allowance according to the Finnish recommended standards (Decree of the Ministry of Agriculture and Forestry 629/2012). During grower and finishing stages, pigs were fed with a standard barley-based liquid meal, restricted in certain production phases. Exceptionally, on Farm 4, dry feed was offered to growing pigs *ad libitum*. The birth to slaughter period was six months, and finishing pigs were slaughtered at an average age of 175 days on Farms 1 and 3, 173 days on Farm 2, and 174 days on Farm 4. The follow-up period is shown in Figure 3.

4.5.2 STUDY DESIGN (III)

On each piglet-producing farm, one farrowing batch was supervised for three to four days by the researchers. Immediately at birth, the sex and birth weight of piglets were recorded, and all viable piglets were individually marked on their back with running numbers. Piglets weighing less than 0.9 kg at birth were excluded due to a poor prediction of survival (Feldpausch et al., 2019) in order to retain the study population until the end of the study. On the following day, a maximum of six piglets of both sexes, including three small and three large piglets according to the birth weight, from each litter were ear-tagged. Out of the initial 163 ear-tagged piglets, six died before the first sampling. Thus, the data were collected from 157 piglets from the birth week onwards, and the final study sample consisted of the records of 83 pigs that were successfully followed until slaughter.

Individual saliva samples were collected three times on piglet-producing farms and once on the finishing farms (Figure 3). Apart from during the first sampling of suckling piglets, all pigs were weighed on sampling (Figure 3).

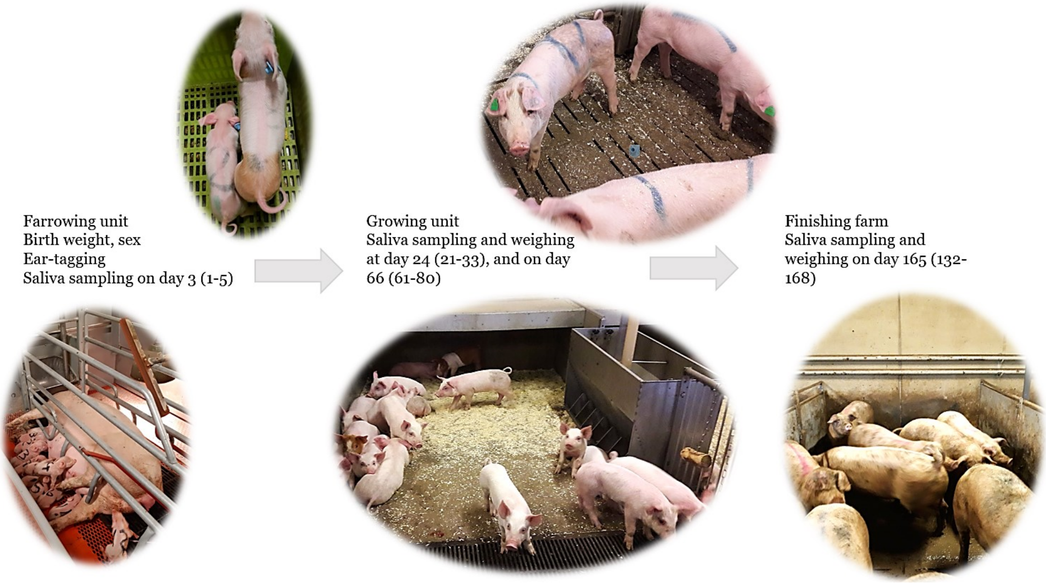

Figure 3 Summary of study procedures during a six-month follow up period for an initial population of 163 pigs from four commercial Finnish piglet-producing and their respective four finishing farms. Farrowing units and growing units were on the piglet-producing farms. After the second farm visit to the growing units, the study pigs were transported to the finishing farms. The age of pigs on consecutive farms visits is presented as the median (range).

Pigs were transported to the slaughterhouse in one batch, except for Farm 1, from which the pigs were slaughtered in two consecutive weekly batches. The interval from the last sampling to slaughter was 7–49 days, and the researchers could not intervene in the time of sending the pigs to slaughter. The individual MI findings were used in the retrospective grouping of finishers into two MI groups: those with (n = 27, Table 3) and those without (n = 56) a finding suggestive of an inflammatory or infectious condition. The official meat inspection veterinarians evaluate the carcasses according to the criteria provided by the Finnish Food Authority (Finnish Food Authority, 2020), and we used these results.

Table 3. Meat inspection (MI) findings considered as infectious or inflammatory, and used for retrospective grouping of study pigs into those with or without an MI finding.

Disease category	Inflammatory/infectious condition	n [1]
Respiratory	Pleuritis	21
	Suppurative pleuritis	1
	Pneumonia	2
Heart	Pericarditis	4
Locomotory system	Arthritis	1
	Bursitis	1
Generalized	Serositis	1
Other	Tail biting [2]	1
	Liver milk spots (*Ascaris suum*)	1

[1] Altogether, 27 pigs with at least one finding considered inflammatory or infectious were identified. One pig could have more than one finding. [2] Tail biting was considered inflammatory, as only the most severe cases are recorded at the slaughterhouse. The official meat inspection veterinarians evaluate the carcasses according to the criteria provided by the Finnish Food Authority (Finnish Food Authority, 2020).

4.6 ANALYTICAL PROCEDURES (II–III)

4.6.1 PROCESSING AND ANALYSIS OF SALIVA SAMPLES (II, III)

Saliva samples were processed immediately after collection (II) or on arrival at the laboratory at the end of the day after the farm visit (III). The cotton pads used for saliva sampling were centrifuged for 5 min at 1000 x g and the extracted saliva was moved to a -80 °C freezer at the end of each sampling day (II) or immediately after processing (III). Saliva samples were analyzed according to previously validated methods for Hp (Gutiérrez et al., 2009b), ADA (Gutiérrez et al., 2017), and IgG (Escribano et al., 2012). A detailed description of analyses is presented in the original publications (II, III). In Study III, the studied pigs were also blood sampled. However, serum dynamics of the studied biomarkers are not included in the present thesis because the primary objective was to assess novel pig health indicators in saliva.

4.7 STATISTICAL ANALYSES (I–III)

In order to examine the relationship of total AMU (mg/PCU and TI) on both farm types and TI of sows (P1–P9) and finishing pigs (F1–F15) with the prevalence of MI findings recorded during one year, non-parametric Spearman correlations were computed (Study I).

A linear mixed model (LMM) approach with repeated measures was used to investigate the salivary biomarker dynamics over a 72-hour period (Study II) and over four production stages from birth to finishing (Study III). LMMs were used to investigate the association between salivary biomarker concentrations of finishing pigs and individual MI findings (Study III). According to a sample size calculation with an assumed 30% prevalence of MI findings, a 95% confidence interval, and a power of 0.8, the desired sample size was 280. Pig was used as an experimental unit in all the models. The models are described in detail in the original publications II and III.

To test whether Hp and ADA responses correlated between baseline (saliva collected prior to injections) and four hours post-injection, non-parametric Spearman correlations were used (Study II). For this purpose, the difference in measured concentrations between four hours post-injection and baseline was calculated for each individual, and these calculations resulted in new outcome variables (Hp response and ADA response, respectively). Furthermore, Spearman correlations were applied to study the relationships between salivary Hp, ADA, and IgG separately in suckling piglets, at the beginning of the growing stage, at the end of the growing stage, and at the end of finishing stage (Study III).

The interpretation of Spearman correlation coefficients (ρ) was as follows: strong correlation (>0.6), moderate correlation (0.4–0.59), weak correlation (0.2–0.39), or very weak correlation (<0.2), according to the web site[1].

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[1] Spearman's correlation. http://www.statstutor.ac.uk/resources/uploaded/spearmans.pdf. Accessed 11 March 2023.

5 RESULTS

5.1 ANTIMICROBIAL USE (I)

Considerable variation in AMU was observed between all farms and between farms of both farm types (Figure 4). Total AMU (as mg/PCU), including all age groups present on farms according to the Swine Registry, was higher on piglet-producing farms (P1–P9) (median, min–max: 13.5, 5.5–47.3 mg/PCU) compared to finishing farms (F1–F15) (2.1, 0.1–34.9 mg/PCU). Overall, 97.9% of all antimicrobial treatments were administered parenterally, while oral treatments comprised 2.9% of all treatments. Oral antimicrobial administration in feed occurred on two piglet-producing farms and on three finishing farms (Figure 4). None of the study farms administered antimicrobials via water.

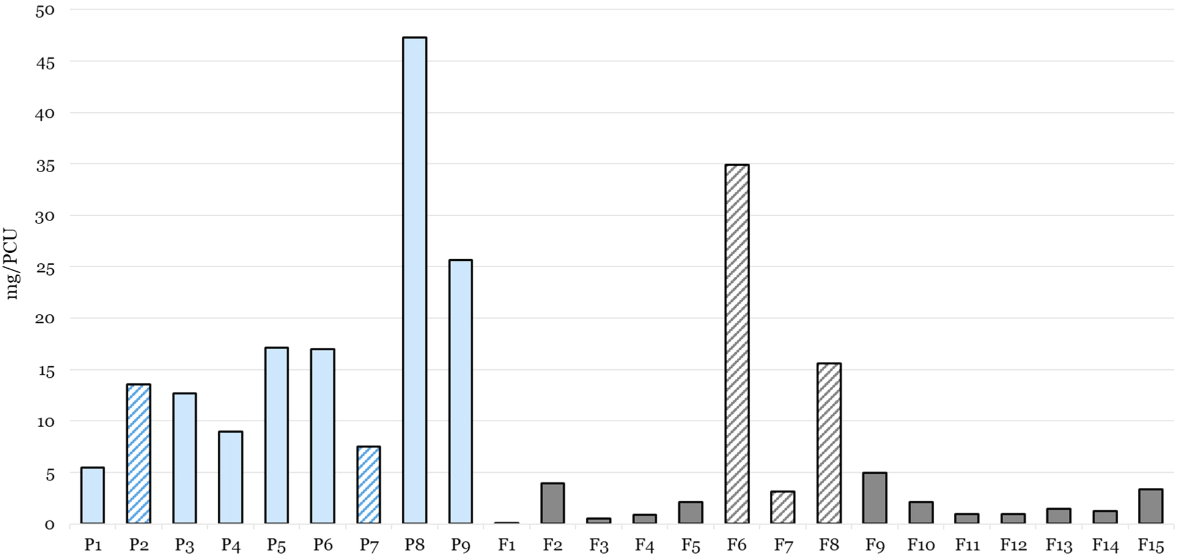


Figure 4 Total antimicrobial use on nine commercial Finnish piglet-producing (P1–P9, in blue) and fifteen finishing (F1–F15, in gray) farms during the year 2018. Total antimicrobial use includes all the age groups present on the farms according to the national Swine Registry during the same year. Hatched columns represent farms on which some antimicrobials had been administered orally in feed, and solid columns represent farms where only injectable antimicrobials were administered to single animals.

As treatment incidences (TIs), the largest amounts of antimicrobials were administered to suckling piglets (median, min–max: 22.5, 1.4–118.5), followed by sows (15.1, 6.2–31.4) and weaned piglets (3.9, 0.2–21.7). In percentage terms, of 419 723 suckling piglets, 21% had received an antimicrobial treatment course during one year. Of 127 068 sows raised on the study farms and of 773 335 weaned piglets, 12.6% and 9.3% had been treated with antimicrobials, respectively. Among finishing pigs, AMU was lowest both as TI (1.04, 0.07–8.0) and in percentage terms, the percentage of all finishers (255 657) treated with antimicrobials being 2.5%.

All the different antimicrobial groups (see Table 2 in the Material and Methods section) were used on both farm types, although the amounts varied between farm types and between individual farms. Antimicrobials grouped as non-CIA accounted for the majority of antimicrobial use on both farm types (Table 4). Penicillin was the most common antimicrobial group used on piglet-producing farms and finishing farms (Table 4). More antimicrobials classified as CIA (WHO, 2019) and all other antimicrobial groups were used on piglet-producing farms compared to finishing farms (Table 4). The CIA group on piglet-producing farms mostly consisted of injectable amoxicillin administered to sows, and on finishing farms of the macrolide antimicrobial tylosin administered in feed.

Table 4. Antimicrobial use according to antimicrobial group during one year on nine commercial Finnish piglet-producing farms (P1–P9) and on fifteen finishing farms (F1–F15) quantified as treatment incidence. The raw values are presented as the median (min–max).

		P1–P9	F1–F15
CIA 1)	BL other than penicillin 2)	4.1 (0.0–7.6)	0.0 (0.0–0.1)
	FQ 3)	0.0 (0.0–0.01)	0.0 (0.0–0.02)
	Macrolides	0.0 (0.0–0.001)	0.0 (0.0–5.7)
	CIA, total	4.1 (0.0–7.6)	0.0 (0.0–5.7)
non-CIA	Penicillin	11.3 (1.8–22.0)	0.6 (0.03–2.4)
	Tetracyclines	0.001 (0.0–4.9)	0.0 (0.0–4.9)
	Su – TMP 4)	0.0 (0.0–5.7)	0.0 (0.0–0.01)
	Lincosamides	0.0 (0.0–0.003)	0.0 (0.0–0.1)
	non-CIA, total	12.4 (4.1–24.6)	0.7 (0.03–5.9)

1) CIA = critically important antimicrobial for human medicine, according to WHO (2019), 2) beta-lactams other than penicillin, solely consisting of injectable amoxicillin, 3) FQ = fluoroquinolones, 4) sulfa-trimethoprim

Altogether, six disease categories were identified on both piglet-producing farms and finishing farms, and two additional categories on piglet-producing farms (Figure 5). Locomotory diseases were the most common indication for antimicrobial treatment in sows, weaned piglets, and finishing pigs, and gastrointestinal diseases were the most common indication for antimicrobial treatment in suckling piglets (Figure 5). Large variation in terms of the different indications registered was found between farms.

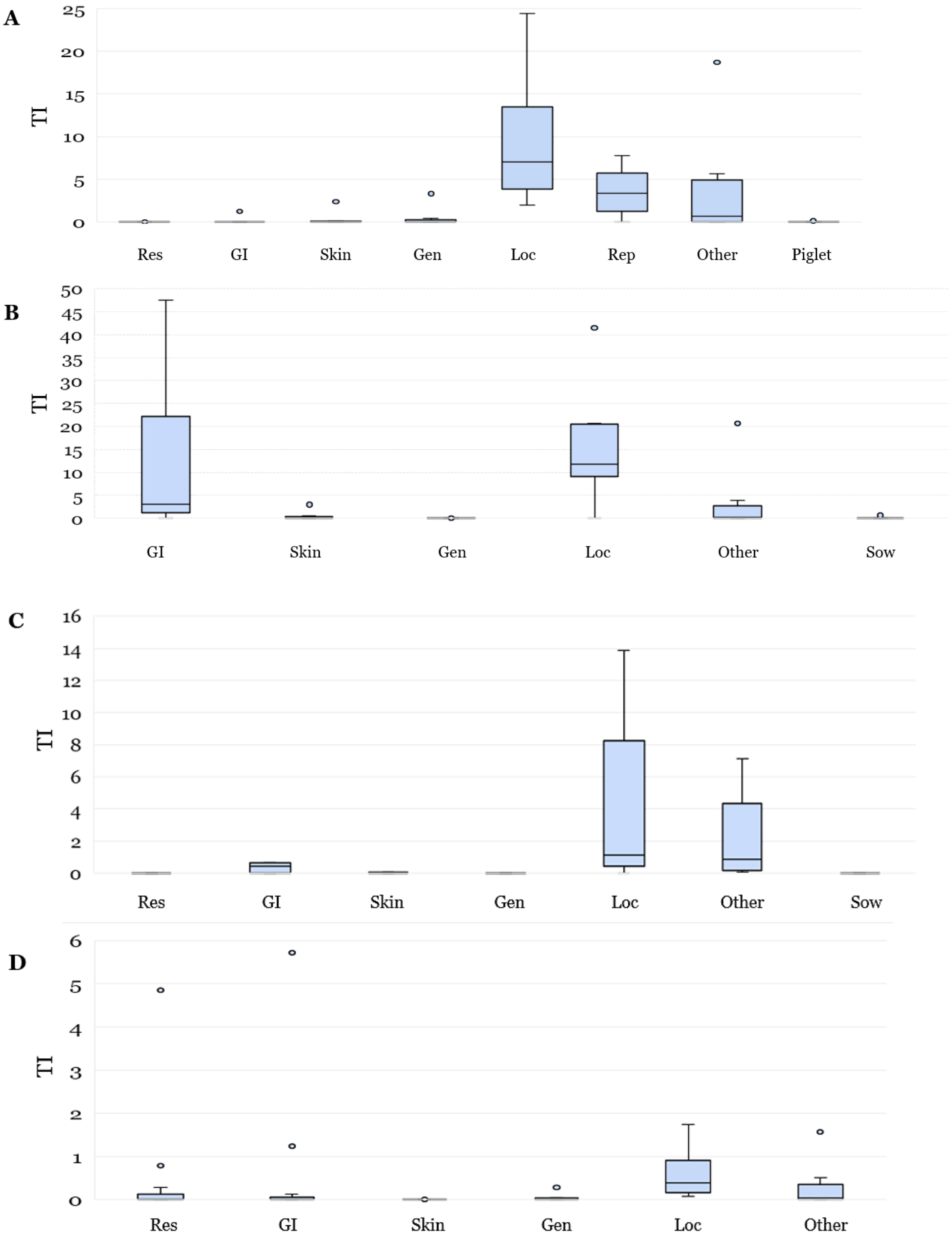

A
TI
25
20
15
10
5
0
Res
GI
Skin
Gen
Loc
Rep
Other
Piglet
B
TI
50
45
40
35
30
25
20
15
10
5
0
GI
Skin
Gen
Loc
Other
Sow
C
TI
16
14
12
10
8
6
4
2
0
Res
GI
Skin
Gen
Loc
Other
Sow
D
TI
6
5
4
3
2
1
0
Res
GI
Skin
Gen
Loc
Other

Figure 5 Treatment indications for A) sows, B) suckling piglets, and C) weaned piglets on nine piglet-producing farms, and D) finishing pigs on fifteen finishing farms during one year. Res = respiratory disease, GI = gastrointestinal disease, Skin = skin disease, Gen = general and nervous system diseases, Loc = locomotory diseases, Rep = reproductive diseases, Piglet = piglet-related indications recorded for sows, Sow = sow-related indications recorded for piglets. Other includes fever without other symptoms, anorexia, cachexia, supportive treatment of a piglet, tail biting, and undefined medicine use marked as a health care visit. Note the different scaling of the y-axis for different age groups.

5.1.1 MEAT INSPECTION FINDINGS AND THEIR RELATIONSHIP WITH ANTIMICROBIAL USE

Overall, 4800 sows on P1–P9 and 85 738 finishing pigs on F1–F15 were slaughtered during one year. The most prevalent MI finding was pleuritis among sows and finishing pigs, being a more common finding in finishing pigs compared to sows (Figure 6). In addition, abscesses and shoulder sores were commonly recorded in sows (Figure 6A), and abscesses and joint inflammation were relatively common in finishing pigs (Figure 6B).

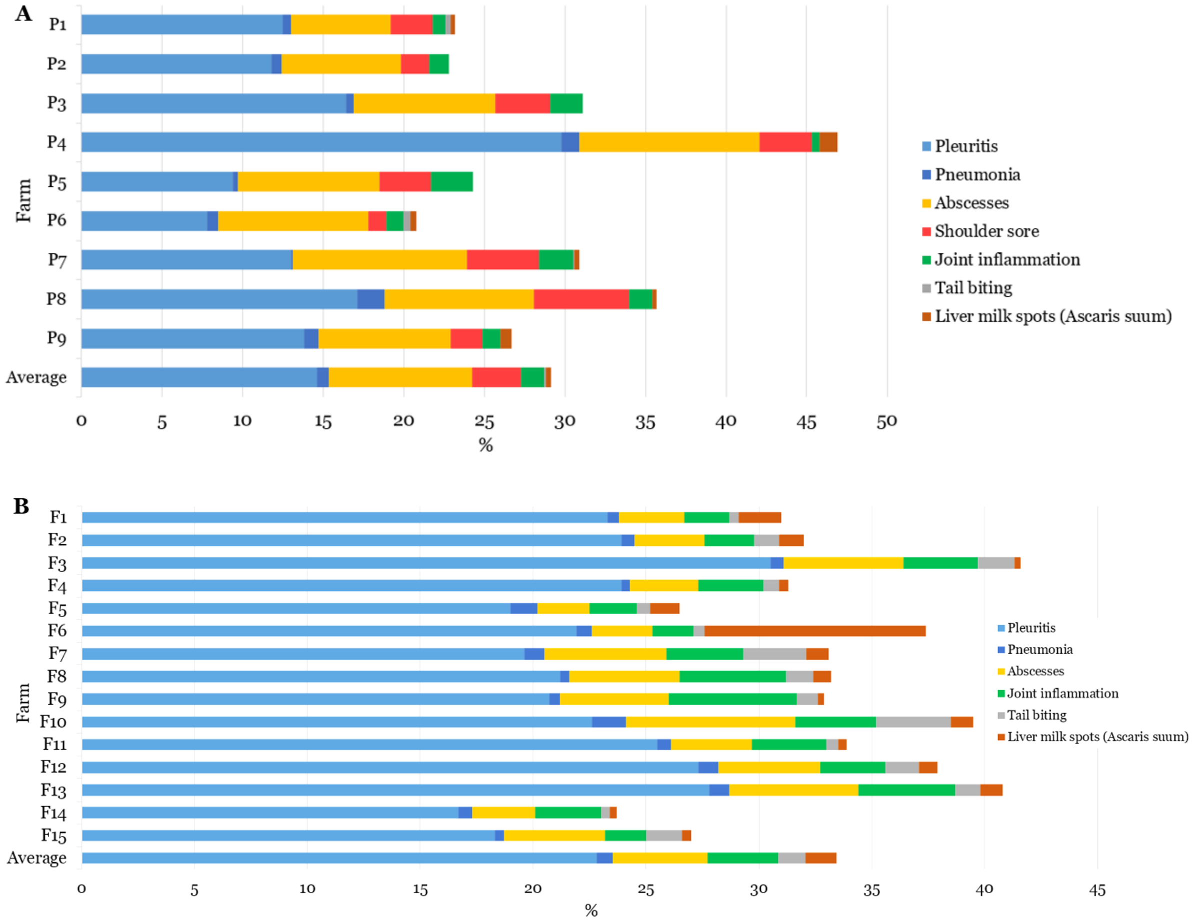


Figure 6 Meat inspection findings A) from 4800 sows on nine piglet-producing farms (P1–P9) and B) from 85 738 finishing pigs on fifteen finishing farms (F1–F15) recorded at the slaughterhouse during one year. Percentages represent the occurrence of each finding out of all pigs slaughtered during the year 2018. Average columns show the average occurrence of each meat inspection finding during one year on all piglet-producing farms (A) and on all finishing farms (B). Mycobacteria and pericarditis were also recorded, but their prevalence was at a maximum 0.3%, and on occasional farms, and the figures are not shown in the graphs. Note the difference in the scaling of the x-axis between figures.

Neither the total TI in sows nor the total AMU (mg/PCU) was correlated with the prevalence of any MI finding in sows. There was a strong and negative relationship between the TI for reproductive diseases of sows and the prevalence of shoulder sores at slaughter (Spearman correlation, $\rho = -0.787$, $p = 0.012$) as well as the prevalence of arthritis at slaughter (Spearman correlation, $\rho = -0.644$, $p = 0.061$).

A moderate negative correlation between the total TI of finishing pigs and the prevalence of pleuritis (Spearman correlation, $\rho = -0.52$, $p = 0.047$), and between the total AMU (mg/PCU) of finishing pigs and prevalence of pleuritis (Spearman correlation, $\rho = -0.50$, $p = 0.056$) were found. There was a moderate negative correlation between the TI of finishers for respiratory diseases and the prevalence of abscesses at slaughter (Spearman correlation, $\rho = -0.541$, $p = 0.037$), and TI for gastrointestinal diseases and the prevalence of pleuritis at slaughter (Spearman correlation, $\rho = -0.530$, $p = 0.042$). Moreover, the TI of finishing pigs for other indications was strongly and positively correlated with the prevalence of arthritis at slaughter (Spearman correlation, $\rho = 0.697$, $p = 0.004$).

5.1.2 ANTIMICROBIAL RESISTANCE OCCURRENCE

The overall occurrence of AMR among fecal indicator *E. coli* bacteria isolated on the study farms was on average 40% (SD 15), ranging from 20% to 70%. On P1–P9, the overall resistance occurrence (mean (SD)) was 50% (20), and on F1–F15 it was 40% (14). On average, the studied *E. coli* isolates were resistant to 30% (SD 10) of the fourteen tested antimicrobials, ranging from 10% to 40%. On piglet-producing and finishing farms, resistance most commonly occurred against tetracycline (median, min–max = 40, 10–70 and 20, 10–60, respectively), followed by sulfamethoxazole (20, 0–50 for both), trimethoprim (20, 0–50 for both), and ampicillin (20, 0–30 and 10, 0–40, respectively). All the indicator *E. coli* isolates were susceptible to azithromycin, colistin, gentamicin, tigecycline, meropenem, and 3rd generation cephalosporines.

Of all the studied isolates (n = 240), 58.8% (n = 141) were fully susceptible to the fourteen tested antimicrobials, and 41.3% (n = 99) were resistant. Of the resistant isolates, 46.5% (n = 46) were resistant to three or four antimicrobials and thus considered MR. On all study farms, altogether 15 resistance phenotypes were found, and one to four different resistance phenotypes were found on single study farms (Figure 7). Phenotypes considered MR displayed resistance against three to four antimicrobials and were found on both farm types (8/9 piglet-producing and 11/15 finishing farms).

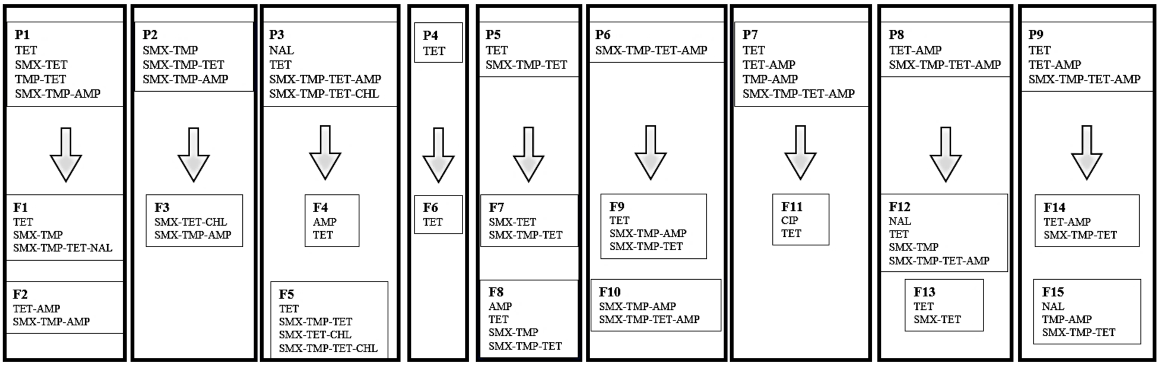
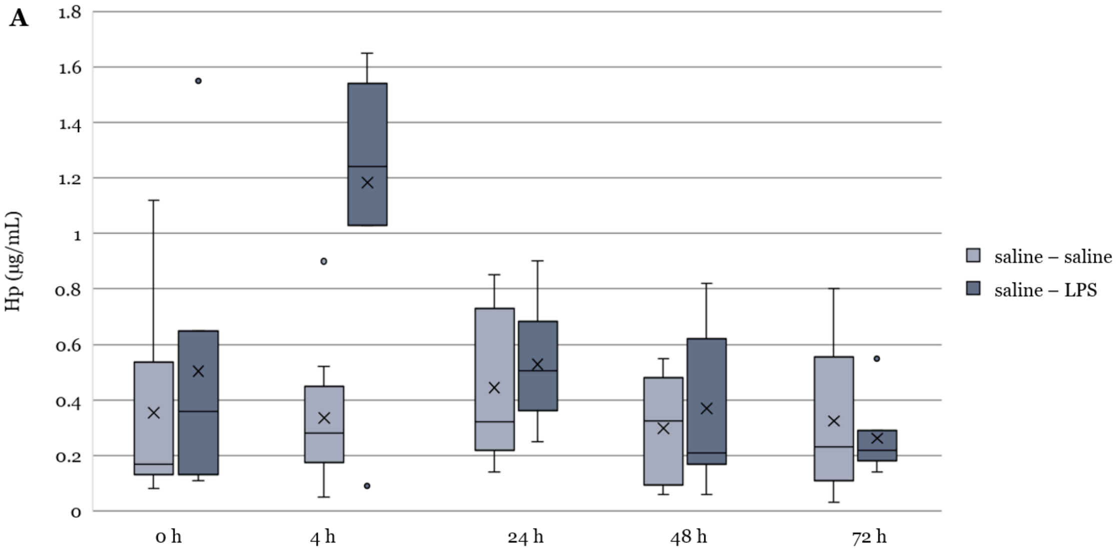


Figure 7 Phenotypic resistance pattern of indicator *Escherichia coli* bacteria isolated from pooled pig fecal samples on nine commercial Finnish piglet-producing farms (P1–P9) and fifteen finishing farms (F1–F15). Arrows within boxes indicate that piglet-producing farms and finishing farms within the same box had integrated production in which the piglet-producing farm sold piglets at an average body weight of 30 kg to the finishing farm or farms. AMP = ampicillin, CHL = chloramphenicol, CIP = ciprofloxacin, NAL = nalidixic acid, SMX = sulfamethoxazole, TET = tetracycline, TMP = trimethoprim.

5.2 SYSTEMIC LIPOPOLYSACCHARIDE MODEL (II)

Intravenous administration of LPS resulted in an inflammatory response, which was seen as a peak in the Hp and ADA concentration at four hours post-injection. Both parameters returned close to baseline soon after this peak (Figure 8).



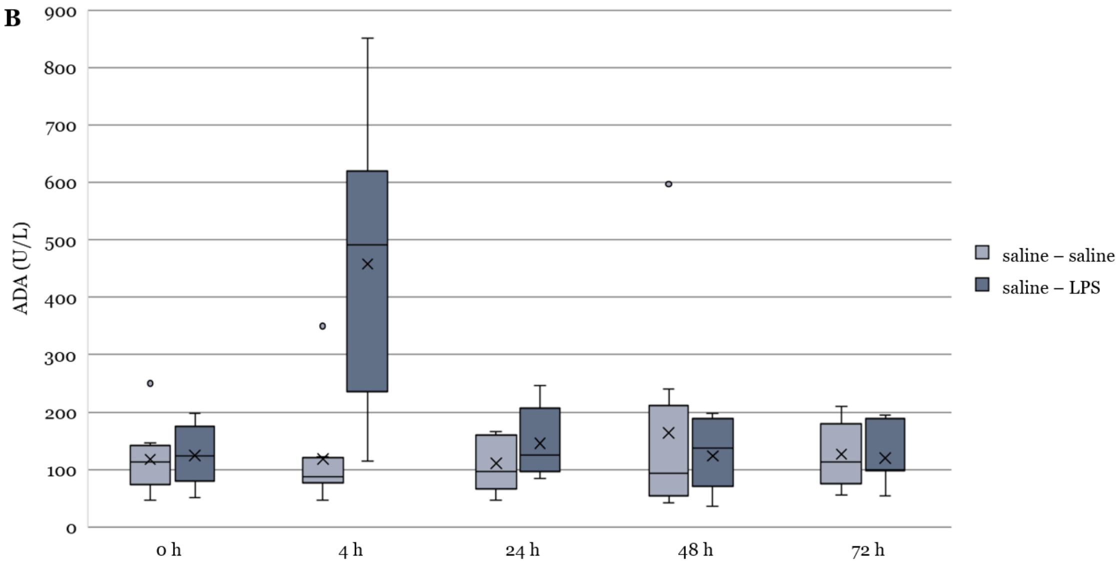


Figure 8 Salivary A) haptoglobin (Hp) and B) adenosine deaminase (ADA) concentration, as raw values, during a 72-hour period in healthy female growing pigs under experimental conditions. Saline – saline = control group (n = 9). Saline – lipopolysaccharide (LPS) = experimental group (n = 7), in which one intravenous dose of LPS was administered. At baseline, pigs had not received either saline or LPS. Cross (x) = mean, horizontal line within the box = median, end edges of the boxes = 25th and 75th percentiles, extreme ends = minimum and maximum concentration, and dots outside the box plots = outliers.

Within the saline–LPS group, the salivary Hp concentration was significantly higher four hours post-injection compared with 48 and 72 hours post-injection (pairwise comparisons, $p < 0.01$ for both). Salivary ADA was significantly higher four hours post-injection compared to all other time points within the saline–LPS group (pairwise comparisons, $p < 0.001$ for all). At baseline, the correlation between salivary Hp and ADA was very weak and non-significant (Spearman correlation, $\rho = 0.09$, $p = 0.7$, n = 16). A strong and significant positive correlation between salivary Hp and ADA responses was found (Spearman correlation, $\rho = 0.86$, $p = 0.01$, n = 7) in the saline-LPS group four hours after LPS treatment.

5.3 DYNAMICS AND RELATIONSHIPS OF IMMUNE AND INFLAMMATORY BIOMARKERS IN COMMERCIAL GROWING PIGS (III)

5.3.1 DYNAMICS OF SALIVARY BIOMARKERS OVER PRODUCTION STAGES

All biomarker concentrations differed significantly between production stages (repeated-measures LMM, $p < 0.001$) in pairwise comparisons ($p <$ 001 for all). Considerable variation between biomarker concentrations was observed between farms in each production stage, and especially in suckling and post-weaning stages (Figure 9).

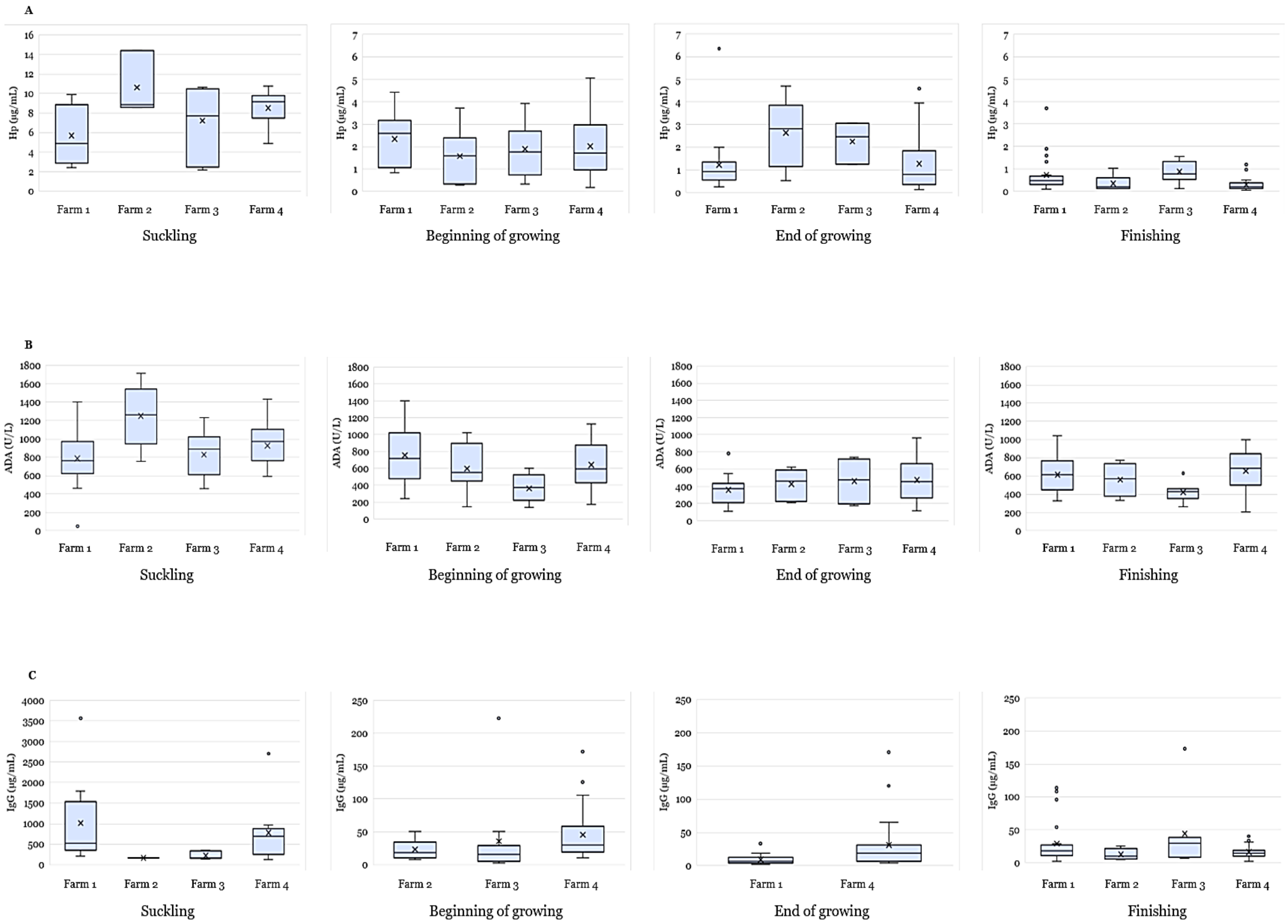

A
Hp (µg/mL)
16
14
12
10
8
6
4
2
0
7
5
3
1
Farm 1
Farm 2
Farm 3
Farm 4
Suckling
Beginning of growing
End of growing
Finishing
B
ADA (U/L)
1800
1600
1400
1200
1000
800
600
400
200
0
C
IgG (µg/mL)
4000
3500
3000
2500
2000
1500
1000
500
250
200
150
100
50

Figure 9 Salivary A) haptoglobin (Hp), B) adenosine deaminase (ADA), and C) immunoglobulin G (IgG) concentration in four consecutive production stages, and separately by farm, in commercial pigs. Note the higher scale of the y-axis for Hp and IgG in suckling piglets compared to other production stages. No salivary IgG results were obtained from Farms 2 or 3 at the end of the growing period. Cross (x) = mean, horizontal line within the box = median, end edges of the boxes = 25th and 75th percentiles, extreme ends = minimum and maximum concentration, and dots outside the box plots = outliers.

5.3.2 RELATIONSHIPS BETWEEN BIOMARKERS AND GENDER DIFFERENCES

The correlations between salivary biomarkers were positive in each production stage, according to the coefficient of correlation. The strength of the agreement between salivary biomarkers varied from very weak to strong (Table 5). Significant correlations were found in all production stages except suckling, and in most cases between salivary Hp and IgG (Table 5).

Table 5. Correlations between salivary haptoglobin (Hp), adenosine deaminase (ADA), and immunoglobulin G (IgG) in four production stages.

	SUCKLING		BEGINNING OF GROWING		END OF GROWING		FINISHING	
	ADA	IgG	ADA	IgG	ADA	IgG	ADA	IgG
Hp	ρ 0.140 P 0.407 n 37	ρ 0.279 P 0.198 n 23	**ρ 0.367** **P 0.005** **n 56**	**ρ 0.400** **P 0.014** **n 37**	**ρ 0.478** **P < 0.001** **n 49**	**ρ 0.661** **P < 0.001** **n 40**	ρ 0.104 P 0.440 n 57	**ρ 0.695** **P < 0.001** **n 57**
ADA		ρ 0.070 P 0.744 n 24		ρ 0.232 P 0.155 n 39		**ρ 0.619** **P < 0.001** **n 40**		ρ 0.169 P 0.209 n 57

Non-parametric Spearman correlations were computed; ρ = Spearman correlation coefficient, P = *p*-value, n = number of available samples for the analysis. Darker blue highlighting denotes a stronger correlation coefficient. Significant correlations are bolded.

The median concentration of most salivary biomarkers was numerically higher in males compared to females, except for salivary IgG in suckling piglets (Figure 10). The only statistically significant difference was found regarding salivary Hp, showing that females have significantly lower concentrations compared to males (repeated measures-LMM, p = 0.045).

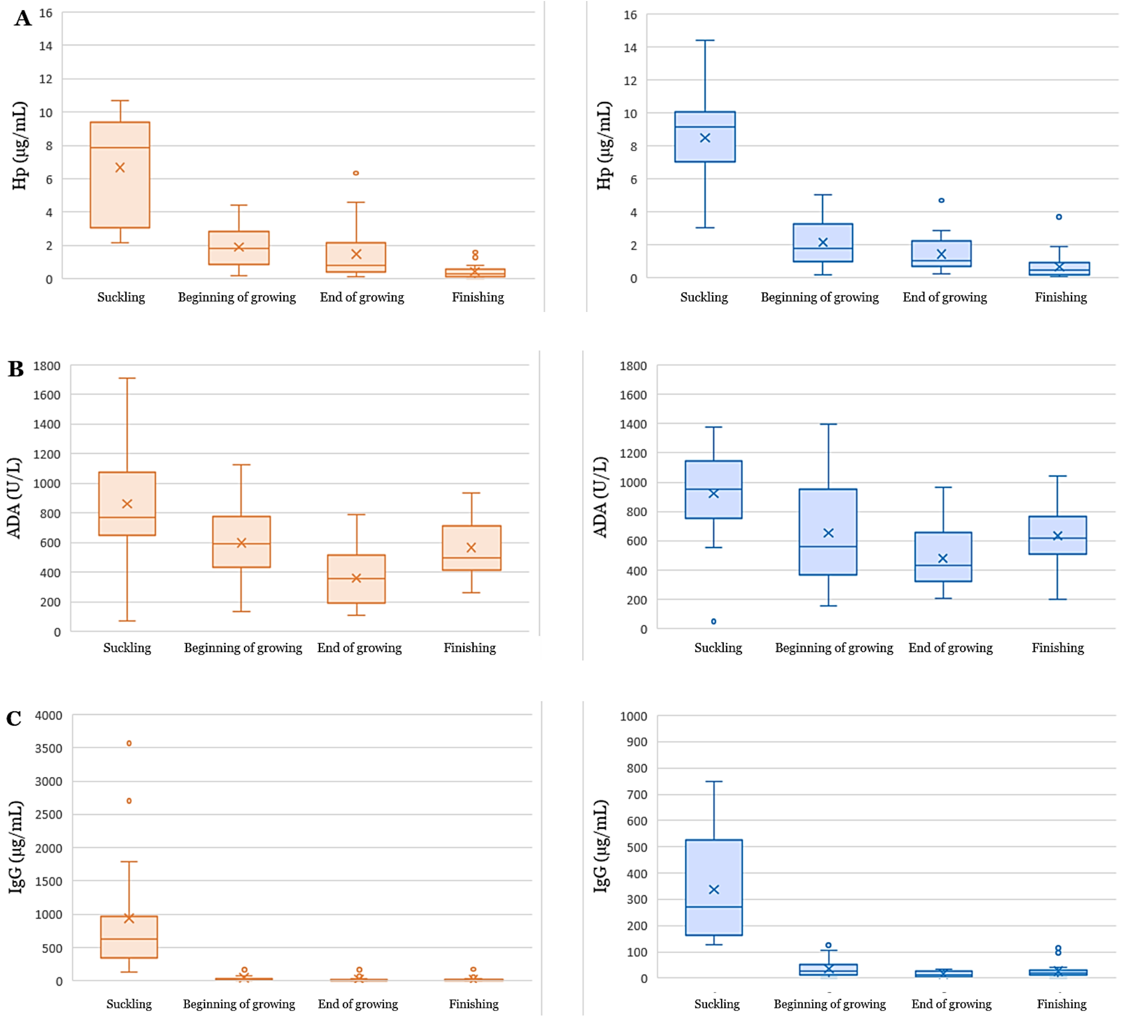


Figure 10 Salivary A) haptoglobin (Hp), B) adenosine deaminase (ADA), and C) immunoglobulin G (IgG) concentration of female (orange) and male (blue) pigs in four consecutive production stages. Note the higher scale for females compared to males on the y-axis for IgG. Cross (x) = mean, horizontal line within the box = median, end edges of the boxes = 25th and 75th percentiles, extreme ends = minimum and maximum concentration, and dots outside the box plots = outliers.

5.3.3 ASSOCIATION OF BIOMARKERS WITH MEAT INSPECTION FINDINGS

Across 83 finishers, the median (interquartile range) concentrations of salivary Hp in the group of pigs without (n = 56) and pigs having a MI finding considered inflammatory or infectious (n = 27) were 0.44 (0.47) µg/mL and 0.31 (0.36) µg/mL, respectively. The corresponding concentrations of salivary ADA were 592.6 (338.1) U/L and 625.3 (279.3) U/L, and for salivary IgG 16.1 (14.1) µg/mL and 14.1 (25.3) µg/mL.

Neither the Hp, ADA, nor the IgG concentration in finishing pigs was associated per se with MI findings (LMM, $p > 0.05$ for all). For ADA, a significant interaction between MI group and gender (LMM $F_{51} = 4.4$, $p < 0.05$) was recorded, and revealed that castrated male pigs with MI findings had the highest salivary ADA concentrations, followed by those without MI findings, and the lowest concentration was measured in female pigs with MI findings.

6 DISCUSSION

The results from Studies I–III are discussed separately below and are summarized at the end of the Discussion.

6.1 STUDY I

In Study I, greater AMU for young pigs compared to sows and finishing pigs was expected, but was not entirely supported by the results obtained, as suckling piglets and sows were the age groups most frequently treated with antimicrobials. As hypothesized, however, total AMU (as mg/PCU) was higher on piglet-producing farms than finishing farms. The hypothesis was based on earlier studies in which suckling piglets and weaned piglets have been reported to receive considerably more antimicrobial treatments than finishing pigs (Jensen et al., 2012; Sjölund et al., 2016; Raasch et al., 2018; Raasch et al., 2020; Yun et al., 2021). Additionally, AMU in sows is usually reported to be low, and even lower than that of finishing pigs (Sjölund et al., 2016; Raasch et al., 2018; Yun et al., 2021). Contrary to this, the median TI of 15.1 for sows in the present investigation was three-fold higher than that of breeders (including sows, gilts, and boars) in a Finnish study by Yun et al. (2021). This relatively high AMU recorded in sows, in particular, requires further investigation in order to better understand and promote prudent AMU in pigs. One explanation could be that the standard weight of a sow of 220 kg is low compared to the actual size of the currently used sows, which may result in an overestimation of sow TI. On the other hand, a previous Finnish study (Yun et al., 2021) used the same formula (Timmerman et al. 2006) and standard weight for sows and obtained results more similar to those reported elsewhere in Europe. In addition to that, the studied farms were recruited by a convenience sampling, being therefore not fully representative of the Finnish pig population and could have some particular disease issues that influenced their AMU.

It is known that AMU differs between farms within a country (van Rennings et al., 2015; Sjölund et al., 2016; Raasch et al., 2018; O'Neill et al., 2020; Yun et al., 2021). As indicated above, variation in AMU between farms in Finland may result from differences in the disease situation on the participating farms, but also from the veterinary guidance on how to treat the pigs, how the farm employees follow the veterinary advice, as well as the ability of farm employees to properly diagnose diseases. Moreover, even though not studied here, farm size may influence AMU, as reported in a Finnish study by Stygar et al. (2020), in which the number of antimicrobial treatments in pigs increased as a function of increasing farm size. The participating farms were on average larger than the average size of Finnish farms (Luke, 2023b). However, some other researchers have not found an association between farm size and antimicrobial use (Collineau et al., 2017b; Matheson et al., 2022).

The vast majority of antimicrobial treatments were administered parenterally, differing greatly from what has been reported in most European countries (O'Neill et al., 2020; EMA, 2022). Benzylpenicillin was the most common antimicrobial administered to all age groups, and was accompanied by a relatively low occurrence of AMR on the study farms, and it could be interpreted that penicillin is still effective in most cases. Benzylpenicillin and aminopenicillins are commonly used antimicrobials in pigs in Finland (Heinonen et al., 2001; Yun et al., 2021) and elsewhere (De Briyne et al., 2014; Sjölund et al., 2016). Tetracyclines were reported to be used in lesser amounts on the studied farms than is generally reported in pigs (Chantziaras et al., 2014; van Rennings et al., 2015; De Briyne et al., 2014; Sjölund et al., 2016). This is presumably due to the low amounts of oral antimicrobials used in Finland, as tetracyclines are often administered orally via feed or water elsewhere (Callens et al., 2012 [Belgium]; O'Neill et al., 2020 [Ireland]).

Antimicrobials considered as CIAs were used on the study farms, but these mostly consisted of injectable amoxicillin administered on piglet-producing farms. Especially in the case of fluoroquinolone and macrolide use, we do not have information on whether this use was based on susceptibility testing results. Therefore, no conclusions can be drawn about the misuse of antimicrobials in this case. Moreover, fluoroquinolones, which had been used in occasional cases on some of the study farms, were nationally banned by the pig industry in 2019 (Animal Health ETT, 2019). De Briyne et al. (2014) summarized the use of CIAs and other antimicrobials according to the treatment indication (respiratory disease, diarrhea, infections caused by *Streptococcus suis*, and postpartum dysgalactia syndrome (PDS) in sows) in pigs in 25 European countries. According to their results, the majority of cases were treated with non-CIAs, primarily penicillins (De Briyne et al., 2014), which is in line with the present study. Macrolides were the most common CIA prescribed for diarrhea and respiratory diseases, whereas fluoroquinolones were prescribed for PDS and also for diarrhea (De Briyne et al., 2014), and these antimicrobials are not considered as first-antimicrobials for such indications in Finland (Finnish Food Authority, 2016). Moreover, contrary to our results, 3rd and 4th generation cephalosporines are widely used in other European countries (De Briyne et al., 2014). However, the current situation regarding AMU between different countries may have changed, possibly due to implementation of national action plans against AMR and due to published international guidelines for prudent AMU (see for example EMA, 2020).

In comparison to CIA –classification intended for AMU in humans, there is more up-to-date classification of veterinary antimicrobials available, which categorizes antimicrobials in order to promote the prudent AMU from both human and animal perspectives (EMA, 2020). According to this categorization, however, no antimicrobials to be avoided (category A) (EMA, 2020) were used on the study farms. Moreover, antimicrobials with restricted use (category B) (EMA, 2020), including fluoroquinolones, were used only occasionally and the vast majority of antimicrobials used on the studied farms belonged to a prudence category (D) (EMA, 2020). As the EMA categorization has built around the topic of prudent AMU, it should be taken into account in further studies where veterinary AMU is assessed.

On the studied farms, locomotory diseases were the most common indication for antimicrobial treatment on both farm types, thus being more common in sows compared to finishing pigs. Previous Finnish studies support the finding that locomotory diseases were among the most common treatment indications in fattening pigs, along with tail biting, gastrointestinal diseases, and respiratory diseases (Heinonen et al., 2001; Stygar et al., 2020). Jensen et al. (2012) also reported that locomotory diseases were amongst the most common treatment indications in sows, but their records additionally included treatments of suckling piglets. In the present study, the indication "Other" was relatively common in weaned piglets, and mainly consisted of antimicrobial treatment for tail biting. In summary, the combination of information on antimicrobial used for different age groups and the indications for treatment is more representative of the true disease situation at the farm level than only quantitative data on AMU. Nevertheless, at the population level, the monitoring of AMU is important in order to follow the pattern of use over time (Collineau et al., 2017a).

Pleuritis and abscesses were the most prevalent MI findings recorded on the study farms in both sows and finishing pigs. Moreover, shoulder sores in sows and joint inflammation in finishing pigs were relatively common. Unexpectedly, the observed associations between AMU and MI findings were negative, indicating that the higher the AMU, the less prevalent were MI findings. It could be speculated that AMU leads to recovery from diseases, and therefore reduces the prevalence of MI findings at slaughter. The treatment threshold might differ between the studied farms, and on some farms, a lower treatment threshold might result in the earlier treatment of acutely sick pigs, which would also result in a better recovery from the diseases, and prevent the spread of disease in the population.

In sows, a strong and negative relationship between the TI for reproductive diseases and the prevalence of both shoulder sores and arthritis at slaughter remains unexplained. It would have been logic if there had been a positive correlation between these variables, because sows suffering from reproductive diseases post-partum are more prone to infections caused by shoulder sores that further predispose to systemic infections and arthritis. However, the finding needs a more thorough assessment. Not surprisingly, pleuritis was the most common MI finding recorded for finishing pigs, in which the negative correlation between AMU and the prevalence of pleuritis also warrants further investigation. As respiratory diseases in finishing pigs are not always clinically apparent but weaken the affected individuals (Zimmerman et al., 2019), they might be more prone to contracting other diseases. This could lead to increased AMU and a higher prevalence of MI findings, which was not the case in the present investigation, although hypothesized. The negative relationship between the TI of finishers for respiratory diseases and the prevalence of abscesses at slaughter might be due to abscess formation being a long process and not always related to respiratory disease, but instead to locomotory diseases and tail biting. Furthermore, the TI of finishing pigs for other indications, mainly tail biting, was strongly and positively correlated with the prevalence of joint inflammation at slaughter. This finding supports a previous Finnish report by Valros et al. (2020).

In summary, register-based data on AMU and MI findings alone are not as useful in farm health management as their combination with other sources of information. Such information includes, for example, practical health management on individual farms, including monitoring of the level of how skilled animal caretakers are in disease detection, farm medication practices and the threshold for starting treatment, and response to treatments and mortality rates. Thus, monitoring and diagnostic tools applicable at the farm level like improvement of educational level of animal caretakers and development of analyses carried out alongside the pigs are needed in the future.

In comparison with other EU countries (EFSA, 2022), the results of national AMR surveillance have revealed a low occurrence of AMR among the indicator bacteria *E. coli* in Finnish slaughter pigs (Finnish Food Authority, 2022a), and the occurrence of resistance was therefore expected to be low on the studied farms. However, resistance occurrence and the occurrence of MR isolates were numerically higher on the studied farms compared to the figures reported nationally (Finnish Food Authority, 2022a). Among the studied isolates, the proportion of fully susceptible isolates was 58.8%, which is lower than the national trend: since 2015, the proportion of fully susceptible indicator *E. coli* isolates has increased from 71% to 78% in 2021 (Finnish Food Authority, 2022a). These result could be due to the relatively low number of farms selected in the present study, differences in sampling between national monitoring and the present study (at slaughter from individuals and on farms at the pen level, respectively), and differences in AMU.

Nevertheless, resistance against the tested antimicrobials chosen based on the standardized test panel (EUCAST, 2021) was similar to the national results, as the most commonly found resistance phenotypes reported have been tetracycline, sulfamethoxazole and trimethoprim, and ampicillin over time (2004–2021) (Finnish Food Authority, 2022a). A similar trend in AMR occurrence has been reported at the European level among *E. coli* indicator bacteria (Österberg et al., 2016; Ceccarelli et al., 2020; EFSA, 2022), although AMR occurrence differs between countries (Chantziaras et al., 2014; Österberg et al., 2016; Ceccarelli et al., 2020; EFSA, 2022). Single isolates were resistant against fluoroquinolones (ciprofloxacin and nalidixid acid) and chloramphenicol, whereas none of the isolates were resistant against azithromycin, colsistin, gentamicin, tigecycline, meropenem, or 3rd generation cephalosporines. This result is in line with the long-term trend reported in Finland (EFSA, 2022).

Tetracycline resistance is common in pigs and other farm animals, primarily due to the widespread use of tetracycline antimicrobials in these species (Chantziaras et al., 2014; Poirel et al., 2018). The MR phenotypes were mostly resistant against tetracycline in combination with sulfamethoxazole and trimethoprim, and ampicillin, which also reflects the occurrence of resistance among MR indicator *E. coli* at the European level (EFSA, 2022). Multi-resistance in bacteria is acquired (Schwarz et al., 2010), and of the various resistance mechanisms described, horizontal gene transfer is important for *E. coli* (Poirel et al., 2018). Similar to tetracyclines, sulfonamide antimicrobials in combination with trimethoprim have been widely used in veterinary medicine, and resistance against both antimicrobials is mainly mediated via mobile genetic elements (Poirel et al., 2018). Selective mechanisms that favor the persistence of resistance in bacteria include antimicrobial selective pressure, resistance coselection via mobile genetic elements such as plasmids, resistance acquisition without costs to bacteria or resistance that is beneficial to fitness, and compensatory mutations that restore bacterial fitness (Andersson & Hughes, 2011). These compensatory mutations, in particular, have been suggested to be a stabilization mechanism in bacteria that allows them to restore their resistance, regardless of the changing antimicrobial exposure in the environment (Andersson & Hughes, 2011). The phenotypic resistance of MR isolates corresponded to the major antimicrobial groups used on the studied farms and are likely a result of resistance co-selection in which bacteria acquire resistance genes via genetic mutations or via gene transfer (Cantón & Ruiz-Garbajosa, 2011). A recent pig-study showed that AMR in *Enterobacteriaceae* is well-maintained regardless of the AMU selective pressure, and besides antimicrobials, metals and biocides contribute to the persistence of AMR (Li et al., 2022). These factors could have influenced the persistence of the described AMR phenotypes also on the studied pig farms irrespective of the generally observed low AMU.

6.2 STUDY II

As expected, LPS administration clearly induced an APR, which was seen as a peak in both the Hp and ADA concentration in the saline–LPS group but not in the saline–saline group at four hours post-injection. In the same pigs, sickness behavior (including decreased general activity and feed intake, and more observed lying on the sternal position with pigs´ heads up) was also observed (Veit et al., 2021), which further confirmed the results. A positive relationship between Hp and ADA responses was confirmed in the present study, as presumed. According to previous reports, a positive correlation between Hp and ADA concentrations in saliva has been reported (Sánchez et al., 2021), thus suggesting that both biomarkers respond to a similar stimulus. Lipopolysaccharide is a strong antigen, validated for use in systemic inflammatory models (Seemann et al., 2017). Comparison with other LPS studies assessing the APR is difficult, because both the LPS dose, serotype and administration routes have differed between studies (Dritz et al., 1996; Escribano et al., 2014; Nordgreen et al., 2018).

At least for Hp, it is known that different triggers result in a Hp response differing in magnitude and duration (Murata et al., 2004; Heegaard et al., 2011), being stronger during infection (Murata et al., 2004) compared to trauma or stress (Murata et al., 2004). In pigs, the ADA concentration has been reported be higher in pigs suffering from respiratory and gastrointestinal diseases than in pigs suffering from local inflammation (Gutiérrez et al., 2017), indicating that ADA possibly also has different dynamics depending on the trigger. Based on the results from Study II, it can be concluded that both salivary ADA and Hp are valid biomarkers of an acute systemic illness.

6.3 STUDY III

Not surprisingly, and in accordance with the results of Gutiérrez et al. (2012a), we observed considerable between-farm differences in salivary biomarker concentrations. As hypothesized, all the salivary biomarker concentrations differed between production stages. The highest concentrations of Hp, ADA, and IgG were measured in suckling piglets, irrespective of gender. Sow colostrum is known to contain bioactive substances (Sauce L., 2018), such as Hp (Hiss-Pesch et al., 2011) and IgG (Curtis & Bourne, 1971). As ADA is present in all mammalian cells, and especially in lymphocytes, which are also known components of colostrum, it could be a potential source of ADA in piglets. Determination of the colostrum ADA concentration would be of interest in further studies. Moreover, colostrum Hp induces Hp production in piglets (Hiss-Pesch et al., 2011), and future studies should therefore especially examine the role of both endogenous and exogenous ADA in the development of the immune system. In general, individual saliva sampling of piglets aged from one to five days was time consuming, and the majority of the missing samples due to an insufficient amount of saliva were from suckling piglets in the present study. A recent study investigated group-level oral fluid collection from 21- and 28-day-old suckling piglets (Boulbria et al., 2020). The results suggested that piglets should be trained prior to saliva collection, and older piglets displayed more spontaneous interaction with the oral fluid collection rope compared with younger ones (Boulbria et al., 2020). Unfortunately, no studies on individual saliva sampling of suckling piglets have been reported. However, based on the results from the study of Boulbria et al. (2020) and practical experience from Study III, we cannot recommend individual saliva sampling of suckling piglets.

In a recent study by Ortín-Bustillo et al. (2022), the dynamics of salivary Hp and ADA were similar to those seen in Study III, but opposing differences were observed between female and male pigs, with females having higher concentrations. Similarly to the results of Study III, the production stage that corresponded to an age of the study pigs was a significant predictor of salivary Hp and ADA concentrations (Ortín-Bustillo et al., 2022). Sánchez et al. (2019) reported that salivary Hp and ADA are dependent on both the production stage and gender, with higher concentrations measured in males in the initial stages of production and in females in later stages. According to Gutiérrez et al. (2018), higher salivary Hp and ADA concentrations were measured in female finishing pigs compared to males, similarly to Sánchez et al. (2019). The contradictory results of different studies may result from differences in the age of the pigs at the time of measurement, but also from breed differences, as the pig breed influences the salivary Hp and ADA concentrations (Sánchez et al., 2019). The study population in Study III included crossbred DanBred x Landrace pigs, as well as pure DanBred and TopigsNorswin pigs. In other studies, Duroc x (Landrace x Large White) (Gutiérrez et al., 2009b; 2011; 2012a; 2017), pure Large White (Contreras-Aguilar et al., 2020; Ortín-Bustillo et al., 2022), Duroc x Large White (Sánchez et al., 2019), or Iberian pigs (Sánchez et al., 2019) have been used, which may also have influenced the results. Besides, different disease pressure on farms may have substantially had an impact on the results. As the disease status of the studied farms was not systematically evaluated, no clear conclusions from the issue can be done.

In case of salivary IgG, no longitudinal studies have been conducted in pigs. However, we found a moderate positive correlation between salivary IgG and Hp at the beginning of the growing stage, and a strong correlation at the end of the growing and finishing stages. Moreover, at the end of the growing stage, there was a strong correlation between salivary IgG and ADA. These new results could be related to both gradually developing immunity of pigs, and also to changes in the environmental infection pressure. However, the links between these biomarkers should be investigated in a larger study population, as the desirable sample size in the present study was not achieved. Furthermore, because similar field studies have not been widely conducted, further studies are needed to strengthen the evidence found.

Another hypothesis was that pigs having inflammatory and/or infectious MI findings at slaughter would have higher biomarker concentrations compared to those with no such findings (III). Individual MI findings were not associated with the concentration of any biomarker in finishing pigs. The MI findings were similar to those published nationally (Finnish Food Authority, 2023), and in line with the findings of Study I. Because the occurrence of pleuritis is clearly a significant disadvantage to the industry, more sensitive methods to detect especially sub-clinical conditions in pigs are needed. The lack of an association between MI findings and biomarkers may be due to the time that had elapsed between saliva sampling and slaughter, as well as due to the relatively low sample size. Acute diseases would have healed by slaughter, while on the other hand, the study pigs did not display any evident clinical signs of disease at the time of sampling.

6.4 IN SUMMARY (I–III)

Study I demonstrated that the combination of quantitative and qualitative data on AMU serves as a good porcine health indicator on farms, but should be combined with other veterinary measures (e.g., clinical inspection of farm animals and sampling) to be more representative the real situation on the farms. Investigation of the treatment indications together with MI findings provides a good overview of the disease situation on individual farms, and could be used as a tool in farm health care planning. According to our results, however, the relationship between AMU and the prevalence of MI findings may be more complex than thought. Nevertheless,, additional monitoring of possible sub-clinical illness via salivary biomarkers at the farm level could improve the health evaluation of pig farms in the future and contribute to prudent AMU. The results of Studies II and III strengthen the evidence that ADA has a role as an acute phase biomarker of systemic disease rather than being a biomarker of chronic illness or local conditions. Biomarkers cannot be used in individual pig treatment decisions as they are general, unspecific Further studies on Hp and ADA are needed in order to develop practical on-farm solutions. The present results, however, strengthen the evidence that in the interpretation of analytical saliva samples, both the production stage (age) and gender need to be considered. In Study II, between-farm differences were marked, which further suggests that biomarker levels should be examined on each individual farm, followed by the preparation of a targeted health management plan as biomarkers alone are probably not enough in assessing the health status of pigs.

6.5 LIMITATIONS

The database used in this research, Sikava, has not been designed to be used in scientific research and it therefore lacks some properties needed. However, this database is the only one in which this type of data can be collected. When interpreting register-based data, it is important to keep in mind possible limitations. The Sikava data contained irrational AMU records of sows and suckling piglets and data was collected via convenience sampling from 24 pig farms only. For AMU quantification, two widely used methods in pig studies (mg/PCU, TI) were chosen, but still the lack of standardized methods to collect data on AMU has also been raised in other studies and recognized as a limiting factor when comparing results between studies (Collineau et al., 2017a; Lekagul et al., 2019). Calculations based on prescriptions only are or may be scarcer, and when the level of quantification is relative to the actual number or biomass of animals treated, the level of quantification becomes more accurate, as reported in experiment I. Thus, the results could be influenced by assumptions such as the defined standard weight and period at risk (length of certain production phases).

Although the methods to quantify AMU chosen in Study I were valid for the investigation of AMR selection pressure as reviewed by Collineau et al. (2017), there is no consensus available for the best method to evaluate the relationships between AMU and AMR. Antimicrobial compounds differ in their molecular weight and it is not therefore feasible to use a weight parameter such as milligrams or kilograms to gain insight into the selection pressure for the development of antimicrobial resistance (Postma et al., 2015). Stygar et al. (2020) argued against the use of treatment incidence in order to evaluate antimicrobial exposure in finisher pigs, because the rapid increase in body weight may result in a calculation (estimation) error. Postma et al. (2015) reported that the recommended doses for the same antimicrobial active substance were highly variable between orally administrable antimicrobial products within a country (e.g., tylosin in Belgium) and between countries (e.g., chlortetracycline in Germany vs. Belgium and Sweden). In general, the dose recommendations for antimicrobial products administered in feed/water deviate from the consensus DDD value for animals more than dose recommendations for parenteral products (Postma et al., 2015). Moreover, the duration of treatment courses for products administered in feed and water was highly variable, whereas less variation was reported for parenteral products between countries (Postma et al., 2015). Pooled fecal samples were collected once and from one room on each farm, and the representativeness of the sample was not therefore optimal.

Although saliva collection is perceived as easy to learn, saliva collection from suckling piglets is difficult compared to post-weaning and older pigs. In Study II, several persons were involved in the sampling procedures, which may have influenced the results. However, the researchers followed a similar protocol for sampling. There is interest in the use of saliva instead of serum for analytical purposes and monitoring. However, there is more emphasis on sampling in later stages of production, because samples from weaned piglets and older pigs are, in contrast, very easy to collect. Saliva sampling of suckling piglets could be reserved for experimental studies, such as the investigation of immune system development.

The study pigs were visually inspected at the time of sampling, but no clinical examination was conducted, which could explain the lack of an association between salivary biomarker concentrations and individual MI findings (Study III). Moreover, half of the study pigs were lost during the follow-up period, resulting in a relatively small sample size in Study III. In addition to that, the study pigs were sent to slaughter within 7 – 49 days after the last saliva sampling, which occurred only once. It is likely, that the health status of some of the pigs changed during that time. The associations between salivary biomarker concentrations and MI findings should be further investigated with a larger number of study animals kept under controlled conditions and examining pigs in the shorter term.

7 CONCLUSIONS

- Finnish pig farms appear to use antimicrobials prudently, as narrow-spectrum benzylpenicillin is the most common antimicrobial used, and to treat usually individual pigs. This presumably helps to sustain the AMR situation, which was generally in line with national monitoring results on the study farms, although resistance occurrence was slightly higher (I).
- Based on the results obtained, the association between AMU and the prevalence of MI findings was against the study hypothesis. In general, MI is a relatively crude measure and alone it may not be specific enough indicator of AMU. Thus the influence of other undefined factors should be explored with a larger number of farms and more thorough investigation of the health management of the study animals (I).
- LPS-induced systemic inflammation triggered an APR prior to any obvious clinical signs of disease, which was seen as an increase in salivary Hp and ADA concentrations in pigs. However, pigs showed decreased general activity and feed intake, and changes in their lying position considered as sickness behavior, which could in practice have gone unnoticed by the pig caretakers. Thus, saliva sampling in this age group of pigs has potential as a sensitive and cost-effective method to detect diseased pigs or pig groups (II). Currently, no tests are available to be applied at the farm-level to measure salivary biomarkers, but this could be the case in the future.
- The interpretation of salivary Hp, ADA, and IgG measurements requires consideration of the production stage that corresponds the age of the pigs, and gender, as well as differences between individual farms (III).
- Individual saliva sampling of suckling piglets in not practical, but the high concentrations of salivary biomarkers in this particular age group would be interesting to investigate further from an immunological research point of view (III).

8 IMPACT AND FUTURE PROSPECTS

Based on the results of the present work, monitoring of AMU and indications for their use can be considered as health indicators of individual farms when combined with other health monitoring measures, such as MI findings. As observed, the medicine use data in Sikava contained clearly erroneous treatments, which might be due to recording errors, and should be taken into account when interpreting the data. In countries, including Finland, where health registers of production animals are relatively well advanced, there is good preconditions to further improve the utilization of register-based data in field conditions. In order to achieve the future needs, more education of animal caretakers is needed together with veterinarians in order to harmonize the overall quality of the work.

To sustain prudent AMU and the relatively low AMR situation on Finnish pig farms in the future, veterinarians are key to ensuring that farm employees are competent at the timing and monitoring of treatments and linking this to the monitoring of clinical signs of the condition. Besides register-based data, other aspects of porcine health will be important to consider. At the farm level, salivary biomarkers, especially Hp and ADA, could be used to detect and monitor diseases at the time when mild signs of disease are present, allowing earlier disease detection than based on clinical signs only. Together with improvements in how skilled the animal caretakers are in observing the signs of disease, this could lead to better recovery of animals and decrease in unnecessary AMU. Currently, the use of salivary biomarkers as a part of practical farm work is not possible, because no rapid assays applicable on farms are available. However, as saliva sampling of pigs is described as easy to learn, farm employees could take samples by themselves and thereby make the veterinarian's job easier. Notably, there is still a lack of information on how to interpret salivary analysis results, although some salivary biomarkers are promising.

The link between individual MI findings and salivary biomarkers measured prior to slaughter remains unclear. The studied biomarkers, however, appear to be more representative of the prevailing disease conditions than past or chronic illness. Data collected at individual level is not necessarily useful in field conditions, because pigs are slaughtered in batches without individual records. However, in future studies similar study setting to ours with greater sample size would be useful to assess the representativeness of MI findings of the health status of finishing pigs, and understanding these associations could further help in the development of health monitoring tools.

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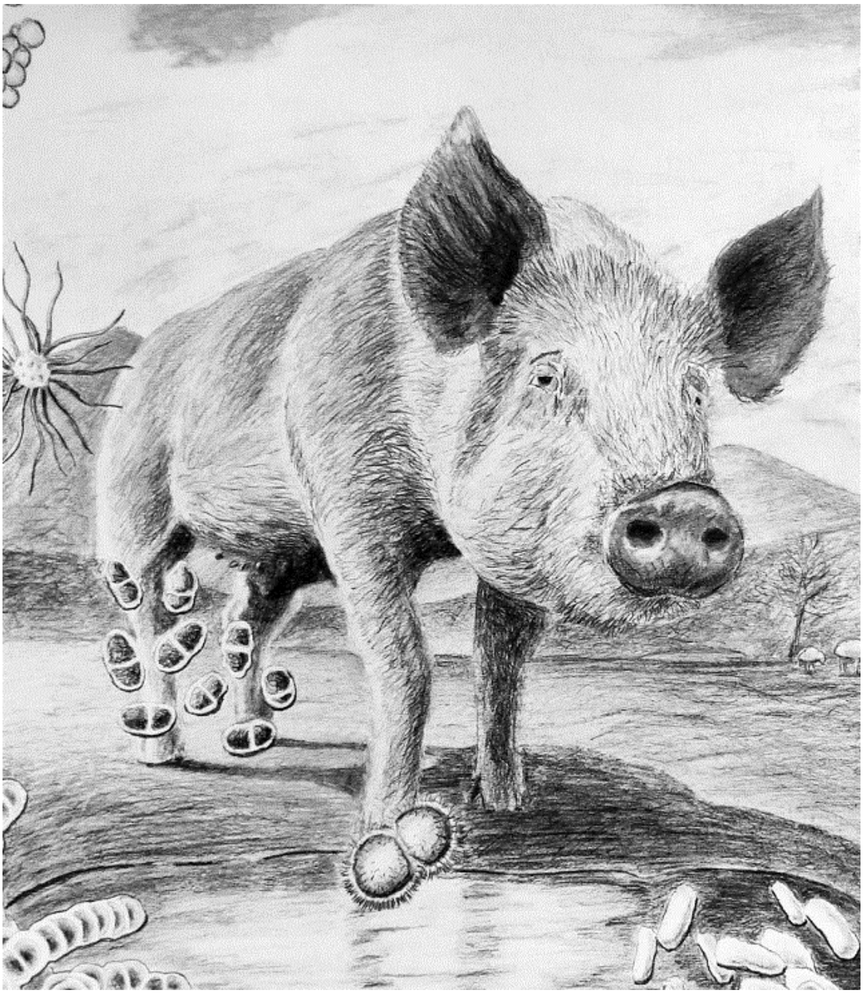

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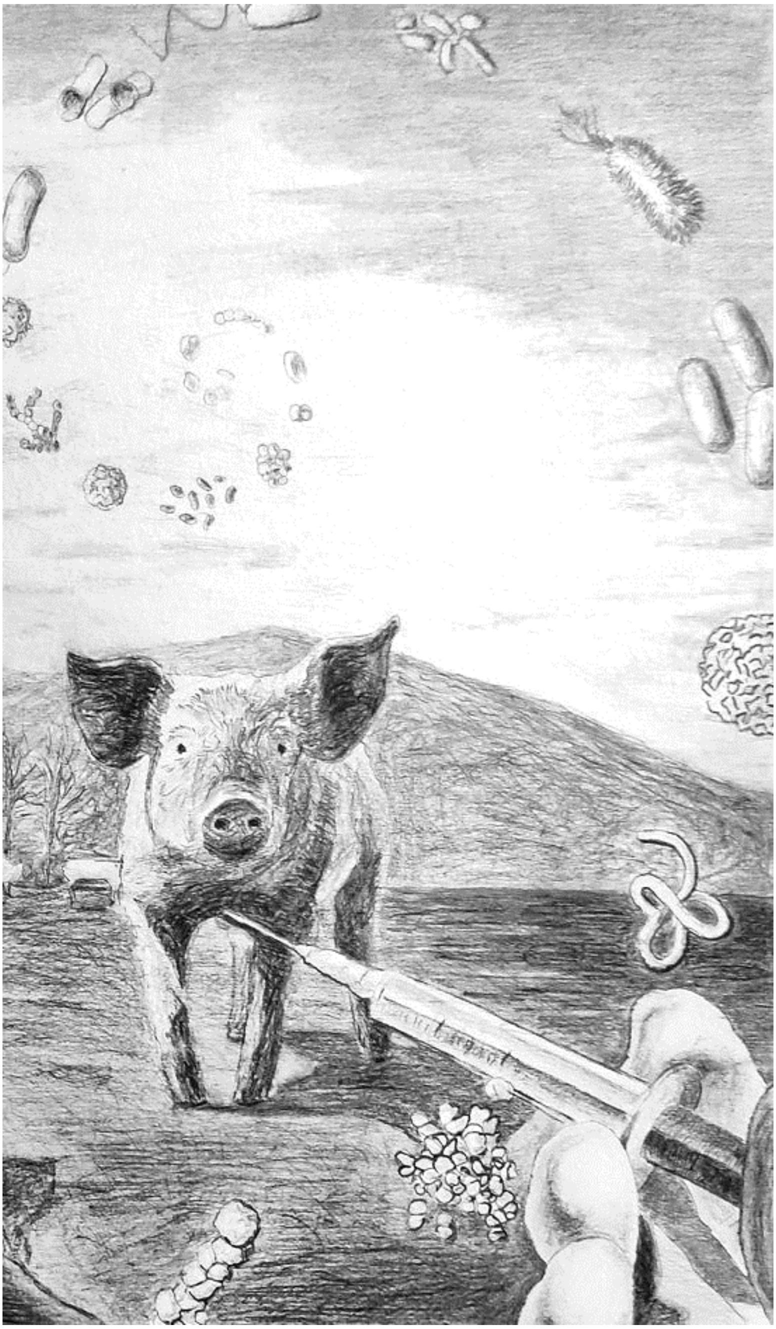

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