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DISSERTAÇÃO DE MESTRADO INTEGRADO EM MEDICINA VETERINÁRIA

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“The difference between ordinary and extraordinary is that little extra”

Jimmy Johnson

To my parents

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ABSTRACT

THE IMPACT OF VACCINATION ON THE CONSUMPTION OF ANTIMICROBIALS IN PIGS

Antimicrobial agents are being used in modern swine production worldwide, generating concern with regards to the development of antimicrobial resistance. Identifying efficient alternatives has therefore become a subject of interest. The aim of this study was to explore the impact of vaccination as an alternative to antimicrobial consumption in pig herds. The hypothesis was that herds with increased use of vaccination would have a lower antimicrobial consumption. Data were obtained from the Danish *VetStat* database in which prescriptions of medication for livestock are recorded as well as the Danish Central Husbandry Register. All Danish one-site pig herds, active in year 2013, with >50 sows and >200 weaners were selected for the study. Initially, data were analysed using a univariable model, and secondly a multivariable linear regression model was applied. The analyses included the use of three different vaccines against Porcine Circovirus Type 2 (PCV2), *Mycoplasma hyopneumoniae* (M_HYO) and *Lawsonia intracellularis* (LAW), respectively, as well as annual production measured as number of weaners produced in a year. The antimicrobial consumption was measured in animal daily doses (ADD). Out of the 1,513 herds selected for the study, 1,415 herds had antimicrobials prescribed for gastrointestinal disorders, and 836 for respiratory disorders. PCV2 vaccine was used in 880 herds, M_HYO vaccine in 787 and LAW vaccine was the least used, with 115 herds using it. The results suggested that antimicrobials, to some extent, were being used for other disease categories than those officially prescribed by the veterinarians. On average, herds using different combinations of vaccines had higher use of antimicrobials than herds not using the vaccines. Information about vaccination protocols, health status, biosecurity, and management practices was not available, limiting the ability to assess causality.

Key words: antimicrobial consumption, alternatives, vaccination, pigs, *VetStat*, Denmark.

RESUMO

O IMPACTO DA VACINAÇÃO NO CONSUMO DE ANTIMICROBIANOS EM SUÍNOS

Os agentes antimicrobianos são mundialmente utilizados na produção suinícola, originando preocupação no que diz respeito ao desenvolvimento de resistência a estes agentes. Nesse sentido, a identificação de alternativas eficientes à utilização de antimicrobianos tem ganho interesse. O objetivo do presente estudo foi explorar a utilização de vacinas como alternativa aos antimicrobianos em porcos de engorda. Os dados para a realização do estudo foram obtidos através do registo nacional dinamarquês de explorações e da base de dados *VetStat*, na qual são registadas as prescrições de medicamentos para as explorações. Todas as explorações suinícolas ativas em 2013, com a produção realizada num único espaço físico, >50 porcas e >200 leitões desmamados foram consideradas para o presente estudo. Inicialmente, os dados foram analisados recorrendo a um modelo univariável, e secundariamente a um modelo multivariável de regressão linear. A análise incluiu os dados correspondentes à utilização de 3 vacinas: circovírus suíno tipo 2 (PCV2), *Mycoplasma hyopneumoniae* (M_HYO) e *Lawsonia intracellularis* (LAW), e a produção anual – medida em número de animais produzidos por ano. O consumo de antimicrobianos foi medido em doses diárias por animal (ADD). Das 1513 explorações incluídas no estudo, 1415 tinham prescrição de antimicrobianos com indicação para afeções gastrointestinais, e 836 para afeções respiratórias. A vacina contra o PCV2 foi utilizada em 880 explorações, a M_HYO foi utilizada em 787 e a LAW, com a menor utilização, em 115. Os resultados sugeriram que os antimicrobianos estavam a ser utilizados, de certa forma, em categorias terapêuticas distintas daquela para a qual foram originalmente prescritas pelo veterinário. Em média, as explorações que utilizaram diferentes combinações de vacinas apresentaram uma maior utilização de antimicrobianos do que aquelas que não vacinavam. No entanto, deve ser tido em conta que informações relativas a protocolos de vacinação, categorização do estado sanitário das explorações, medidas de biossegurança e ainda práticas de maneio, não estavam disponíveis, constituindo uma limitação ao acesso da causalidade.

Palavras-chave: consumo de antimicrobianos, alternativas, vacinação, suínos, *VetStat*, Dinamarca.

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LIST OF ABBREVIATIONS, ACRONYMS AND DEFINITIONS

AC	Antimicrobial Consumption
ADD	Animal Daily Dose
AGPs	Antimicrobials as Growth Promoters
AR	Antimicrobial Resistance
CHR	Central Husbandry Register
DAFC	Danish Agriculture & Food Council
DANMAP	Danish Integrated Antimicrobial Resistance Monitoring and Research Program
DVFA	Danish Veterinary & Food Administration
EC	European Commission
ECDC	European Centre for Disease Prevention and Control
EFSA	European Food Safety Authority
EMA	European Medicines Agency
EP	Enzootic Pneumonia
EU	European Union
FAO	Food and Agriculture Organisation of the United Nations
HMA	Heads of Medicines Agency
OECD	The Organisation for Economic Co-operation and Development
OIE	World Organisation for Animal Health
PCV2	Porcine Circovirus Type 2
PCVDs	Porcine Circovirus Diseases
PE	Proliferative Enteropathy
PMWS	Postweaning Multisystemic Wasting Syndrome
PRDC	Porcine Respiratory Disease Complex
RUMA	Responsible Use of Medicines in Agriculture Alliance
USDA	United States Department of Agriculture
WHO	World Health Organisation

“**Antibiotic –** generally used in the past to mean antimicrobials. However, it is now more often used to mean antibacterials and is understood by the public and professionals in this way” (Heads of Medicine Agency [HMA], 2012, p. 2).

“**Antimicrobial –** a general term for natural or synthetic compounds which at certain concentrations inhibit growth of, or kill, micro-organisms. The term antimicrobial is a collective for anti-virals, anti-bacterials, anti-fungals and anti-protozoals**”** (HMA, 2012, p. 2).

Antimicrobial Resistance – is the resistance of a microorganism to an antimicrobial medicine to which it was originally sensitive. Resistant microorganisms (bacteria, fungi, viruses and some parasites) are able to withstand the attack by antimicrobial medicines, so that standard treatments become ineffective and infections persists increasing risk of spread to others (World Health Organisation [WHO], 2015b).

I – INTRODUCTION

1 – INTRODUCTION AND OBJECTIVES

It is estimated that 80% of the total global antimicrobial use is applied to livestock (Stärk, 2013; Ungemach, Müller-Bahrdt, & Abraham, 2006). The undisciplined use of antimicrobials has created a strong selective pressure in bacteria which has contributed for the survival and spreading of resistant bacteria (Barbosa & Levy, 2000). Therefore, the persistence and spreading of antimicrobial resistance (AR), in conjunction with decreased profitability of new antibiotics, have created the dangerous prospect of a post-antibiotic era, where the effectiveness of antimicrobials in human and livestock health is being threatened (Parisien, Allain, Zhang, Mandeville, & Lan, 2008; Stanton, 2013).

The World Health Organisation (WHO) has classified AR as a global health problem in need of urgent action (World Health Assembly, 1998). Consequently, reduced antimicrobial usage in livestock is promoted as a public health measure to reduce AR (WHO, 2001). The focus is both in promoting prudent usage and improving non antimicrobial disease prevention (Jensen, Knegt, Andersen, & Wingstrand, 2014). The identification of efficient alternatives to the use of antimicrobials has gathered global interest (Seal, Lillehoj, Donovan, & Gay, 2013).

The aim of this dissertation was to evaluate the impact of routinely used vaccination as an alternative to antimicrobial consumption in pig herds. It is a result of a project which started with a traineeship period of three months in Denmark, which represented the fulfilment of requirements for obtaining the degree of Master of Veterinary Science at the Faculty of Veterinary Medicine – University of Lisbon.

This dissertation is composed by three main parts. The first is the present one, with an introduction to the theme, aims of the dissertation and a description of the curricular training. In part II, a comprehensive literature review was performed according to the aims of the dissertation. It provided important background for this work. Chapter III corresponds to the project – research work. It was written as a manuscript for a peer reviewed journal, so it is possible for the reader to read this part independently from the literature review.

2 – CURRICULAR TRAINING DESCRIPTION

The introductory part of the curricular training took place at the Faculty of Veterinary Medicine – University of Lisbon, at the Department of Animal Production and Food Safety. A training period of one month under the supervision of Dr. Telmo Nunes, introduced the author to various important topics of veterinary epidemiology, data management and software.

From March to June 2014, the second part of the training was held at the University of Copenhagen, Faculty of Health and Medical Sciences, Department of Large Animal Sciences, Sector for Animal Welfare and Disease Control, with the supervision of Dr. Liza Rosenbaum Nielsen and at the Danish Agriculture & Food Council (DAFC), with the supervision of Dr. Lis Alban. Several meetings were arranged with Dr. Liza Rosenbaum Nielsen, Dr. Lis Alban and Dr. Ken Steen Pedersen, in order to designate the project and to discuss its progress throughout the weeks and different phases. An intensive literature review was devised concerning the different vaccines and diseases chosen for the study, the challenge of antimicrobial resistance, the legislation and initiatives of Danish government to address high antimicrobial consumption in pig production and the alternatives to antimicrobial consumption which are currently in use, as well as future prospects in these different areas. Moreover, throughout the entire training period, special focus was given on the development of competences in data analysis as well as software learning – R (version 3.1.2 of 2014 – The R Foundation for Statistical Computing).

Beyond the project itself, another aim of the curricular training was to understand the overall scenario of pig production in Denmark. In consequence, several visits to different pig herds were carried out. The aim of these visits were to provide the author with an overview of the diverse methodologies used in herds; different health status; biosecurity measures; management practices; routines, beliefs and behaviour of farmers and owners. In all visits, the author accompanied the veterinary practitioner Frede Keller in his work. This provided the opportunity to participate in the clinical activities developed by the veterinarian. Frede was exceptional in finding a way to include the author in all moments of his work, ranging from meetings with farmers and clients, to performing necropsies, helping achieving a diagnosis based on evidence and learning how to collect important data in order to perform visit reports. Together with Frede's patience, these visits were essential to develop the project and to gain knowledge about swine production in the country, as he clarified the reality and the practical side of diverse important topics. For example, the author understood the practical reasons that lead an owner of a pig herd or a veterinary to use certain vaccines or to use antimicrobial agents; comprehended which "type" of herd owners would be more willing to invest extra money implementing a complete plan of vaccination or to improve biosecurity; became conscious about the "negative" side of strict government policies concerning the producers and the veterinary practitioners; recognised several personalities of herd owners and farmers and its implication on animal welfare, investment and technology use; gained knowledge of the production cycles, the management practices and the differences, advantages and disadvantages of one-site and multi-site pig herds.

Within the clinical part of pig production, a one day visit to a laboratory that receives only pig samples was carried out. The laboratory is run by the Danish Pig Industry and located in Kjellerup in Jutland, Denmark. The laboratory receives a large number of samples every day and performs microbiological and pathological analysis. The author had the opportunity to meet with the different departments and to understand the logistics of the laboratory, also participating in laboratory activities, essentially in pathology, performing necropsies.

Several other activities were carried out during the training period. Dr. Lis Alban planned and scheduled numerous meetings within the DAFC departments, in order to give the author the opportunity to meet with different employees and to understand the different research projects and activities they were involved in. For example, the author had a meeting with Ejvind Pedersen, concerning the Danish organic market. This allowed the author to understand that Denmark is a pioneer country in organic farming, having implemented the first organic rules in 1987 and the Danish organic logo in 1989. Since then, these organic rules are looked upon as being a high priority political issue and nowadays, an “Organic Action Plan 2020” is in effect with the aim to achieve a minimum of 60% of organic food in public canteens. Moreover, Denmark was in the top 5 European countries in 2013 concerning the production of organic pork. Another example of learning within the DAFC departments is the meeting with Elisabeth Okholm Nielsen, which occurred with the aim of addressing *MINAPIG* project, which is a multi-country project including: Germany, Sweden, Belgium, France, Denmark and Switzerland; with the aim of evaluating strategies to reduce the use of antimicrobials in pig production, while assuring the animal health and welfare and offering sustainable solutions for farmers. A recent publication of this project is available (Postma *et al*., 2015).

In the context of the curricular training, the author attended two seminars hosted by Dr. Lis Alban in Copenhagen University. The first seminar: “Managing the risk associated with the use of antimicrobials in pigs”, consisted of a review of the initiatives adopted by Denmark in order to control the use of antimicrobials in pigs and its effects. The second seminar: “Use of risk assessment within the area of antimicrobial resistance”, consisted in a risk assessment presentation, concerning the risk to Danes human health from the veterinary use of macrolides in Danish pigs (Alban, Nielsen, & Dahl, 2008).

This curricular training came to a conclusion with the final presentation of the project. It took place at the Sector for Animal Welfare and Disease Control, Faculty of Health and Medical Sciences in the University of Copenhagen.

This training period contributed greatly to the personal and academic development of the author. The author was given the opportunity to work with some of the greatest specialists in epidemiology, public health and research field, which resulted in a greater interest in scientific research.

As well as challenging, these three months were especially motivating as the work which was developed and carried out is relevant in a global scale. The author feels that the continuous study and implementation of alternatives to antimicrobials already has had positive impacts on local economies, Denmark being a good example of this. With further research and collaboration between farmers, veterinarians and governments, this project can be taken to a global scale and have a positive impact on pig production and public health.

The abstract of this project was submitted and accepted for poster presentation at the 11th International Conference on the Epidemiology & Control of Biological, Chemical and Physical Hazards in Pig and Pork (Safe Pork 2015) this September in Porto (Portugal) and it will also be submitted to a peer-reviewed journal in the coming months. The proceeding for the conference is posted in Annex 2.

II – LITERATURE REVIEW

1 – WORLDWIDE OVERVIEW OF PIG PRODUCTION

The constant increase of the human population has been accompanied by a raise in demand for animal protein (Food and Agriculture Organisation of the United Nations [FAO], 2009). According to FAO (2009), by 2050 the world population will reach 9.1 billion, which corresponds to an increase by 34% compared to 2009. Therefore, the technological, industrial and economic development associated with the need to feed a growing population, led to profound changes in food animal production over the past years (Boschma, Joaris, & Vidal, 1999; Roguet *et al*., 2015).

Pig production has evolved to greater intensity and scale of production, improved infectious disease management and better nutrition (McEwen & Fedorka-Cray, 2002). The last decade was characterized, in the European Union (EU), by a marked concentration of animal production in the most competitive production areas and on farms becoming larger (Roguet *et al*., 2015). Denmark is an example of that: in 1993 there were 28,860[1] registered pig farms in the country, in contrast to 3,855[1] registered in 2013 (Danish Agriculture & Food Council [DAFC], 2014). This means that smallholders on mixed farms have gradually given way to larger scale, and specialised livestock holdings (Eurostat, 2013). This downward trend in the EU is explained mainly by the restructuring processes and the need to adapt to new welfare regulations, as well as increased productivity, higher feed costs and lower profitability in the sector (European Comission [EC], 2013). However, in spite of the decline in the number of herds, more efficient farming methods have led to higher meat yields (EC, 2013; Eurostat, 2013). According to the United States Department of Agriculture (USDA, 2015), 110,476 million tons of pork were produced worldwide in 2014. From these, China produced 56,710 million tons and the EU 22,400 million tons (USDA, 2015). In regards to the EU, the following six countries were responsible for around 73% of the swine production in 2013: Germany (25%), Spain (16%), France (10%), Poland (8%), Denmark (7%) and Italy (7%) (Danish Agriculture & Food Council [DAFC], 2014).

The Danish pig industry is amongst the world leaders in areas such as breeding, food safety, animal welfare and traceability (DAFC, n.d.). For this, swine production is and has been a major source of income for this country, being Denmark one of the World's largest exporters of pig and pork with approximately 90% of the pork produced in the country being exported (Aarestrup, Jensen, Emborg, Jacobsen, & Wegener, 2010; Danish Integrated Antimicrobial Resistance Monitoring and Research Programme [DANMAP] 2012, 2013). During the year 2013, a total of 1,902,693 tons of pork were exported from Denmark (DAFC, 2014). In the

[1] Notice that farms with pigs less than 50kg and without sows are not included in these numbers.

same year, the country produced 29.1 million pigs, of which around 10 million live pigs were exported, 92% of those being, pigs with a weight of 15-50kg (DAFC, 2014). The largest exporting EU markets for Danish pork, in terms of volume, are Germany, United Kingdom, Poland, Italy and Sweden (DAFC, 2014). Concerning the exports of pork for third countries, the largest markets correspond to China, Japan, Russia and United States of America (DAFC, 2014).

It is important to note that pork is the most consumed animal source of protein in the world. According to USDA (2015), during the year 2014, 109,954 million tons of pork were consumed, 84,668 million tons of broiler meat and 57,629 million tons of beef. According to FAO and The Organisation for Economic Co-Operation and Development (OECD) (2014) the global meat production will continue to increase through until 2023. This is mainly due to improved productivity from the genetic progress, the improvement of animal health and evolution of feeding practices of the pig sector, whose production cycles are short, allowing fast changes in practical techniques. Global meat consumption per capita is expected to increase and to be higher in developing countries, while for developed countries the increase will be less noticeable, mainly due to an aging population, lifestyle changes and diets (FAO & OECD, 2014). This increase in food animal production imposes for veterinary monitoring, in order to ensure the health and welfare of animals, guaranteeing the required productivity without neglecting food security (Marabelli, 2003).

2 – ANTIMICROBIAL USE IN PIG PRODUCTION

2.1 – PURPOSES OF USING ANTIMICROBIAL AGENTS

The introduction of antimicrobial agents in human and veterinary medicine has been one of the most significant achievements of the twentieth century (Aarestrup, 2015). Since the introduction of the first antimicrobial agents in the 1930s, a large number of new compounds were discovered in the following decades (Aarestrup, 2015; Pagel & Gautier, 2012; Stanton, 2013). Antimicrobials have become indispensable in decreasing morbidity and mortality and, since their introduction into veterinary medicine, animal health and productivity have improved significantly (National Research Council, Institute of Medicine, 1999).

Pig production ranges from the intensive and large-scale production, to small-scale with few pigs per farm. In this sector, especially in highly intensive production, which is the most common in Denmark, the use of antimicrobials has become an integrated part of the production system (Aarestrup, Duran, & Burch, 2008). Antimicrobial agents are used in pigs for therapeutic purposes, as metaphylactic treatment, as prophylaxis to prevent disease –

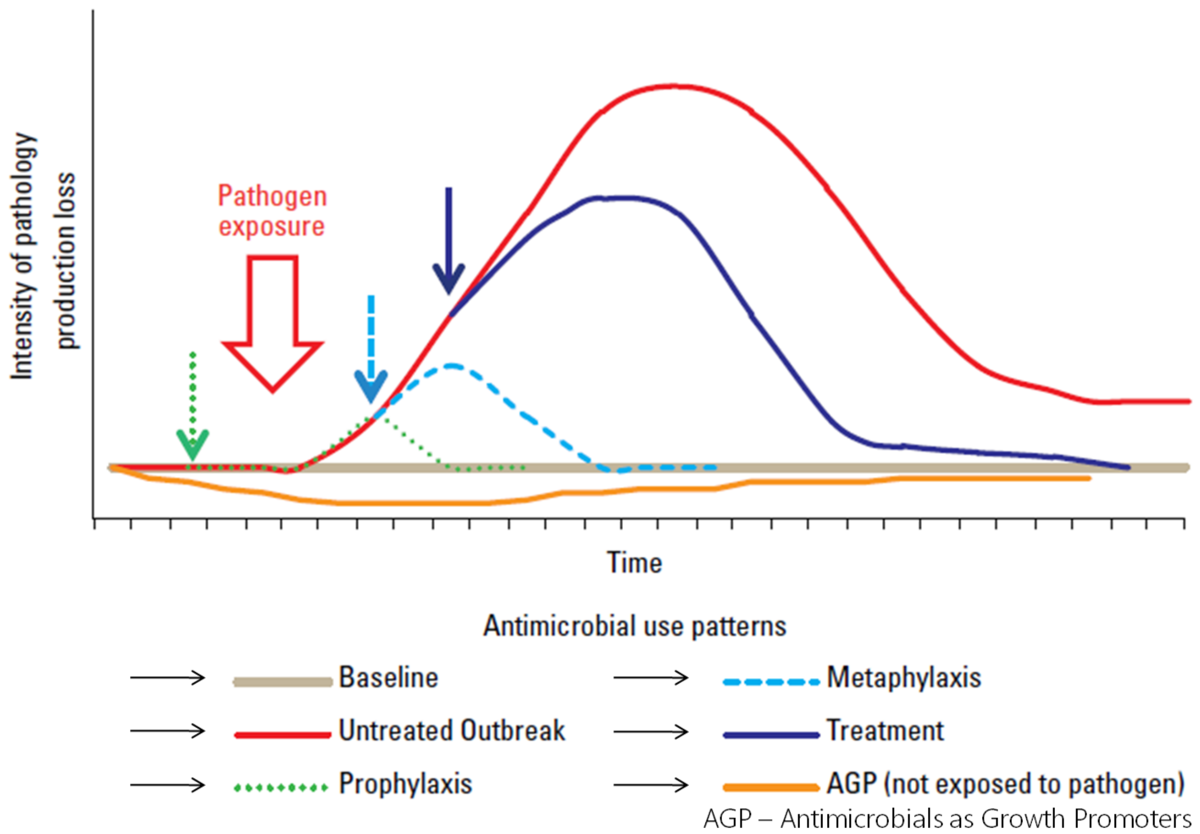
and previously also as growth promoters (Aarestrup *et al*., 2008; Barton, 2014) (Figure 1). These four major ways of administrating antimicrobial agents are illustrated in Figure 1 as a blue solid line, a blue dotted line, a green dotted line and a brown line respectively.

The therapeutic use of antimicrobial agents is intended to control infection (McEwen & Fedorka-Cray, 2002; Schwarz, Kehrenberg, & Walsh, 2001). In the specific case of pig production, an individual animal treatment is sometimes impracticable, since animals are kept in larger groups, with preference being given to group treatment (McEwen & Fedorka-Cray, 2002; Schwarz *et al*., 2001). Moreover, veterinary intervention in large animal groups usually occurs when only some animals of the group present clinical signs of disease (Schwarz *et al*., 2001). This mass treatment of apparently healthy animals, aiming at preventing the spread of infectious disease to animals in close contact and at considerable risk, is referred to as metaphylaxis (Schwarz *et al*., 2001; The Responsible Use of Medicines in Agricultures Alliance [RUMA], 2014). In contrast, prophylaxis consists of a preventive treatment applied to healthy animals considered to be at risk, prior to the onset of disease and for which no etiologic agent has been confirmed by any detection method (Pagel & Gautier, 2012; RUMA, 2014). The prophylactic application of antimicrobials can be to both individual animals and to groups, a preventive measure which should be used with caution (Schwarz *et al*., 2001). One example of a prophylactic application is the weaning period (Schwarz *et al*., 2001). Piglets in commercial intensive industry are weaned abruptly and at a young age for economic reasons (Oostindjer, Kemp, Van Den Brand, & Bolhuis, 2014). As a result, many weaners are poorly adapted to ingest solid food, often resulting in a period of underfeeding (Oostindjer *et al*., 2014). The underfeeding normally leads to low food intake after weaning, accompanied by a poor growth or weight loss and reduced welfare (Bolhuis, Oostindjer, Van Den Brand, Gerrits, & Kemp, 2009). In fact, there can be a malabsorption in the gut associated with the period of fasting and, when piglets start to eat again, diarrhoea might result from it (Oostindjer *et al*., 2014; Zhu, Zhao, Chen, & Xu, 2012). The weaning can also result in inflammation and increased permeability of the gut, which can ease the entrance of toxins and bacteria to the organism (Moeser & Blikslager, 2007). Diarrhoea and low food intake are major contributors to a low performance around weaning (Oostindjer *et al*., 2014). It is likely that, applying antimicrobial prophylactic measures at this stage is unavoidable in most cases in the industrial pig producing systems, in order to evade reduced profitability (Schwarz *et al*., 2001). Furthermore, stressors to which animals can be subjected to, as for example, new environments, relocation and the mixing of animals from different litters in certain moments throughout production, might be a reason to employ prophylactic treatments (Barcellos, Marques, Mores, Coelho, & Borowski, 2009; Schwarz *et al*., 2001).

Another purpose for which substances exhibiting antimicrobial activity is used in food producing animals is growth promotion. This administration is not allowed in the EU since the year 2006, according to the European Parliament and Council Regulation EC No.1831/2003

(Official Journal of the EU, 2003). Where it is allowed, it is usually practiced through use of feed additives over a period of time, resulting in an improved physiological performance: increasing the weight gain by 3.3 – 8.8%, improve feed efficiency by 2.5 –7.0% (Doyle, 2001). It is used to a greater extent in young pigs other than in older animals (Gaskins, Collier, & Anderson, 2002). The aim is to reduce production costs (Aarestrup, 2000) (Figure 1). The mode of action of antimicrobials as growth promoters (AGPs) is not fully understood (Barcellos *et al*., 2009). Early studies demonstrated that oral antimicrobials did not have growth promoting effects when given to germ-free animals (Coates, Fuller, Harrison, Lev, & Suffolk, 1963). Therefore, it is likely that growth promotion is linked with changes in the gut microbiota (Kim *et al*., 2012). Several mechanisms of how growth promoters act have been presented and discussed. The main effects are believed to be two: a growth reduction of bacteria in the intestinal track and thereby less microbial degradation of useful nutrients; and the prevention of infections with pathogenic bacteria (Aarestrup, 2000; Dibner & Richards, 2005; Gaskins *et al*., 2002). Also, this decreased competition for nutrients might result in a reduction in microbial metabolites that depress animal growth (Gaskins *et al*., 2002).

Figure 1 – Antimicrobial use patterns (Adapted from Pagel & Gautier, 2012)



2.2 – THE CHOICE OF THE ANTIMICROBIAL AGENT

The selection of a suitable antimicrobial agent is a crucial step in any therapeutic regime (Pagel & Gautier, 2012; Schwarz *et al*., 2001; RUMA, 2014). Ideally, an effective therapy requires laboratory identification of the bacterial pathogen and an accurate antimicrobial sensitivity test (Schwarz *et al*., 2001). In veterinary practice, waiting for a laboratory result is time consuming and a lingering process, as well as a reason for increased laboratory costs (Schwarz *et al*., 2001). Thus, in many cases a choice has to be made, mainly based on empirical data and clinical experience (Schwarz *et al*., 2001).

Antimicrobial agents can be categorized in two groups: the broad-spectrum substances, which can inhibit growth or even kill a wide range of bacteria; and the narrow-spectrum substances, which are more specific for treatment of bacterial pathogens of certain genera or species (Schwarz *et al*., 2001; Ungemach, Müller-Bahrdt, & Abraham, 2006). Preferably, narrow-spectrum substances should be the first choice when the causative bacteria is identified (Federation of Veterinarians of Europe [FVE], 2000; Ungemach *et al*., 2006). In situations where it is not possible to perform an antimicrobial sensitivity test or the information is not immediately available and the treatment needs to begin, a broad-spectrum substance can be used (Schwarz *et al*., 2001). The therapy should be adjusted with use of a narrow-spectrum substance targeting the causative bacteria whenever information about it is provided (Schwarz *et al*., 2001). Moreover, the route of administration, time of therapy, doses, and aspects concerning pharmacological and pharmacokinetic properties, and withdrawal periods should be taken into account (FVE, 2000; Schwarz *et al*., 2001; Ungemach *et al*., 2006). It is important to keep in mind that choosing the most suitable antimicrobial agent requires careful evaluation.

2.3 – ANTIMICROBIAL RESISTANCE: MECHANISMS, TRANSMISSION AND EPIDEMIOLOGY

For bacteria, antimicrobials act as threats whose lethal effects have to be neutralised (Boerlin & White, 2013). In consequence, they have developed powerful DNA modification strategies, facilitating the adaptation process, allowing their survival and growth (Bennett, 2008). Antimicrobial resistance has been acknowledged as a potential consequence of the use of antimicrobial agents since the discovery of these compounds, being recognized in the late 1950s (Aryal, 2011; Törneke, Torren-Edo, Grave, & Mackay, 2015). The development of bacterial strains resistant to antimicrobials has been attributed primarily, but not exclusively, to the non-therapeutic use of antimicrobials and AGPs in feed (Aryal, 2011). Additionally, various stress factors, including weaning and transportation, have been taken as instigators of the prevalence of bacterial resistance in pigs, regardless of these antimicrobials being used or not (Mathew, Cissell, & Liamthong, 2007).

There is a wide spectrum of biochemical and physiological mechanisms that can be responsible for resistance (Davies & Davies, 2010). Boerlin & White (2013), classify the resistance mechanisms into four major categories: (1) the antimicrobial agent is prevented from reaching its target as the bacterial cell reduces its penetration capability; (2) general or specific efflux pumps expel antimicrobial agents from the cell; (3) the antimicrobial agent can be inactivated by modification or degradation, before or after penetrating the cell; or (4) the antimicrobial cannot act because the target has been modified, or the microorganism's acquisition or activation of an alternate pathway may render the target dispensable (For further information it is suggested to consult the United States Food and Drug Administration video about the resistance mechanisms described above, available at: http://www.fda.gov/AnimalVeterinary/SafetyHealth/AntimicrobialResistance/ucm134359.htm).

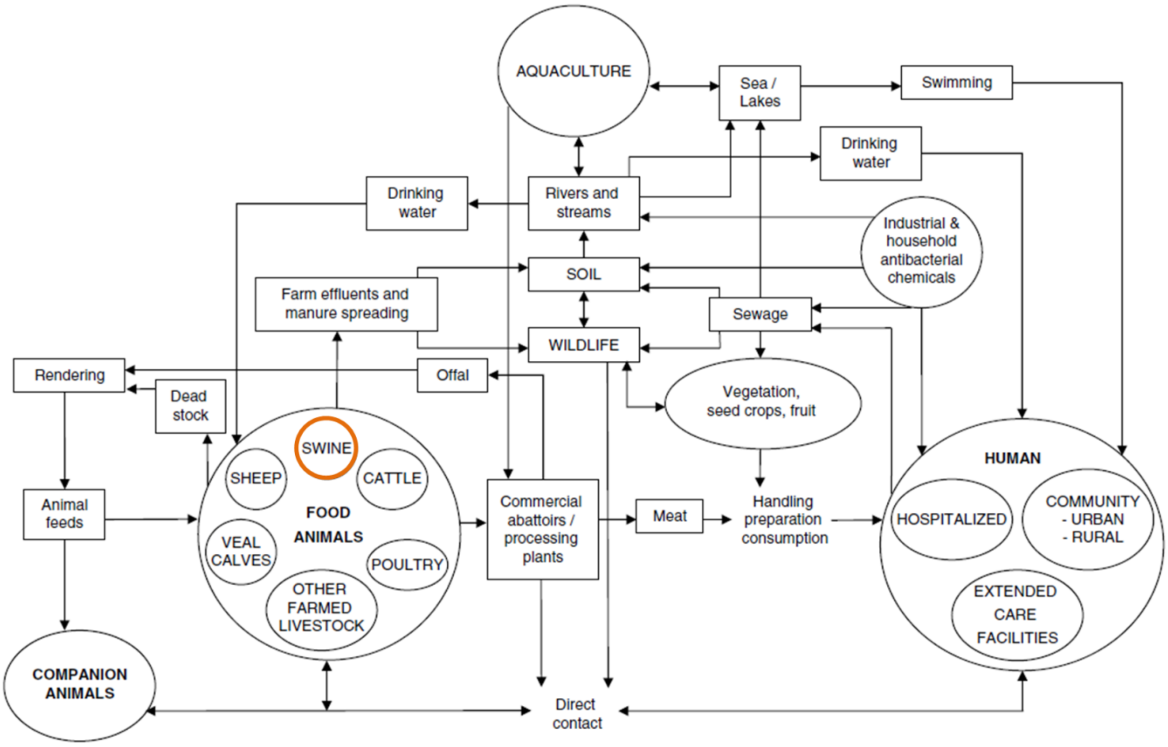
Moreover, concerning AR, bacteria display three fundamental phenotypes: susceptibility, intrinsic resistance or acquired resistance. Intrinsic resistance is natural to all the members of a specific bacterial taxonomic group, such as a bacterial genus, species or subspecies, and results from structural or biochemical characteristics inherent to the native microorganism (Boerlin & White, 2013). For example, the aminoglycoside class of antimicrobials are known to have reduced activity against anaerobic bacteria due to poor drug penetration into the cells under anaerobic conditions. Characteristically, Gram negative bacteria are intrinsically resistant to macrolides, since these chemicals are too large to cross the cell wall of these bacteria (Aryal, 2011; Boerlin & White, 2013).

Acquired resistance can be manifested to agents of several different classes, to a whole class of antimicrobial agents, to some agents within an antimicrobial class, or even to a single agent (Boerlin & White, 2013). This type of resistance may result from the mutation of genes involved in normal physiological processes and cellular structures, from transfer of an extrachromosomal genetic material followed by the selection of resistant organisms, or from a combination of these mechanisms (Almond *et al*., 2005; Aryal, 2011; Boerlin & White, 2013). Antibacterial resistance genes can be present in (1) bacteriophage, (2) plasmids, (3) transposons and (4) self-splicing molecular parasites (Siefert, 2009). These four classes are generically called *mobilome*, an expression that represents all mobile genetic elements, particularity allowing them to move freely between bacteria (Boerlin & White, 2013; Siefert, 2009).

Mutations are spontaneous events during bacteria replication, involving changes in chromosomal nucleotide sequences (Aryal, 2011). In general, these mutations result in the modification of the target of the antimicrobial and therefore, the antimicrobial is unable to bind and inhibit bacterial growth (Aryal, 2011; Boerlin & White, 2013). However, the mutation persists only occasionally (Boerlin & White, 2013; Willey, 2008). It is important to notice that the development of mutational resistance is favoured by low and intermittent dosage of antimicrobial agents (Aryal, 2011).

The transfer of genetic material between bacteria is viewed by veterinary science as a major cause of AR (Hirsh, 2003). If the resistance genes become integrated into mobile genetic elements (*mobilome*) they might be transmitted between bacteria by transduction, conjugation, mobilization or transformation, which are the mechanisms of horizontal transfer of genetic material between bacteria (Boerlin & White, 2013; Schwarz *et al*., 2001). It is important to keep in mind that this circulation of genetic elements can occur between pathogens, as well as between commensal bacteria and pathogens (Boerlin & White, 2013). Antimicrobial resistant bacteria and resistance genes may disseminate through several routes, as represented in Figure 2. Examples include the environment, animal waste containing resistance genes, migrating animals, international trade of food and feed products, transference to humans via direct contact and ingestion of contaminated food and/or water (Aarestrup, 2015;Boerlin & Reid-Smith, 2008; McEwen & Fedorka-Cray, 2002). The importance of the represented multiple routes is not easy to quantify, however, the animal reservoir of AR can have negative effects on AR in bacteria and pathogens from humans (Aarestrup, 2015; Boerlin & Reid-Smith, 2008; Garcia-Graells *et al*., 2011; Marshall & Levy, 2011). Consequently, more research is needed to quantify the risk associated with the use of antimicrobial agents in animals and their responsibility in AR in humans (Boerlin & White, 2013).There is also a major need for interventions and continued research in order to limit the use of antimicrobial agents and transmission of resistance (Aarestrup, 2015).

Figure 2 – Schematic representation of the spread of antimicrobial resistance and of resistance genes across the multiple ecological compartments. (Adapted from Boerlin & White, 2013)



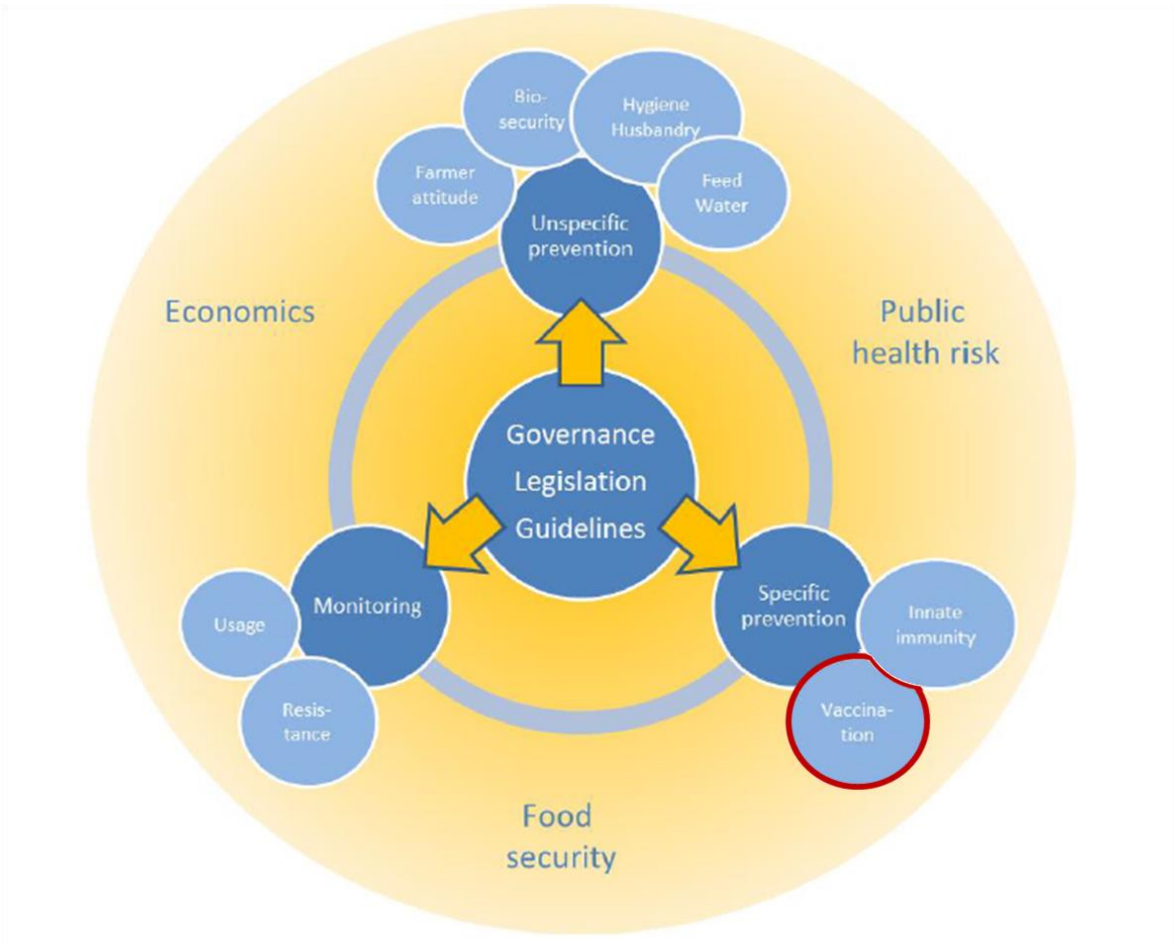
3 – APPROACHES TO REDUCE ANTIMICROBIAL USAGE IN PIG PRODUCTION

Reduced antimicrobial usage in livestock is promoted in Europe and worldwide as a public health measure to reduce AR. The EC, the World Organisation for Animal Health (OIE), FAO and WHO have described strategies in order to address this issue (FAO, OIE, & WHO, 2015; WHO, 1997, 2001, 2012; WHO & FAO, 2009; OIE, 2015a, 2015b). The aims of this section are to provide a review on the different options to reduce antimicrobial usage in pig production and draw attention for the various dimensions of this issue, which are summarized in Figure 3. As it is possible to distinguish in Figure 3, the first step in addressing the reduction of antimicrobial use is to apply strategies designed by organisations such as WHO, OIE and FAO to a national and local level, by implementing adequate governance rules, legislation and guidelines (WHO, 1997). Furthermore, it is an issue that has potential implications in terms of public health risk, food security and economics and that is why there is a need for an interdisciplinary coordination between organisations from human and animal health (Stanton, 2013). Moreover, it is important to take into account animal health, welfare and economic viability in pig farms (Postma *et al*., 2015). Thus, different plans of action concerning effective and efficient approaches to reduce antimicrobial usage in pig production (Figure 3) will be discussed throughout this section.

3.1 – UNSPECIFIC PREVENTION

In general, good farming practices, especially when it comes to hygiene are very important towards preventing the spread of diseases among livestock (Doyle, 2001). Attention to efficient cleaning methods; effective sanitary measures; the maintenance of an appropriate ventilation rate and environmental temperatures; adequate stocking rates; anticipating high risk moments in particularly stressful times for animals, as well as keeping records, are examples of practices that can be adopted within farms, in order to reduce the use of antimicrobials (Doyle, 2001). It is also possible to take advantage of technological progress and new tools that continuously become available in order to reduce antimicrobial use. For example, a free online application, called *ABcheck* developed by Ghent University – available at http://www.abcheck.ugent.be/v2/home/. This tool allows for the calculation of antibiotic use in pig, poultry and turkey farms and also provides free access to literature involving information on antibiotics and antimicrobial resistance. Another example is an advisory tool named *Farm Facts* described by Bak (2011). It assists on the implementation of alternatives to antibiotics in pig herds and the visualisation of results to veterinarians and pig producers (Bak, 2011). It consists on a spreadsheet composed by three different parts: the first, identifying the source of main antimicrobial consumption within the herd; the second part giving an action plan taking into account a defined goal; and the third part providing elements for an appropriate follow up according to and after the implementation of the action plan. Bak (2011) also provides an example of success in a Danish finishing herd using these *Farm Facts.* The result was a reduction of the use of antibiotics by 64%, with reduced mortality and maintenance of the daily weight gain (Bak, 2011).

Figure 3 – Overview of approaches to reduce antimicrobial usage in livestock (Adapted from Stärk, 2013).



However, when required, antimicrobials should be used. Guidelines concerning the prudent use of antimicrobials have been developed by several organisations, representing another approach to reduce antimicrobial usage in pig production (FVE, 2000; WHO, 2011; OIE, 2015a). A major principle of prudent use of antimicrobials' guidelines is that antimicrobials should not be used to compensate for poor or deficient husbandry methods. The guideline principles cover points such as: administering antimicrobials only when prescribed by veterinarians; avoiding economic incentives that facilitate the inappropriate prescriptions;

need for diagnosis, selection of the most suitable substance, formulation and spectrum, correct dosages; restricted usage of antimicrobials identified as critically important for human medicine and use of antibiotics therapeutically, based on resistance testing, as well as clinical experience (FVE, 2000; WHO, 2011). International guidelines should be adapted at a national level for different classes of food animals by veterinary associations and stakeholder platforms (Stärk, 2013; WHO, 2011). An example is the manual on good antibiotic practice, developed by the Danish Pig Research Center (2013), in order to help farmers, staff and advisors in their work to reduce antibiotic use on their pig farms. Ungemach *et al*. (2006) findings indicated a change on the prescribing attitude of veterinarians and a reduced consumption of antimicrobials after the implementation of guidelines for prudent use of antimicrobials in Germany, suggesting that guidelines are an important tool to reduce the use of antimicrobials.

Biosecurity embraces all aspects that reduce the risk of disease agents being introduced and spread within a group of animals (Amass & Clark, 1999). It can be subdivided into two different categories: (1) external biosecurity – prevention of pathogens entering a herd, and (2) internal biosecurity – reducing the spread of pathogens within herds (FAO, 2010). In literature, there are several documented relationships between the management practices and the incidence of specific diseases in pig herds, biosecurity importance and producers attitudes towards biosecurity in todays food-livestock production systems (Boklund, Alban, Mortensen, & Houe, 2004; Casal, De Manuel, Mateu, & Martín, 2007; Lambert, Arsenault, Poljak, & D'Allaire, 2012; Maes *et al*., 2008; Meyns *et al*., 2011). High levels of biosecurity have been associated with improved animal health and productivity, as well as with a reduction of the use of antimicrobials (Laanen *et al*., 2013; Ribbens *et al*., 2008). It is important to alert each producer to be concerned with biosecurity and treat it as a collective responsibility. Continued education of producers and employees is essential to increase awareness towards the importance of this topic in pig farms (Lambert & Dallaire, 2009). Being biosecurity a complex concept, it is often difficult to measure, and efforts are essential in evaluating and classifying its effects (Stärk, 2013). An example is a risk-based weight biosecurity scoring system that consists in a free online tool that allows for evaluation of the quality of biosecurity of each herd (can be found at: http://www.biocheck.ugent.be/). Laanen (2011) used this *Biocheck* system in her study with Belgian pig herds. It was concluded that improved biosecurity levels on a farm are a useful method in reducing the use of antimicrobial drugs and improving production parameters. The study also showed that biosecurity was higher amongst younger farmers and in more modern herds. This brings us to the idea that beliefs and attitudes of farmers and veterinarians are important when it comes to antimicrobial usage in pig production. Recent surveys conducted in Belgium, France, Germany, Sweden and Switzerland revealed that pig farmers were more worried about financial issues rather than antimicrobial use and resistance within their farms

(Visschers *et al*., 2014, 2015). Possible explanations discussed by Visschers *et al*., (2015) for the relatively low concern towards AR from farmers, were the current global financial developments and the closeness with which farmers experience financial and legal consequences, rather than negative effects from AR. There is enough room for educating and promoting risk awareness amongst farmers related to the use of antimicrobials. Marvin *et al*. (2010) conducted a study in Canada regarding the risk of AR and concluded that farmers had less knowledge compared to veterinarians. Garforth, Bailey, & Tranter (2013) showed that veterinarians were the preferred source of information regarding pig farming in a study performed in the United Kingdom. Thus, it is possible to assume that veterinarians play an important role when it comes to antimicrobial use in pig production, being an important channel of information towards farmers, in a local context.

As represented in Figure 3, another area of unspecific prevention is water and feed. Feed composition is extremely important, and should be adjusted to animals in different production phases (Lewis & Southern, 2000). Furthermore, performing dietary supplementation with different feed additives and adjusting nutritional components, is an approach to prevent the need to use antimicrobials (Allen, Levine, Looft, Bandrick, & Casey, 2013; Thacker, 2013). Probiotics are an example of feed additives, which consist of live cultures of microbes, such as *Lactobacillus*, *Streptococcus* and *Bacillus* (Cho, Zhao, & Kim, 2011). They are known to be able to destroy pathogenic microorganisms by producing compounds that are toxic to the pathogens; to stimulate the immune system and to improve gastrointestinal microbial balance (Cho *et al*., 2011; Musa, Wu, Zhu, Seri, & Zhu, 2009). However, the use of these compounds in animal feeds is limited and the results obtained have been inconsistent (Cheng *et al*., 2014). Prebiotics are non-digestible food substances, which have a beneficial effect to the host, through selective growth of bacteria in the intestinal tract (Jacela *et al*., 2010b). Prebiotics can also promote immune functions and show anti-viral activity (Cheng *et al*., 2014). Still, the effectiveness of these compounds is unpredictable and they are also associated with a high cost of production (Cheng *et al*., 2014). Organic acids, as for example formic, propionic and lactic acids, have shown favourable effects in improving the performance in weaned piglets (Doyle, 2001). Benefits from using these acidifiers are seen in the growth performance and health status of the pigs (Papatsiros, Cristodoulopoulos, & Filippopoulos, 2012). Furthermore, the use of zinc oxide as a feed additive in high dietary levels is associated with a beneficial effect in reducing the severity of diarrhoea and increasing weight gain in recent weaners (Hill *et al*., 2000; Jacela *et al*., 2010a). European Food Safety Authority (EFSA, 2012) published a scientific opinion where they state that when taking into account the maximum limits for total zinc in feedingstuffs set by the EU legislation, the substance does not pose a risk for animal species, consumer safety or for agricultural soil. However, there is a potential environment concern related to groundwater, drainage and the run-off of zinc to surface water (EFSA Panel on Additives and Products or Substances used in Animal Feed [FEEDAP], 2012). Therefore, this information needs to be taken into consideration when making use of this compound.

These are some of the examples of available additives with potential to be used as an approach to reduce antimicrobial usage. Indeed, other compounds are also available. Parisien *et al*. (2008) describe the result of an extensive research in three alternatives to antibiotics: bacteriophages, bacterial cell wall hydrolases and antimicrobial peptides. Thacker (2013) focuses on less traditional alternatives such as essential oils, recombinant enzymes, clay minerals and others. Cheng *et al*. (2014) present a recent and complete review that summarizes aspects as mechanism of action, application and prospective of alternatives to antibiotics, such as, inhibitors targeting bacterial pathogenicity, plant extracts, bacteriophages, feed enzymes and others.

3.2 – SPECIFIC PREVENTION

Before introducing the measures aimed at specific disease prevention, it is important to introduce the concept of immune response. The exposure of an animal to a foreign agent or a pathogen results in the activation of the immune system recognising that exposure and subsequently, reacting to neutralize it (Babiuk, 2006). There are two types of immune response: (1) innate or non-adaptive and (2) acquired or adaptive (Male, 2001). The second type of response is highly specific for a particular pathogen and, as the name implies, it improves with each successive exposure with the same specific pathogen (Male, 2001). The first type of response, on the contrary, does not alter with several exposures to a given pathogen (Male, 2001).

3.2.1 INNATE IMUNITY

There is a wide genetic variation amongst animals for disease resistance (Holden *et al*., 2002). In the context of different approaches to reduce the use of antimicrobials, animals that have developed resistance against specific pathogens could be an asset in developing breeding programmes in order to improve the performance of animals in disease resistance through heritability (Holden *et al*., 2002; Stärk, 2013). Also, future developments in genomic programs might help and complement the actual breeding programs (Stärk, 2013). It is important to keep in mind that these programmes should not be implemented without a verification of the production system, environmental conditions and goals of each farm, as they will not compensate for inappropriate management practices (Holden *et al*., 2002).

Rowland, Lunney, & Dekkers, (2012) findings support this approach, showing that it is possible to develop breeding programs that will produce pigs with increased resistance to porcine reproductive and respiratory syndrome.

3.2.2 VACCINATION

Vaccination is one of the most important immunomodulators (Cheng *et al*., 2014). The basic principle behind it is the promotion of natural acquired immunity by exposing the animal to different components of a certain pathogen that composes the vaccine (Babiuk, 2006; Meeusen, Walker, Peters, Pastoret, & Jungersen, 2007). The different compositions of vaccines are well described by Meeusen *et al*., 2007 and will not be a subject of study in the present dissertation. Much progress has been made since the initial development of vaccines (Poole et. al 2010). The emergence of new diseases, the rise in AR and the technological advances in vaccine developments allowed for the expansion in the range of veterinary vaccines and increased effectiveness of existing ones (Meeusen *et al*., 2007; Törneke *et al*., 2015). However, there is still room and need for research in this sector as recommended by WHO (2001).
Vaccines increase animal resistance against a specific pathogen (Törneke *et al*., 2015). They may be used in a prophylactic way to prevent disease, to prevent clinical signs of a disease after the infection, as an emergency plan to control an outbreak of a disease or even to eliminate an infection at the population level (Border & Amund, 2013;Meeusen *et al*., 2007). Vaccines can improve animal health and welfare by reducing the disease burden (Allen *et al*., 2013; Border & Amund, 2013; Meeusen *et al*., 2007). Moreover, they can have a significant impact on public health by reducing the need for antimicrobial treatment and therefore constrain the spread of AR and also, when used, to prevent zoonotic diseases (Allen *et al*., 2013; Cheng *et al*., 2014; WHO, 2012). Brockhoff, Cunningham, & Misutka (2009) performed a retrospective study in a pig herd in Canada, showing improved animal performance, reduced antibiotic cost per pig and a return on investment after the implementation of PCV2 vaccine. Brockhoff *et al*. (2009) confirms the possibility of the reduction of antimicrobial consumption after vaccination usage and also that there is a possibility of return on investment for the producer. Similar findings are confirmed in other reports (Aerts & Wertenbroek, 2011; Koenders & Wertenbroek, 2012). In several countries, more case studies and meta-analysis concerning one or a combination of two vaccines, confirm the reduction of antimicrobial use after applying vaccination (Adam, 2009; Bak & Havn, 2011; Bak & Rathkjen, 2009; Coube, Serrano, Pottier, Jagu, & Adam, 2012; Dommelen & Wertenbroek, 2011; Kristensen, Baadsgaard, & Toft, 2011; Tebar, Caravaca, Coll, & Celma, 2012).

3.3 – MONITORING

As presented in this literature review, there is evidence of transfer of resistant bacteria or resistant determinants amongst livestock, environment and humans. In order to monitor the antimicrobial use and the AR levels, monitoring programmes are applied in several EU countries, such as Sweden, Netherlands, Belgium, France, Spain and Denmark (Törneke & Boland, 2013). According to WHO (1997), these programmes should facilitate the identification of resistance in bacteria from humans, animals and food of animal origin as it occurs. Furthermore, these programmes can be seen as an incentive to promote the prudent use of antimicrobials and guidance for their clinical management. In general, the programmes should help providing information to several institutions such as governmental legislative authorities, public health authorities, pharmaceutical companies, laboratories and veterinarians; allowing for the identification of areas for more research and to promote collaboration among the different sectors involved (WHO, 1997, 2001).

Regarding the monitoring of antimicrobial usage, there are significant differences in the methodology adopted in different countries, even at the European level (Stärk, 2013; Törneke & Boland, 2013). Data is usually not directly comparable between countries and regions and there is a need for a greater harmonisation of the methodology (Barbosa & Levy, 2000; Silley, Jong, Simjee, & Thomas, 2011; Törneke & Boland, 2013; OIE, 2011). For example, Grave, Torren-Edo, & Mackay (2010), compared the sales of veterinary antimicrobial agents between 10 European countries and found a wide variation between them, concluding that the disparities could not be explained only by differences in animal species demographics. Moreover, Silley *et al*. (2011) compared several veterinary antimicrobial resistance surveillance and monitoring programmes in detail, emphasizing the need for harmonisation at the national and international level. The European Medicines Agency (EMA) started a monitoring project named European Surveillance of Veterinary Antimicrobial Consumption (ESVAC) in April 2010 (EMA, 2015). The ESVAC project collects information on how antimicrobial medicines are used in animals across the EU (EMA, 2015). The first report of historical data from nine countries was published in 2011 (EMA, ESVAC, 2011). Presently, there are more reports available, including information from more countries (EMA, ESVAC, 2012, 2013, 2014). All of them concluded that, in order to establish a more accurate picture of antimicrobial use, to identify the development and occurrence of resistance and the impact of interventions across Europe, it is important to obtain data with a sufficient level of detail from all countries. A leading country in implementing measures of antimicrobial usage registration and reduction is Denmark. A detailed description of the Danish monitoring system is present in the next subsection (3.3.1.).

With regards to resistance monitoring, EFSA and European Centre for Disease Prevention and Control (ECDC) have been collecting information on antimicrobial resistance in zoonotic bacteria affecting humans, animals and food from monitoring programs from each EU country (EFSA & ECDC, 2015). This work assists the EC as it develops its proposals for action to fight AR. Moreover, EFSA, ECDC and EMA published a joint report and associated the use of certain antimicrobials in animals and humans, to the occurrence of resistance to these antimicrobials (ECDC *et al*., 2015). Moreover, WHO (2015a) published a report providing an analysis, by region and globally, of the initiatives under way to address AR and identifying areas in which more work is needed.

3.3.1 DANISH MONITORING SYSTEM OF ANTIMICROBIAL USE AND RESISTANCE

In 1995, the Danish Integrated Antimicrobial Resistance Monitoring and Research Program (DANMAP) was established by the Danish Ministry of Food, Agriculture and Fisheries and the Danish Ministry of Health, being Denmark the first country to establish systematic and continuous monitoring of antimicrobial agents consumption and resistance in animals, food and humans (Hammerum *et al*., 2007). The aims of the programme are to (1) provide data on trends of antimicrobial resistance, (2) monitor consumption of antimicrobial agents for food animals and humans, (3) explore associations between the occurrence of resistance and the use of antimicrobials and (4) identify routes of transmission and areas for further research studies (Bager, 2000; Hammerum *et al*., 2007). The results of the DANMAP programme are published annually and are available at: http://www.danmap.org/Downloads.aspx.

For DANMAP, data regarding veterinary use of antimicrobials was collected from the pharmaceutical industry and importers during the years 1996-2000 (Hammerum *et al*., 2007). In 1998, the Danish government decided that a monitoring system of all the veterinary use of prescription medicine on a detailed level should be developed and consequently, the implementation of the program *VetStat* was initiated in the year 2000 (Frimodt-Møller, 2004).

Data on medicine consumption are submitted to *VetStat* from three different sources: pharmacies, veterinarians and feed mills, having each of them a unique identification (Dupont & Stege, 2013; Stege, Bager, Jacobsen, & Thougaard, 2003). Taking into account some legislative regulations imposed by the Danish Government and the EU, it is possible to assume that the majority of therapeutic drugs are prescription-only in this country (DANMAP 2010, 2011; Dupont & Stege, 2013). According to Stege *et al*. (2003), pharmacies provided 95% of the total weight of antimicrobial compounds used in production animals in Denmark, being 80% of these sold directly by the pharmacies to the farmer. Jensen, Jacobsen, & Wegener, 2003 mentioned that 2% of the antimicrobial drugs used in pig production were purchased at feed mills. Therefore, virtually all sale of veterinary medicine in Denmark is made through pharmacies, veterinary practitioners or feed mills (Dupont & Stege, 2013).

All entries from these entities in *VetStat* must include information on date, the identity of the farm where the animals are located, which corresponds to the Central Husbandry Register (CHR) number; the identity of the prescriber, official product number, the amount used and, when possible, the target animal species, the age group and the diagnostic category (Stege *et al*., 2003). Pharmacies report individual records of sale of drugs for veterinary use. All Danish pharmacies have electronic and standardized billing systems, which ensure the extraction of information during the electronic process of sales at the pharmacy (Dupont & Stege, 2013). This improves the validity of the data, which is reported to *VetStat* on a monthly basis (Dupont & Stege, 2013). Veterinarians have to report, at least once a month, the administrated drugs to production animals, including the ones that are handed out to herd owners for follow up treatments (Stege *et al*., 2003). It is possible to record the usage directly into *VetStat* via internet, or using software that extracts information automatically (Jensen *et al*., 2003; Stege *et al.*, 2003). Feed mills with a license to manufacture and sell medicated feed are required to report all sales of medicated feed and coccidiostats to *VetStat*, providing the same type of information as the other sources of data (Stege *et al*., 2003). Feed mill records on sales can also be registered directly into the database or transferred once a month (Jensen *et al*., 2003). All this data is subject to full logic validation within the database (Jensen *et al*., 2003). Dupont & Stege (2013) also describe some typing errors by the reporting entities and the non-existence of an automatic linking between animal species, age group and diagnostic group in *VetStat* during data entry. An example of that, provided by Dupont & Stege (2013) is the possibility to make an entry with animal species “cattle”, age group “broilers” and diagnostic group “furunculosis”, at the time of the study. Despite that, *VetStat* data provides great opportunity to assess antimicrobial usage at a national and herd level, concerning the monitoring of antimicrobial use, providing information for research, being a tool that helps veterinary practitioners in their work and allowing for risk assessment of resistance development at the population level (Dupont & Stege, 2013; Jensen *et al*., 2003).

III – RESEARCH WORK

ASSOCIATIONS BETWEEN VACCINATION AND ANTIMICROBIAL CONSUMPTION IN DANISH PIG HERDS, 2013

1 – INTRODUCTION

Since the introduction of the first antimicrobial agent, a large number of new compounds were discovered in the following decades (Aarestrup, 2015; Pagel & Gautier, 2012; Stanton, 2013). Antimicrobials became indispensable, improving animal health and productivity significantly (National Research Council, Institute of Medicine, 1999). From then onwards, antimicrobials have been used in EU's veterinary medicine for therapeutic purposes, as metaphylactic treatment, as prophylaxis to prevent disease – and early on also as growth promoters, in order to enhance the production performance of animals (Aarestrup *et al*., 2008; Barton, 2014; Wegener, Aarestrup, Jensen, Hammerum, & Bager, 1999). The excessive use of AGPs led to concerns about the potential risk of the development of AR in animals and the transfer of resistance factors from animals to humans, through zoonotic bacteria or through the consumption of animal products (Aarestrup *et al*., 2008; Agersø & Aarestrup, 2013; Allen, Levine, Looft, Bandrick, & Casey, 2013; Jensen, Jacobsen, & Bager, 2004; Seal, Lillehoj, Donovan, & Gay, 2013; Wierup, 2001).

In Denmark, there has been political and public focus on this issue since the 1990s (Bager, 2000). As a result, a series of events emerged: (1) an industry initiative leading to the phasing out of AGPs for finisher pigs in 1998 and for weaners in 1999 (effective from January 1, 2000) (DANMAP 2012, 2013); (2) mandatory limited veterinary profit from sales of antimicrobials to 5-10% (DANMAP 2010, 2011); (3) increased oversight and regulation of veterinary practice and prescriptions (Jensen & Hayes, 2014), as well as (4) recommendations and guidelines for prudent use of antimicrobials (Ungemach *et al*., 2006). Moreover, in 2010 the authorities adopted the Yellow Card initiative, a scheme that sets threshold limits to antimicrobial use on swine farms (Alban, Dahl, Andreasen, Petersen, & Sandberg, 2013; Jensen & Hayes, 2014). That same year, an industry ban was implemented by the Danish pig industry in order to stop the use of cephalosporins (Alban *et al*., 2013; Jensen & Hayes, 2014).

Danish policies are supported by DANMAP, initiated in 1995 to monitor the consumption of antimicrobials and the occurrence of AR (Bager, 2000). These resulted in the collection of data regarding medical consumption for production animals in a national database called *VetStat* (Bager, 2000; Stege *et al*., 2003). This database has four objectives: "(1) monitor veterinary usage of drugs in animal production; (2) help practitioners in their work as farm advisors; (3) provide transparency as a basis for ensuring compliance with rules and legislation and (4) provide data for pharmaco-epidemiological research" (Stege *et al*., 2003, p. 106). *VetStat* collects information from pharmacies, feed mills and veterinary practitioners (Bager, 2000; Hammerum *et al*., 2007; Stege *et al*., 2003). Information from pharmacies is generated during the electronic process of their sales, containing data on the targeted animal species, age group, disease category and the characteristics of the farm and of the prescriber, which are reported on a monthly basis to *VetStat* (DANMAP 2010, 2011; Dupont & Stege, 2013). Veterinarians and feed mills report the same type of information related to their prescriptions and sales (DANMAP 2010, 2011). After visiting a herd, the veterinarian provide a prescription to a pharmacy to order medicine to use in practice (DANMAP 2001, 2002). Veterinarians have the choice of recording usage directly into *VetStat* database, which can be accessed via the internet (DANMAP 2001, 2002). Pharmacies and veterinary drug companies provided the majority of antimicrobial compounds in Denmark and only 2% was provided through feed mills in 2012 (DANMAP 2013, 2014).

The quantification of antimicrobial consumption (AC) in food animals has been described in the literature with various measurement units (Chauvin, Madec, Guillemot, & Sanders, 2001). The *VetStat* approach uses Animal Daily Dose (ADD) which is defined as the average maintenance dose per live animal for the main indication of an antimicrobial in a specified species (Dupont & Stege, 2013; Jensen *et al*., 2004). This measure takes into account the different effectiveness of antimicrobials. In order to consider the large variation in weight of production animals, the standard animal bodyweight measure was introduced, per age group (Dupont & Stege, 2013; Jensen *et al*., 2004). On average a weaner weighs 15 kg, a finisher pig 50 kg and a sow with piglets weighs 200 kg (Dupont & Stege, 2013).

In Denmark, the pig production results in a total of 28 million pigs produced annually (Alban *et al*., 2013). Between 2008 and the first half of 2010 the AC in pigs increased, pointing to a potential overuse and leading to a public debate (Alban *et al*., 2013). Consequently, the Yellow Card scheme adopted by the Danish Veterinary authorities began operating in 2010 (DANMAP 2010, 2011). According to this scheme, farmers that have antimicrobial usage above the pre-established threshold per age group are given a yellow card that is translated into an order to reduce the antimicrobial use below the threshold within 9 months. If these requirements are not met during that period, the farmer is subject to a visit by a second opinion veterinarian, additional monitoring and is responsible for all related costs. If there is still no resolution, the farmer is given a red card compelling the owner of the holding to implement more initiatives as the ones mentioned above. As a last resort, the owner is forced to reduce the stocking density with a suitable percentage, to ensure that the AC is reduced below the threshold levels. The red card will remain in force until the goals are achieved (Alban *et al*., 2013; Andreasen, Alban, Dahl, & Nielsen, 2011; DANMAP 2010, 2011). A

description of the Yellow Card initiative by the Danish Veterinary & Food Administration (DVFA) can be found here:
http://english.foedevarestyrelsen.dk/english/SiteCollectionDocuments/25_PDF_word_filer%20til%20download/Yellow%20Card%20Initiative.pdf
This reflects the increasing political pressure that is forcing Danish pig producers to reduce the usage of antimicrobials on their farms. Additionally, consumer expectations and, above all AR is a concern that contributes even more to the "social pressure" in this sector. Therefore, efficient alternatives to routinely applied antimicrobials have become crucial. Vaccines are being considered as a means to decrease the burden of animal diseases and also to reduce the need for antimicrobials with therapeutic purposes (Allen *et al*., 2013; Cheng *et al*., 2014). However, the extent of using vaccination as an alternative to antimicrobials will depend on its cost, effectiveness and ease of use (Allen *et al*., 2013).

To explore the impact of further use of vaccination as an alternative to antimicrobial usage, the present study was carried out using data from *VetStat* and the Danish CHR covering Danish pig herds in the year 2013. The aim was to investigate overall associations between vaccination against *Lawsonia intracellularis, Mycoplasma hyopneumoniae* and Porcine Circovirus Type 2 (PCV2) and AC. The three infections that these vaccines deal with are considered very important in modern pig production, taking into account the ubiquitous nature of these agents as well as the impact they can have in triggering/predisposing for other disorders in different organism departments (Aarestrup et al., 2008; Segalés, 2011; Segalés, Allan, & Domingo, 2012; Thacker & Minion, 2012).

Lawsonia intracellularis is the causative bacterium of proliferative enteropathy (PE), which is a common intestinal infection with high prevalence worldwide: in the specific case of Denmark, Stege *et al*. (2000) verified by Polymerase Chain Reaction that 94% of the Danish pig herds are infected with these bacteria. This may have a direct impact on pig production and herd economics as it can affect growing pig performance due to the decrease in growth rates and feed conversion (Bak & Rathkjen, 2009; Smith & McOrist, 1997). During the study period, the only vaccine available in Denmark against PE was Enterisol®Ileitis, produced by Boehringer Ingelheim – licensed to improve weight gain and reduce growth variability associated with the disease.

Mycoplasma hyopneumoniae is the primary agent responsible for swine enzootic pneumonia (EP), which is a chronic respiratory disease that causes significant economic losses worldwide and is highly prevalent in most areas of pig production (between 38 to 100%) (Thacker & Minion, 2012). This agent predisposes the infected animals to secondary infections, typically with bacteria such as *Actinobacillus pleuroneumoniae*, *Pasteurella* *multocida, Mycoplasma hyorhinis, Streptococcus suis, Haemophilus parasuis, Bordetella bronchiseptica* and *Trueperella pyogenes* (Thacker & Minion, 2012). These can increase the severity of the disease (Thacker & Minion, 2012). When there is an interaction between bacterial (those listed before) and viral agents (such as PCV2, swine influenza viruses, porcine respiratory coronavirus, Aujeszky's disease virus or porcine reproductive and respiratory syndrome virus), the syndrome is called Porcine Respiratory Disease Complex (PRDC) (Sibila *et al*., 2009). This is why the notorious performance of *Mycoplasma hyopneumoniae* to enhance the effect of other pulmonary pathogens needs to be taken into account in pig herds. Vaccination is considered the most adequate measure for controlling the infection with *Mycoplasma hyopneumoniae* (Mateusen, Maes, Verdonck, Kruif, & Goubergen, 2002). Furthermore, it has been associated with reduced clinical signs, fewer treatment costs and with increased average daily weight gain (Jensen, Ersbøll, & Nielsen, 2002; Maes et al., 1999).

Porcine Circovirus Type 2 has a causal role in a large number of clinical syndromes, which are collectively named as Porcine Circovirus Diseases (PCVDs) (Segalés et al., 2012). The most economically significant condition within PCVDs is postweaning multisystemic wasting syndrome (PMWS) (Segalés, 2011). For the pig industry in Europe, PMWS represents a cost of approximately €600 million per year (Segalés *et al*., 2012). However, PCV2 can also play a role in the occurrence of reproductive failure, porcine respiratory disease complex (PRDC), enteritis, porcine dermatitis and nephropathy syndrome and proliferative necrotizing pneumonia (Chae, 2004; Opriessnig, Meng, & Halbur, 2007; Segalés, Allan, & Domingo, 2005). PCV2 is ubiquitous all over the world, while prevalence of clinical disease is much lower (Segalés, 2011; Segalés *et al*., 2005).This attests the multifactorial component and complex causality of the PCVDs: in addition to PCV2 infection, triggering factors that induce immune stimulation are needed in order to cause expression of the disease (Segalés *et al*., 2012). Therefore, PCVDs control has traditionally been based on preventive measures such as (1) improved management practices in order to control risks or triggering factors, (2) control of co-infections and (3) changes of the boar genetic background (Fraile *et al*., 2012a). Currently, disease control is mainly based on vaccination, which has been shown to be very effective in reducing viraemia, improving production parameters (reducing mortality and increasing average daily weight gain) and the probability of co-infection by other pathogens (Gerber, Garrocho, Lana, & Lobato, 2011; Kristensen *et al*., 2011; Segalés *et al*., 2009).

The aim of the present study was to investigate associations between three different types of vaccination on the AC in pig herds. Considering the hypothesis that herds which use vaccination routinely would have a lower AC.

2 – MATERIAL AND METHODS

2.1 PRELIMINARY DATA MANAGEMENT

The data used in this study were obtained from *VetStat* and Danish CHR. All herds which were active in 2013 were selected for the study if they fulfilled the following inclusion criteria: a) one-site system herds b) herds with more than 50 sows and c) herds with more than 200 weaners. In total 1,518 herds met these three criteria.

In this dataset there were four herds with negative values of recorded AC most likely caused by an error in *VetStat*. As all the herds in the dataset were anonymised, it was not possible to assess the reasons for the AC-values being negative. Consequently, these four herds were excluded from the study.

To test the hypothesis regarding an association between the use of vaccines and antimicrobials, a treatment index was initially developed for the three types of vaccination: a) PCV2 vaccination; b) *Mycoplasma hyopneumoniae* vaccination; c) *Lawsonia intracellularis* vaccination. The index was used to assess the level of protection present on the herds. Each index was created taking into account different vaccination protocols that could be applied, the number of doses prescribed for the herds and the number of animals in each herd. However, this approach was subsequently abandoned as it was difficult to assess which protocol would have been most appropriate for each herd as well as there were no data available regarding the applied protocols. Moreover, one vaccine prescription could cover up to an entire year of use and this could lead to erroneous information about the level of protection within the herd in the year of the study. For example, a herd could have stored vaccines for more than a year, or have a vaccine prescription for an entire year starting halfway through the year.

It was therefore decided to employ a dichotomous approach instead, which meant splitting the herds into two different groups: zero – corresponding to the herds not using the vaccine; and group 1 – corresponding to the herds using the vaccine. This approach was chosen to minimize the probability of falsely-allocating the herds to the two groups.

In the process of analysing the average AC in the different groups of indexes for each vaccination, one herd was removed from the dataset as it was identified as an outlier due to the AC being the double of the maximum value of the remaining herds, and hence, considered very unlikely to be true. Consequently, 1,513 herds were included in the analysis.

2.2 SUBSEQUENT DATA MANAGEMENT

The age groups defined in *VetStat* are 'boars, sows and 'piglets' (200 kg), 'weaners' (7 – 30kg) and 'finishers' (30kg – slaughter) (Stege *et al*., 2003). On average, from the antimicrobial agents used for the production of a pig until slaughter, 22% was used in the sow section, 36% during weaning period and 42% in the finisher section (DANMAP, 2012). Taking into account that weaners only cover the production from 7 to 30 kg, which corresponds to around 4 weeks, the treatment proportion[2] is much higher in weaners when compared with finishers and sows (DANMAP, 2012). This is the reason why only the AC for the age group 'weaners' (7 – 30 kg) was taken into account for this study.

In Denmark there are eight weeks between batches in the nursery (K. S. Pedersen, personal communication, March 10, 2014). This implies that 52/8 = 6.5 weaners can be produced per year per pen place[3] (K. S. Pedersen, personal communication, March 10, 2014). Therefore, a conversion factor of 6.5 was used to go from the number of pen places to the number of weaners produced per year.

For each of the 1,513 herds, a variable was created in order to estimate the average AC per weaner per year (2013). This variable was calculated using the total administered daily doses (ADD) in weaners in year 2013, for each herd, dividing it by the number of pen places for weaners, multiplied by 6.5.

$$\text{AC per weaner per year} = \frac{\text{ADD used (for all weaners in the herd)}}{\text{Number of pen places } \times 6.5}$$

On the other hand, to be able to assess if the production level had an influence on the AC in weaners, the annual production of weaners was estimated for each herd by using the number of pen places for weaners multiplied by 6.5.

$$\text{Annual production} = \text{Number of pen places } \times 6.5$$

Taking into account that weaners may receive more than one type of vaccine, all two-way combinations between vaccination groups were constructed to assess the possible association between different vaccinations and AC. Moreover, for the final models, one

[2]Refers to DAPD which is calculated as the number of standard doses for one kg of animal divided by the estimate live biomass in the age group (DANMAP, 2012).
[3]Number of pen places – data that corresponds to number of animals present in the herd (Dupont & Stege, 2013).

variable was developed to take into consideration all combinations of use of the three different vaccines within the herds. Resulting in eight different groups, as shown in Table 4 and Table 6.

For this study, the AC in weaners is presented by treatment indication. This allows the assessment of biologically meaningful correlations of the AC in weaners and the different vaccinations applied. Three different approaches were analysed: (1) total AC covering all the diagnostic groups as applied in *VetStat* – a) reproduction, urogenital system; b) udder; c) gastrointestinal system; d) respiratory system; e) joints, limbs, hooves, central nervous system, skin and f) metabolism, digestion, circulation; (2) AC only with gastrointestinal indication and (3) AC only with respiratory indication.

2.3 DATA ANALYSES

All data analyses were carried out in R (version 3.1.2 of 2014 – The R Foundation for Statistical Computing). Univariable analyses were performed for the total AC (Table 1), AC with gastrointestinal indication (Table 2) and AC with respiratory indication (Table 3). A t-test was conducted for each of the three types of vaccination comparing the AC for herds which used the vaccine with herds which did not use the vaccine.

For each of the two-way combinations, a t-test and a one-way ANOVA were conducted. Following the one-way ANOVA, a post hoc comparison, using the TUKEY HSD test, was performed to assess the statistical difference between the individual combinations (appendix 1).

Finally, multivariable analyses were performed for (1) total AC, (2) AC with gastrointestinal indication and (3) AC with respiratory indication as three separate outputs. The variable annual production (measured as number of weaners), as well as use of vaccine (divided into the eight different combinations of vaccine use) were the explanatory variables. It was tested whether annual production was significantly associated with the response and whether it acted as a confounder by being associated with the vaccine use. A P-value < 0.05 was used as threshold for statistical significance.

3 – RESULTS

3.1 RESULTS OF UNIVARIABLE ANALYSES

Out of the 1,513 herds selected for the study, 1,415 herds had antimicrobials prescribed for gastrointestinal disorders, and 836 herds had antimicrobials prescribed for respiratory disorders, corresponding to 94% and 55% of the herds, respectively.

Concerning the different vaccines, 58% (n=880) of the herds used the PCV2 vaccine and 42% (n=633) did not. *Mycoplasma hyopneumoniae* vaccination was used in 52% (n=787) of the herds and not used in 48% (n=726). *Lawsonia intracellularis* vaccination was the least used vaccine, with just 8% (n=115) of the herds using it and 92% (n=1,398) not using it.

In relation to the results of the univariable analyses regarding the total AC: herds using PCV2 and *Mycoplasma hyopneumoniae* vaccine had a significantly higher mean of AC than herds not using the vaccination (Table 1), whereas herds using *Lawsonia intracellularis* vaccination had a significantly lower mean of AC (Table 1). Concerning the impact of annual production, it explained 0.5% of the variance in the total AC (R^2=0.005, F=8.6, p=0.004) (Table 1, Figure 4).

Regarding the AC with gastrointestinal indication (Table 2), the results demonstrate that herds using the PCV2 vaccine had a notably higher mean of AC than those not using the vaccination. Furthermore, herds using the vaccine had on average a higher annual production than those not using PCV2 vaccination. The annual production had no influence on the AC with gastrointestinal indication (Table 2).

Relating to AC with respiratory indication the results revealed that herds using *Mycoplasma hyopneumoniae* vaccination had a significantly higher mean of AC than herds not using the vaccine (Table 3). On the contrary, herds using *Lawsonia intracellularis* vaccination had a significantly lower mean of AC with respiratory indication than those not using the vaccine – about half of the AC (Table 3). Concerning the variable annual production, herds using *Mycoplasma hyopneumoniae* vaccine or herds using vaccination against *Lawsonia intracellularis* had a higher annual production compared to the herds that did not use the vaccines (Table 3). However, the results show that annual production had no influence on the AC with respiratory indication (Table 3).

The results of the univariable analyses, as well as descriptive statistics, for the two-way combinations of three different vaccines, are presented in the appendix 1: for total AC

(Appendix 1 – Table 1); AC with gastrointestinal indication (Appendix 1 – Table 2) and AC with respiratory indication (Appendix 1 – Table 3). These intermediate results support the results listed above.

3.2 RESULTS OF MULTIVARIABLE ANALYSES

The final models are presented in Table 4 for total AC, in Table 5 for AC with gastrointestinal indication and in Table 6 for AC with respiratory indication.

With respect to total AC (Table 4), the results of multiple regression analyses indicated that the variable annual production and the variable representing different combinations of vaccine use explained 3% of the variance in the AC (R^2=0.03, F=7.2, p<0.001). The results of the model also showed that, when the annual production increased by 1000 weaners, the average ADD per weaner per year (total AC), increased by 4 ADD (Table 4). The use of *Mycoplasma hyopneumoniae* vaccination alone (group 4, n=221) or together with PCV2 vaccination (group 5, n=507) was associated with a statistically higher AC than the use of no vaccine at all (group 0, n=380); an increase of 19.9 and 31.8 ADD per weaner per year (AC) comparing with group 0, respectively; or the use of only PCV2 vaccination (group 1, n=290); 15.3 ADD per weaner per year (AC) more when compared to group 0. The rest of the groups, reflecting the remaining vaccine combinations, were not associated with statistically different levels of AC (p>0.05).

In regards to AC with gastrointestinal indication, PCV2 vaccination was the only significant variable, explaining 0.4% of the variance in the AC (R^2=0.004, F=6.1, p=0.01) (Table 5). Herds using PCV2 vaccination (n=830) were associated with a statistically higher (7.6 ADD per weaner per year) AC with gastrointestinal indication, than herds not using this vaccination (n=585).

In relation to the AC with respiratory indication (Table 6), the results of multiple regression analyses indicated that the variable different combinations of vaccines explained 2% of the variance in the AC (R^2= 0.02, F=3.5, p=0.001). This final model suggests that the routine use of *Mycoplasma hyopneumoniae* vaccination alone (group 4, n=134) or with PCV2 vaccination (group 5, n=349) was associated with a significantly higher AC with respiratory indication, compared to the herds that did not use any of the three vaccines (group 0). Group 4 was associated with more 24.7 ADD per weaner per year (AC) compared to group 0; and group 5 with more 17.3 ADD per weaner per year (AC) when compared to group 0. The changes on AC associated with the rest of the combinations were not statistically significant (p>0.05).

Table 1 – Descriptive statistics (mean and 95% confidence intervals (CI) and univariate analyses of the association between the use of different vaccines and the total antimicrobial consumption in 1,513 Danish one-site pig herds, 2013.

Vaccination	Total Antimicrobial consumption (ADD/weaner/year)			Annual production (Number of weaners/year)		
	Mean	**95% CI**	**P-value**	**Mean**	**95% CI**	**P-value**
PCV2			< 0.0001			< 0.0001
No use (n=633)	86.3	(83.4 – 89.2)		10,714	(10,336 – 11,092)	
In use (n=880)	103.9	(101.5 – 106.3)		12,745	(12,407 –13,083)	
***Mycoplasma hyopneumoniae* (Myc)**			< 0.0001			0.01
No use (n=726)	85.9	(83.3 – 88.5)		11,031	(10,696 – 11,366)	
In use (n=787)	106.4	(103.9 – 108.9)		12,692	(12,317 – 13,067)	
***Lawsonia intracellularis* (LI)**			0.04			0.09
No use (n=1398)	97.4	(95.5 – 99.3)		11,690	(11,448 – 11,932)	
In use (n=115)	85.6	(80.1 – 91.1)		14,392	(12,825 – 15,959)	

	Total antimicrobial consumption		
	Estimate	**Se** (Standard error)	**P-value**
Intercept	24.5	2.9	< 0.001
Annual Production	0.1	0.03	0.003
Adjusted R-square		0.005	
F-Statistic	8.6		0.004

Figure 4 – Scatter plot explaining the effect of the annual production in the total antimicrobial consumption

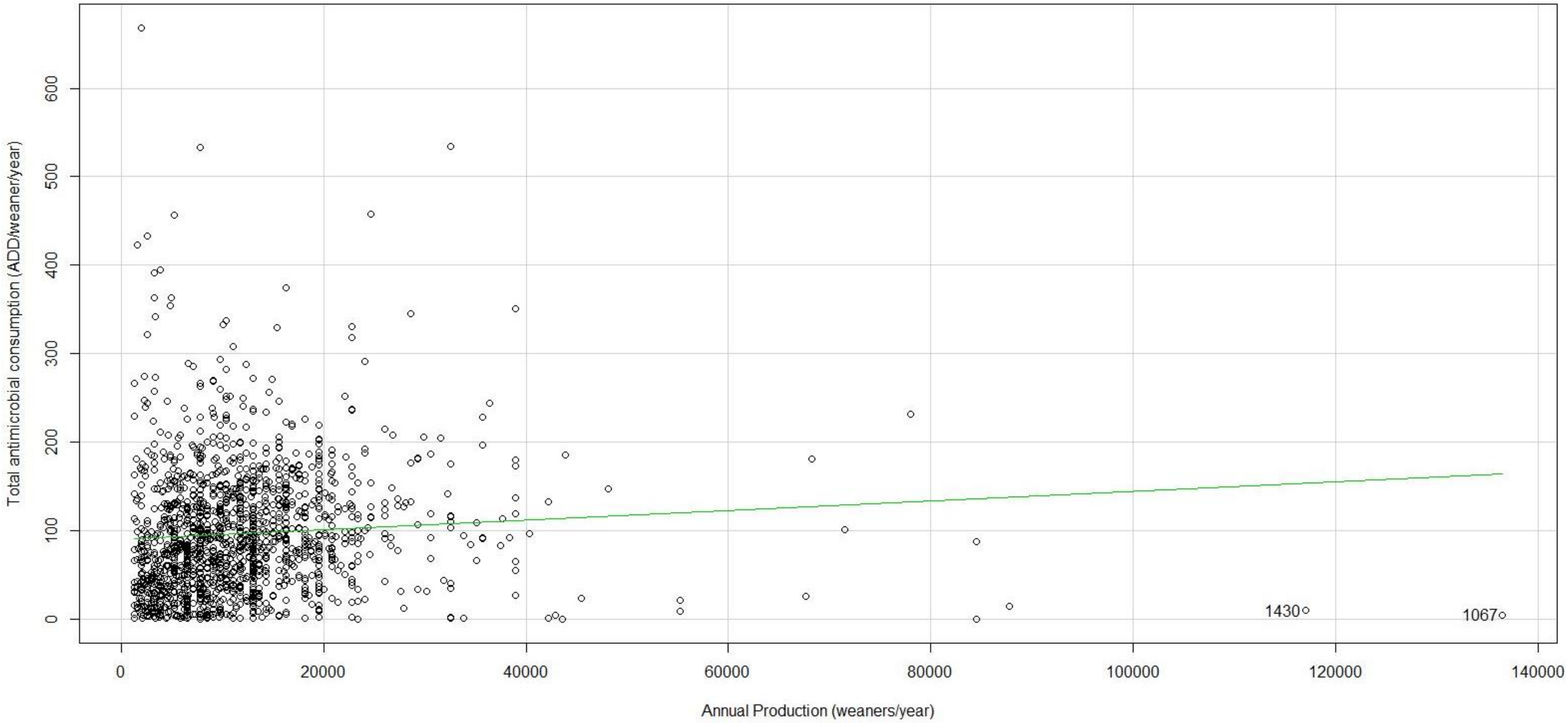


Table 2 – Descriptive statistics (mean and 95% confidence intervals (CI) and univariate analyses of the association between the use of different vaccines and antimicrobial consumption with gastrointestinal indication in 1,415 Danish one-site pig herds, 2013.

Vaccination	Antimicrobial consumption with gastrointestinal indication (ADD/weaner/year)			Annual production (Number of weaners/year)		
	Mean	**95% CI**	***P*value**	**Mean**	**95% CI**	***P*value**
PCV2			0.01			< 0.0001
No use (n=585)	64.6	(62.3 – 66.9)		10,819	(9,441 – 12,196)	
In use (n=830)	72.2	(70.2 – 74.2)		12,979	(11,629 – 14,329)	
***Mycoplasma hyopneumoniae* (Myc)**			0.7			0.0004
No use (n=681)	68.3	(66.0 – 70.6)		11,147	(10,817 – 11,478)	
In use (n=734)	69.7	(67.7 – 71.7)		12,957	(12,564 – 13,349)	
***Lawsonia intracellularis* (LI)**			0.8			0.06
No use (n=107)	68.9	(67.3 – 70.5)		11,845	(11,601 – 12,090)	
In use (n=1308)	70.6	(65.1 – 76.1)		15,030	(13,365 – 16,694)	

	Antimicrobial consumption with gastrointestinal indication		
	Estimate	**Se** (Standard error)	**P-value**
Intercept	18.4	2.4	<0.001
Annual Production	0.02	0.03	0.5

Table 3 – Descriptive statistics (mean and 95% confidence intervals (CI) and univariate analyses of the association between the use of different vaccines and the antimicrobial consumption with respiratory indication in 836 Danish one-site pig herds, 2013.

Vaccination	Antimicrobial consumption with respiratory indication (ADD/weaner/year)			Annual production (Number of weaners/year)		
	Mean	**95% CI**	***P*value**	**Mean**	**95% CI**	***P*value**
PCV2			0.6			0.001
No use (n=305)	41.3	(37.6 – 45.0)		11,820	(11,244 – 12,396)	
In use (n=531)	43.8	(41.3 – 46.3)		14,081	(13,648 – 14,514)	
***Mycoplasma hyopneumoniae* (Myc)**			0.0006			0.3
No use (n=314)	34.0	(30.9 – 37.1)		12,805	(12,227 – 13,384)	
In use (n=522)	48.2	(45.5 – 50.9)		13,527	(13,092 – 13,962)	
***Lawsonia intracellularis* (LI)**			< 0.0001			0.1
No use (n=774)	44.5	(42.3 – 46.7)		13,041	(12,697 – 13,384)	
In use (n=62)	22.2	(19.0 – 25.4)		15,947	(14,070 – 17,822)	

	Antimicrobial consumption with respiratory indication		
	Estimate	**Se** (Standard error)	**P-value**
Intercept	44.4	3.4	<0.001
Annual Production	-0.001	0.0002	0.6

Table 4 – Final model for the association between the use of vaccines and the total antimicrobial consumption in 1,513 Danish one-site pig herds, 2013.

Total Antimicrobial Consumption

	Estimate	Se	*P*-value
Intercept	74.2	4.1	<0.001
Annual Production	0.0004	0.0002	0.01
Combination of Vaccine use			<0.001
Group 0: Myc=0 & LI=0 & PCV2=0 (n=380) [c]	0	0	
Group 1: Myc=0 & LI=0 & PCV2>0 (n=290) [b]	15.3	5.5	0.005
Group 2: Myc=0 & LI>0 & PCV2>0 (n=35)	4.9	12.4	0.7
Group 3: Myc=0 & LI>0 & PCV2=0 (n=21)	8.9	15.7	0.6
Group 4: Myc>0 & LI=0 & PCV2=0 (n=221) [a, b]	19.9	5.9	0.0008
Group 5: Myc>0 & LI=0 & PCV2>0 (n=507) [a]	31.8	4.8	<0.001
Group 6: Myc>0 & LI>0 & PCV2=0 (n=11)	-5.6	21.5	0.8
Group 7: Myc>0 & LI &>0 PCV2>0 (n=48)	5.3	10.9	0.6
F-Statistic	7.2		<0.001
Adjusted R–Square		0.03	

[a], [b], [c] – different letters indicate levels that are significantly different.
Se – Standard error

Table 5 – Final model for the association between the use of vaccines and the antimicrobial consumption with gastrointestinal indication in 1,415 Danish one-site pig herds, 2013.

Antimicrobial Consumption with Gastrointestinal Indication

	Estimate	**Se** (Standard error)	P-value
Intercept	64.6	2.4	
PCV2 vaccination			0.01
No use of PCV2 (n=585)	0	0	
Use of PCV2 (n=830)	7.6	3.1	
F-Statistic	6.1		0.01
Adjusted R-Square		0.004	

Table 6 – Final model for the association between the use of vaccines and the antimicrobial consumption with respiratory indication in 836 Danish one-site pig herds, 2013.

Antimicrobial Consumption with Respiratory Indication

	Estimate	Se	P-value
Intercept	30.7	4.7	<0.001
Combination of Vaccines			0.001
Group 0: Myc=0 & LI=0 & PCV2=0 (n=155) [b]	0	0	
Group 1: Myc=0 & LI=0 & PCV2>0 (n=136)	9.6	6.9	0.2
Group 2: Myc=0 & LI>0 & PCV2>0 (n=13)	-19.4	16.9	0.3
Group 3: Myc=0 & LI>0 & PCV2=0 (n=10)	-2.4	19.1	0.9
Group 4: Myc>0 & LI=0 & PCV2=0 (n=134) [a]	24.7	6.9	0.0004
Group 5: Myc>0 & LI=0 & PCV2>0 (n=349) [a]	17.3	5.7	0.002
Group 6: Myc>0 & LI>0 & PCV2=0 (n=6)	12.6	24.4	0.6
Group 7: Myc>0 & LI &>0 PCV2>0 (n=33)	-5.3	11.2	0.7
F-Statistic	3.5		0.001
Adjusted R–square		0.02	

[a], [b], [c] – different letters indicate levels that are significantly different.
Se – Standard error

4 – DISCUSSION

The 1,518 one-site pig herds covered in this study represent all one-site pig herds registered in Denmark, in 2013, with a minimum of 50 sows and 200 weaners. By following these criteria, the selected herds were a representation of the tendency, registered from 1993 onwards, which reveals that pig herds in Denmark have integrated and evolved into larger pig herds (DAFC, 2014).

4.1 *LAWSONIA INTRACELLULARIS* VACCINATION

Bak & Rathkjen (2009) undertook a study in a Danish Specific Pathogen Free[4] herd where they showed positive effects of preventing PE with the use of vaccination such as improved growth rate and a reduction in the use of antimicrobials after the administration of vaccines. Also, Thaker & Bilkei (2006) concluded that oral vaccination reduced Lawsonia-associated losses as well as improved health and the immune state of pigs in a large Hungarian pig production unit with a high prevalence of *Lawsonia intracellularis* infection. These findings are in agreement with the results of the univariable analyses of the present study, which showed a statistically significant association between the use of *Lawsonia intracellularis* vaccination and a reduction in the amount of AC with respiratory indication in weaners, when compared to herds that did not use this vaccination. This may suggest a direct causal association.

As *Lawsonia intracellularis* vaccination is used to prevent PE (Stege *et al.*, 2000), it would be expected that AC with gastrointestinal indication would have reduced in herds using the vaccination instead of the AC with respiratory indication, as shown in the results. This could suggest that – to some extent – antimicrobials are being used for other disease categories than those officially prescribed by the veterinarians. Certain antimicrobials, as for example some doxycyclines, are not registered for treating infections with gastrointestinal indication in Denmark, although they are known to be effective in everyday practice (Danish pig veterinarians, personal communication, April 28, 2014). Consequently, it might be that practitioners used the antimicrobials officially prescribed for gastrointestinal infections to treat respiratory infections (according to *VetStat* rules).

However, the results of the multivariable analyses revealed that when taking all three vaccines into account simultaneously, the differences in AC between the groups that use *Lawsonia intracellularis* vaccination and those that did not use it, were no longer statistically

[4] Specific Pathogen Free – i.e.: free of infection with *Mycoplasma hyopneumoniae*, *Actinobacillus pleuropneumoniae* (serotypes: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12) Toxigenic *Pasteurella multocida, Brachyspira hyodysenteriae*, Porcine Reproductive and Respiratory Syndrome virus (type 1 and 2), *Sarcoptes scabiei* var. suis and *Haematopinus suis*.

significant. These results can be explained by the fact that, with regards to the AC with respiratory indication, only 62 herds (7.4%) used this vaccine. It would be better to gather data from a larger number of herds using the *Lawsonia intracellularis* vaccine in order to address this issue in detail as this could be too small of a sample to have relevance in the overall multivariable analysis. Moreover, the final model only accounted for 2% of the variance in the AC. This indicates that other unknown factors explain the variance in the AC, such as: other vaccines applied in each herd; management practices; biosecurity measures; herd health status and others, which are discussed in detail in section 4.5 (Considerations and limitations).

4.2 *MYCOPLASMA HYOPNEUMONIAE* VACCINATION

Infections with *Mycoplasma hyopneumoniae* are highly prevalent in swine producing areas worldwide, causing significant economic losses, mainly due to the reduced performance and increased susceptibility to other infections (Maes *et al*., 1998, 2008; Maes, Pasmans, & Haesebrouck, 2011; Simionatto, Marchioro, Maes, & Dellagostin, 2013). Although vaccination is still considered the most adequate measure for controlling the infection (Mateusen et al., 2002), the results of the univariable and multivariable analyses indicate that, on average, herds using *Mycoplasma hyopneumoniae* vaccination had a higher use of antimicrobials with respiratory indication in weaners than herds not using the vaccine. In this particular situation, one possibility for what might have occurred is that herds using *Mycoplasma hyopneumoniae* vaccination were those that had already detected this pathogen within the herds. Subsequently, these herds were using more antimicrobials with respiratory indication in order to control *Mycoplasma hyopneumoniae* and, simultaneously using the vaccine in order to avoid the predisposition of the infected animals to secondary invaders, especially other pulmonary pathogens (Thacker & Minion, 2012).

Another alternative is that herds could have been using antimicrobials with the aim of preventing respiratory disorders and, after detecting *Mycoplasma hyopneumoniae*, started the vaccination as a complementary tool. This approach makes sense, since pigs treated with antimicrobials such as tetracyclines and macrolides have less clinical signs and a lower number of secondary infections (Ciprián *et al*., 2012; Vicca *et al*., 2004). Even if treatment with antimicrobials was ongoing within the herds towards other respiratory disorders, vaccination was a good practice to use as a complement, since the presence of *Mycoplasma hyopneumoniae* is associated with (1) reduced average daily weight gain, (2) reduced feed efficiency and (3) increased mortality (Maes, Verbeke, Vicca, Verdonck, & Kruif, 2003). Vaccination is allied to increase average daily weight gain as shown by Jensen *et al*. (2002) in a meta-analysis comparing the effect of vaccines against *Mycoplasma hyopneumoniae*.

Furthermore, vaccination is associated with reduced clinical signs, diminished lung lesions and fewer treatment costs (Maes *et al*., 1999).

On the contrary of what was expected, these results indicates that herds which used *Mycoplasma hyopneumoniae* vaccination had a higher AC for respiratory indication than herds not using the vaccine. As already mentioned, this may result from the presence of health problems prior to the initiation of vaccine use or from the need to control the predisposition towards respiratory disorders as a consequence of the presence of *Mycoplasma hyopneumoniae*. These are two brief possible scenarios of what might have occurred in these herds, however, there could be other possible circumstances for these results.

4.3 PORCINE CIRCOVIRUS TYPE 2 VACCINATION

The weaning period is associated with inflammation, increased permeability of the gut, and high occurrence of diarrhoea that may lead to high antimicrobial use (Moeser & Blikslager, 2007; Oostindjer *et al.*, 2014). In addition, PCV2 is associated with enteritis, which can justify the results of the univariable and multivariable analyses that showed a higher use of antimicrobials with gastrointestinal indication in herds using PCV2 vaccination, compared to those not using the vaccine. A possibility is that herds using PCV2 vaccination could have enteritis associated with the presence of PCV2 and therefore turned to this strategy as a resort to control the problem. Furthermore, it could be a coincidence that PCV2 vaccination was used at the same time as a high amount of antimicrobials to treat weaners against gastrointestinal disorders, as these disorders can be related with various pathogens other than PCV2. Moreover, PCV2 has been linked with various clinical syndromes in different organic departments. This might justify the application of a major amount of antimicrobials (Chae, 2004; Opriessnig *et al*., 2007; Segalés *et al*., 2005).

Being PCV2 considered a necessary but not sufficient factor to develop clinical disease (Tomás, Fernandes, Valero, & Segalés, 2008), practitioners could have decided to routinely use PCV2 vaccination in these herds, in order to avoid this “factor” and consequently, the occurrence of PCVDs. A published meta-analysis, comparing the effect of PCV2 vaccines on average daily weight gain and mortality rate in pigs, revealed that the mortality rate decreased and the average daily weight gain increased significantly for nursery-finishing pigs in response to PCV2 vaccination (Kristensen *et al*., 2011). Also, Aerts & Wertenbroek (2011), Brockhoff *et al.*(2009), Dommelen & Wertenbroek (2011) and Koenders & Wertenbroek, (2012) findings demonstrate an improvement in production and reduced antimicrobial use in pig herds, after the implementation of PCV2 vaccination.

4.4 COMBINATIONS OF DIFFERENT VACCINES

Vaccination can improve pig health, welfare and productivity, being a power tool towards controlling diseases on pig farms (RUMA, 2006). Especially after some restrictions in the use of antimicrobials agents, the importance of vaccines has increased (Lee & Yoo, 2015). Therefore, implementation of effective vaccination programs is needed (RUMA, 2006). Taking into account the implementation of pig vaccination programs adjusted to the reality of each farm, it makes sense to assume that several vaccines are administered to the animals in order to increase the individual immunity and therefore, the immunity within the herds; with the purpose of reducing the need for subsequent treatment.

Regarding total AC and all possible combinations of vaccines; this was the only model where the annual production was statistically significant (p=0.01). However, according to the results, when the production increases 1000 weaners per year, the total AC increases only by 4 ADD (Table 4), which can be considered as not biologically meaningful.

In addition, all the F-statistics for the final models suggest a strong association between the AC and the use of the vaccines. However, the adjusted square of the correlation coefficient (R^2) for all the final models was low. This means that many more unknown factors are important to describe the AC and hence, some of the possible unknown factors are discussed in detail in section 4.5 (Considerations and Limitations)

In the final models, the use of *Mycoplasma hyopneumoniae* and PCV2 vaccines were the protagonists in respect to the consumption of antimicrobials. The use of *Mycoplasma hyopneumoniae* vaccine alone, or combined with PCV2 was associated with a higher AC, compared to the herds that did not use any of the three vaccines.
Regarding the AC with respiratory indication, it is important to notice that herds which used *Mycoplasma hyopneumoniae* vaccine alone (n=134) were associated with 24.7 ADD per weaner per year (AC) more than the ones not using any vaccine (n=155). On the other hand, herds that used PCV2 combined with *Mycoplasma hyopneumoniae* (n=349) were associated with 17.3 ADD per weaner per year (AC) more compared to those not using any of the three vaccines (n=155). Even if herds that used both vaccines were linked with a higher AC when compared to those not using the vaccines, when *Mycoplasma hyopneumoniae* and PCV2 were used together, the AC with respiratory indication was lower than the ones just using *Mycoplasma hyopneumoniae*. These results suggest that the use of both vaccines might provide a larger immune protection to the animals than the use of one vaccine alone and therefore, reduce the need for the use of antimicrobials. Interaction between both of these agents has been described; some field investigations suggest that PCV2 plays an important

role in some cases of PRDC (Harms, Halbur, Sorden, & others, 2002; Kim, Chung, & Chae, 2003). Moreover, Opriessnig *et al*. (2004) undertook a study to investigate the interactions between *Mycoplasma hyopneumoniae* and PCV2. Among other results, the study concluded that *Mycoplasma hyopneumoniae* potentiates the severity of PCV2-associated lung and lymphoid lesions, increases the amount and prolongs the presence of PCV2-antigen, and increases the incidence of PMWS in pigs. The results of the study mentioned above might also support some of the results for total AC. For instance, the herds that used PCV2 in combination with *Mycoplasma hyopneumoniae* vaccines (n=507) were associated with 31.8 ADD per weaner per year (AC) more compared to the ones not using any of the three vaccines (n=380). In this situation, it is possible that these herds were infected with both agents, and the presence of *Mycoplasma hyopneumoniae* increased the persistence of PCV2 and the incidence of PMWS within the herds. Thus, being the ones with a higher total AC, in order to control these infections and the predisposition of the infected animals to secondary invaders. Another possibility is that herds could have been using antimicrobials with the aim of preventing/treating early health problems and, after detecting *Mycoplasma hyopneumoniae* and PCV2, started using vaccination against both of these agents.

However, it is important to notice that the results for total AC also showed significant differences between the herds that used *Mycoplasma hyopneumoniae* and PCV2 vaccines together (n= 507) and the ones that only used PCV2 vaccine (n=290). It is characterised by a difference of less 17.3 ADD per weaner per year (AC), in the herds that only used PCV2 vaccine, when compared to the ones using both. These findings are in agreement with an experimental study undertaken by Seo, Park, Park, & Chae (2014), with the aim to determine the effects of both these vaccines on disease severity. The results demonstrated that vaccination with PCV2 alone decreased the likelihood of PCV2-induced lesions by *Mycoplasma hyopneumoniae* but, the vaccination against *Mycoplasma hyopneumoniae* alone did not decrease the chances of PCV2-induced lesions by *Mycoplasma hyopneumoniae* in dually infected pigs (Seo *et al*., 2014). This study shows one possibility of what may have occurred in these herds.

For instance, the herds using both vaccines could have primarily started by using *Mycoplasma hyopneumoniae* vaccine; however this vaccination could not have been able to control possible clinical PCV2 infection and the infection of the animals by other agents. Therefore, these herds might have used PCV2 vaccine and a higher amount of antimicrobials with various indications (total AC).

Herds that used the PCV2 vaccine alone had a lower total AC than those using both vaccines; this could be explained by this virus being associated with several syndromes in different organic departments of the pigs, thus, needing to use less antimicrobials with various indications (total AC).

Obviously, each herd has its own identity, daily routines, vaccination programs and strategies to a thriving pig production that was unknown at the time of the study. A lot of questions remain to be addressed and more information besides the use of different vaccines is important to attend to these topics of vaccination use as an alternative to antimicrobials. In section 4.5, several of the possible unknown factors will be discussed.

4.5 CONSIDERATIONS AND LIMITATIONS

It is important to take into consideration that the information regarding what was happening in each herd was partially out of reach as there was no knowledge about (1) vaccination protocols applied (2) time of vaccination (3) other vaccines that were part of the general plan of vaccination (4) herd health status, (5) internal and external biosecurity (6) management practices (7) turnover of animals in each herd (8) export of live animals.

In regards to points (1), (2) and (3) it would be advised to routinely monitor these topics in each herd and each vaccination; this information was not retrievable from *VetStat*. At the beginning of the study an intensive literature review was carried out and field recognition about the three types of vaccines. While for *Lawsonia intracellularis* there was only one commercial vaccine available in the market, for *Mycoplasma hyopneumoniae* and PCV2, there was a much larger variety of available vaccines. Thus, a wide diversity of protocols, in different animals and different doses applied could be present. Prescription of vaccines and therefore the number of doses for a specific herd could be available in *VetStat* but still not providing absolute certainty about the precise time of vaccination.

Information about point (4) would provide knowledge about disease outbreaks and the majority of pathogens present or absent within each herd. This would allow for a better association between the applied vaccines and the AC.

Point (5) corresponds to biosecurity, which is defined as the implementation of measures that reduce the risk of disease agents being introduced and spread within a group of animals. External biosecurity relates to the prevention of pathogens entering a herd, whereas internal biosecurity is how the spread of pathogens within a herd is reduced (FAO, 2010). Examples include: access control, barriers for wildlife and rodents, special hygiene protocols as ‘all-in all-out’ system of production and avoiding the mixing of animals within the herd, or quarantining stock for an appropriate period prior to mixing (McEwen & Fedorka-Cray, 2002; Official Journal of the European Union, 2015; Stärk, 2013; Vaarten, 2012). It would be truly important to have information on this point, since it has been stated that high levels of biosecurity are associated with reduced use of antimicrobials and increased animal health and productivity (Laanen *et al.*, 2013; Ribbens *et al.*, 2008). Thus, obtained knowledge of biosecurity within each herd could be associated with the need for more or less intake of antimicrobials by weaners and the application of vaccines.

In respect to point (6) – different management practices – there might be heterogeneity within different herds, namely with regards to: environmental temperatures; cleaning methods; general hygiene; ventilation rates; stocking density; feeding practices; feed strategies for individual age groups; records concerning different areas of the herd; amongst others. For example, poor air quality in housing, inadequate light, space and reduced quality of water can result in an increased susceptibility of animals to various kinds of disease and may decrease production of pigs (McEwen & Fedorka-Cray, 2002; Official Journal of the EU, 2015; Vaarten, 2012). All the examples mentioned above, may or may not contribute to the onset and establishment of syndromes/infections and, consequently the recurrence to prescriptions for antimicrobials and vaccination. Therefore, information about management practices regarding each herd would be valuable in order to study the association between vaccination and antimicrobials.

Concerning point (7), it would be relevant to have information about this topic for each herd, ensuring that the AC was the closest possible to reality. For example, it would be helpful to further explore the association between antimicrobials and vaccination if the replacement rate of animals was provided instead of the number of pen places and, therefore the correspondent herd size and annual production.

Point (8) would be important to measure and take into consideration for this study, since Denmark exported a total of 10,034,078 live pigs in the year 2013, of which 387,406 were pigs from 0-15 kg and 9,223,980 were pigs from 15-50 kg (DAFC, 2014). In this particular situation there was no knowledge about the amount of exports for each herd. Also, the pigs produced for export may or may not have received similar amounts of antimicrobials as the other pigs in the same age groups. Moreover, the administration of vaccines in pigs for export might, or might not be a requirement of the destination of exportation.

Considering that this is a preliminary study based on register data, there were several important topics that have not been carried out due time. Besides the inclusion of all points mentioned earlier in this section, the study of AC with regards to active substance used would be the next step along this project. Furthermore, it would have been of interest to include substances which may be administered or produced as medicated feeding stuff and study its association with other ways of antimicrobial consumption. It would also be interesting to test for presence of regional differences concerning the AC with different active substances administered.

This study enabled a first approach about a possible association between vaccination and antimicrobial use in pig herds in Denmark. More information is needed to assess to which extent vaccinations can reduce the need for the use of antimicrobials. Further studies need to be carried out in order to take into consideration the issues mentioned in this section. It is important to also take into account the economic aspect of this matter: is vaccination a good alternative to antimicrobials economically? If in the future we get an affirmative answer to this question and farmers can see return on their investment, improvement of pig herds´ health and productivity will occur through a wider application of routine vaccination instead of routine antimicrobial treatments.

5 – CONCLUSIONS

This was a pioneer study, as it included a large number of herds, providing an overview of the use of vaccination as an alternative to antimicrobials in the pig industry in Denmark. In general, herds applying routine vaccination had a higher use of antimicrobials compared to herds not using the vaccines – probably as a result of existing health problems in the herds prior to the use of vaccination. These results suggest that vaccination alone is not able to reduce the use of antimicrobials, despite being an asset in this regard. Further studies need to be carried out in order to take into consideration other factors towards the prevention of diseases, which are of extreme importance, such as biosecurity measures and management practices within the herds.

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V – APPENDICES

APPENDIX 1

Table 1 – Descriptive statistics (mean and 95% confidence intervals (CI) and analyses of the association between the use of different combinations of vaccines, the total antimicrobial consumption and the annual production in 1,513 Danish one site pig herds, 2013.

	Antimicrobial consumption (ADD/weaner/year)			Annual production (Number of weaners/year)		
Combinations of different vaccines	**Mean**	**95% CI**	***P*-value**	**Mean**	**95% CI**	***P*-value**
PCV2 + *Lawsonia intracellularis* (LI)			< 0.0001			< 0.0001
Group 0: LI=0 & PCV2=0 (n=601)	86.4 [a]	(83.5 – 89.3)		10,716 [a]	(10,321 – 11,110)	
Group 1: LI=0 & PCV2>0 (n=797)	105.7 [b]	(103.1 – 108.3)		12,424 [b]	(12,125 – 12,724)	
Group 2 LI>0 & PCV2=0 (n=32)	83.0 [a,b]	(72.0 – 94.0)		10,683 [a,b,c]	(9,662 – 11,704)	
Group 3: LI>0 & PCV2 >0 (n=83)	86.5 [a,b]	(80.2 – 92.8)		15,822 [c]	(13,703 – 17,941)	
PCV2 + *Mycoplasma hyopneumoniae* (Myc)			< 0.0001			< 0.0001
Group 0: Myc=0 & PCV2=0 (n=401)	79.6 [c]	(76.2 – 83.0)		10,727 [a]	(10,225 – 11,230)	
Group 1: Myc =0 & PCV2>0 (n=325)	93.6 [a]	(89.5 – 97.7)		11,406 [a]	(10,987 – 11,825)	
Group 2: Myc>0 & PCV2=0 (n=232)	97.8 [a,b]	(93.0 – 102.6)		10,691 [a]	(10,134 – 11,249)	
Group 3: Myc>0 & PCV2>0(n=555)	109.9 [b]	(106.9 – 112.9)		13,529 [b]	(13,055 – 14,003)	

[a], [b], [c] – different letters indicate levels that are significantly different

Table 2 – Descriptive statistics (mean and 95% confidence intervals (CI) and analyses of the association between the use of different combinations of vaccines, the antimicrobial consumption with gastrointestinal indication and the annual production in 1,415 Danish one site pig herds, 2013.

	Antimicrobial consumption (ADD/weaner/year)			Annual production (Number of weaners/year)		
Combinations of different vaccines	**Mean**	**95% CI**	***P*-value**	**Mean**	**95% CI**	***P*-value**
PCV2 + *Lawsonia intracellularis* (LI)			0.09			< 0.0001
Group 0: LI=0 & PCV2=0 (n=555)	64.2	(61.9 – 66.5)		10,815 [a]	(10,421 – 11,210)	
Group 1: LI=0 & PCV2>0 (n=753)	72.4	(70.2 – 74.6)		12,604 [b]	(12,297 – 12,912)	
Group 2: LI>0 & PCV2=0 (n=30)	70.9	(59.6 – 82.2)		10,875 [a,b]	(9,802 – 11,948)	
Group 3: LI>0 & PCV2>0 (n=77)	70.6	(64.2 –77.0)		16,648 [c]	(14,395 – 18,901)	
PCV2 + *Mycoplasma hyopneumoniae* (Myc)			0.1			< 0.0001
Group 0: Myc=0 & PCV2=0 (n=372)	65.4	(62.4 – 68.4)		10,770 [a]	(10,282 – 11,258)	
Group 1: Myc=0 & PCV2>0 (n=309)	71.8	(68.3 – 75.3)		11,601 [a]	(11,170 – 12,033)	
Group 2: Myc>0 & PCV2=0 (n=213)	63.2	(59.8 –76.6)		10,903 [a]	(10,310 – 11,496)	
Group 3: Myc>0 & PCV2>0(n=521)	72.4	(69.9 – 74.9)		13,796 [b]	(13,304 – 14,289)	

[a], [b], [c] – different letters indicate levels that are significantly different

Table 3 – Descriptive statistics (mean and 95% confidence intervals (CI) and analyses of the association between the use of different combinations of vaccines, the antimicrobial consumption with respiratory indication and the annual production in 836 Danish one site pig herds, 2013.

	Antimicrobial consumption (ADD/weaner/year)			**Annual production (Number of weaners/year)**		
Combinations of different vaccines	**Mean**	**95% CI**	***P*value**	**Mean**	**95% CI**	***P*value**
PCV2 + *Lawsonia intracellularis* (LI)			0.03			0.0007
Group 0: LI=0 & PCV2=0 (n=289)	42.2 [a,b]	(38.4 – 46.0)		11,884 [a]	(11,283 – 12,486)	
Group 1: LI=0 & PCV2>0 (n=485)	45.9 [a]	(43.3 – 48.5)		13,729 [a]	(13,317 – 14,142)	
Group 2: LI>0 & PCV2=0 (n=16)	24.5 [a,b]	(17.1 – 31.9)		10,658 [a,b]	(9,066 – 12,251)	
Group 3: LI>0 & PCV2 >0 (n=46)	21.4 [b]	(17.8 – 25.0)		17,785 [b]	(15,367 – 20,203)	
PCV2 + *Mycoplasma hyopneumoniae* (Myc)			0.003			0.002
Group 0: Myc=0 & PCV2=0 (n=165)	30.6 [a]	(26.7 – 34.5)		12,645 [a,b]	(11,710 – 13,580)	
Group 1: Myc=0 & PCV2>0 (n=149)	37.8 [a,b]	(32.8 – 42.8)		12,983 [a,b]	(12,335 – 13,631)	
Group 2: Myc>0 & PCV2=0 (n=140)	53.8 [b]	(47.4 – 60.2)		10,848 [a]	(10,255 – 11,440)	
Group 3: Myc>0 & PCV2 >0 (n=382)	46.1 [b]	(43.3 – 48.9)		14,509 [b]	(13,964 – 15,054)	

[a], [b], [c] – different letters indicate levels that are significantly different

Important highlights of the Appendix 1:

Relating to the PCV2 and *Lawsonia intracellularis* combination, in the herds where these two vaccines were used, the mean of the AC with respiratory indication was lower than the herds not using both of them (Appendix 1 – Table 3). Concerning the PCV2 and *Mycoplasma hyopneumoniae* combination, in the herds where *Mycoplasma hyopneumoniae* vaccination was used, the mean of the AC with respiratory indication was particularly higher than the herds not using either this vaccination and PCV2 (Appendix 1 – Table).

APPENDIX 2 – PROCEEDING OF SAFEPORK 2015 CONFERENCE

Associations between vaccination and antimicrobial consumption in Danish pig herds, 2013

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Abstract

Antimicrobial agents are being used in modern swine production worldwide, generating concern in regards to the development of antimicrobial resistance. Identifying efficient alternatives has therefore become a subject of interest. The aim of this study was to explore the impact of routinely used vaccination as an alternative to antimicrobial consumption in weaner pig herds. The hypothesis was that herds with increased use of vaccination would have a lower antimicrobial consumption. Data were obtained from the Danish VetStat database in which prescriptions of medication for livestock are recorded as well as the Danish Central Husbandry Register. All Danish one-site pig herds, active in year 2013, with >50 sows and >200 weaners were selected for the study. Initially, data were analysed using a univariable model, and secondly a multivariable linear regression model was applied. The analyses included use of three different vaccines against Porcine Circovirus Type 2 (PCV2), *Mycoplasma hyopneumoniae* (M_HYO) and *Lawsonia intracellularis* (LAW), respectively, as well as annual production measured as number of weaners produced in a year. The outcome was the average antimicrobial consumption measured in animal daily does (ADD) per weaner pig. Out of the 1,513 herds selected for the study, 1,415 herds had antimicrobials prescribed for gastrointestinal disorders, and 836 for respiratory disorders. PCV2 vaccine was used in 880 herds, M_HYO vaccine in 787 and LAW vaccine was the least used, with 115 herds using it. The results suggested that antimicrobials to some extent were being used for other disease categories than those officially prescribed by the veterinarians. On average, herds using the different combinations of vaccines had higher use of antimicrobials than herds not using the vaccines – probably as a result of health problems in the herds existing prior to the initiation of vaccine use. Information about vaccination protocols, health status, biosecurity, and management practices was not available limiting the ability to assess causality.

Introduction

In the European Union (EU), antimicrobials (AM) are used extensively in veterinary medicine for therapeutic purposes, as metaphylactic treatment, or as prophylaxis to prevent disease (Aarestrup *et al*., 2008; Barton, 2014). This has led to concerns about the potential risk of the development of antimicrobial resistance (AR) in animals and the transfer of resistance factors from animals to humans, through zoonotic bacteria or consumption of animal products (Aarestrup *et al*., 2008; Allen *et al*., 2013; Seal *et al*., 2013). In Denmark, there has been political and public focus on this issue since the 1990s (Bager, 2000). As a result, a series of events emerged: phasing out of the use of AM as growth promoters; limited veterinary profit from sales of AM to 5-10% and development of guidelines for prudent use of AM. Moreover, in 2010 the Danish veterinary authorities adopted the Yellow Card initiative, a scheme that sets threshold limits to AM use in swine farms (Alban *et al*., 2013). That same year, an industry ban was implemented to stop the use of cephalosporins (Alban *et al*., 2013; Jensen & Hayes, 2014). The measures listed above reflect the increasing political pressure that is

forcing Danish pig producers to reduce the usage of AM in their farms. Therefore, efficient alternatives to routinely applied AM have become crucial. Vaccines are being considered as means to decrease the burden of animal diseases and also to reduce the need of AM for therapeutic purposes (Allen *et al*., 2013). However, the extent of using vaccination as an alternative to AM will depend on its cost, effectiveness and ease of use (Allen *et al*., 2013).
To explore the impact of further use of vaccination as an alternative to AM, the present study was carried out using data from Danish pig herds covering the year 2013. The aim was to investigate overall associations between vaccination against PCV2, M_HYO and LAW and antimicrobial consumption (AC). The hypothesis was that herds with increased use of vaccination would have a lower AC.

Material and Methods

The data used in this study were obtained from VetStat and the Danish Central Husbandry Register. All Danish one-site pig herds, active in the year 2013, with >50 sows and >200 weaners were selected for the study (N=1,513 herds). In VetStat, the AC is divided into three age groups: sows with piglets, weaners (7 – 30 kg), and finisher pigs. Moreover, the indication the AM were prescribed for is recorded. Only the AC for the weaners was taken into account in the present analysis. In Denmark there are 8 weeks between batches in the nursery. This implies that 52/8 = 6.5 weaners can be produced per year per pen place. Therefore, a conversion factor of 6.5 was used to go from the number of pen places to the number of weaners produced per pen place per year.

For each of the 1,513 herds, a variable was created to reflect the AC per weaner produced per year (2013). This variable was calculated using the total administered animal daily does (ADD) in weaners in 2013, for each herd, divided by the number of pen places for weaners, multiplied by 6.5. Also, to be able to assess if the production level had an influence on the AC in weaners, the annual production (AP) of weaners was estimated for each herd by using the number of pen places for weaners multiplied by 6.5.

For this study, the AC in weaners is presented by treatment indication. This allows for investigation of biologically meaningful correlations of the AC in weaners and the different vaccinations applied. Three different outcomes were analysed: (1) total AC (TAC) covering all diagnostic groups as applied in VetStat: a) reproduction, urogenital system; b) udder; c) gastrointestinal system; d) respiratory system; e) joints, limbs, hooves, central nervous system, skin and f) metabolism, digestion, circulation); (2) AC with gastrointestinal indication only (ACGI) and (3) AC with respiratory indication only (ACRI). All analyses were carried out in R (version 3.1.2). Univariable analyses were performed for TAC, ACGI, and ACRI, respectively. A t-test was conducted for each of the three types of vaccination comparing herds using the vaccine versus herds which did not use the vaccine. Subsequently, multivariable analyses were performed for 1) TAC, 2) ACGI, and 3) ACRI as three individual outcomes. The explanatory variables were AP and vaccine use. The latter variable had eight levels which reflected all combinations between the three different vaccines. It was tested whether AP was significantly associated with the response and whether it acted as a confounder by being associated with the vaccine use. A P-value < 0.05 was used as threshold for statistical significance.

Results

Out of the 1,513 herds selected for the study, all of them had AM prescribed for the total of diagnostic groups, 1,415 had AM prescribed for gastrointestinal disorders, and 836 for respiratory disorders. Concerning the different vaccines, 880 herds used the PCV2 vaccine, whereas M_HYO vaccination was used in 787 herds, and LAW vaccination was the least used vaccine, with just 115 herds using it. In the following the mean ADD per weaner per year will be reported as ADD.

Univariable analysis
Concerning TAC: herds using PCV2 vaccination (TAC=103.9 ADD, p<0.0001) or M_HYO vaccination (TAC=106.4 ADD, p<0.0001) had a higher mean of AC per weaner than herds not using each of these vaccines (TAC= 86.3 ADD and TAC=85.9 ADD, respectively) whereas herds using LAW vaccination had a lower mean of AC (TAC=85.6 ADD, p=0.04) than the ones not using it (TAC= 97.4 ADD). The AP explained 0.5% of the variance in TAC (R^2=0.005, F=8.6, p=0.004).

Regarding ACGI: herds using PCV2 vaccine (ACGI=72.2 ADD, p=0.01) had a notably higher mean of AC than those not using it (ACGI=64.6 ADD). The AP had no influence on the ACGI (p=0.5).

Regarding ACRI: herds using M_HYO vaccination (ACRI=48.2 ADD, p=0.0006) had a substantially higher mean ACRI than herds not using the vaccine (ACRI=34.0 ADD). On the contrary, herds using LAW vaccination (ACRI=22.2 ADD, p<0.0001) had only half mean ACRI compared to those not using the vaccine (AC=44.5 ADD). AP had no influence on ACRI (p=0.6).

Multivariable analysis
Regarding TAC, the results revealed that a model containing AP and different combinations of vaccines explained a total of 3% of the variance in the AC (R^2=0.03, F=7.2, p<0.001). The output of the model also showed that, when the AP increases by 1000 weaners, the ADD per weaner per year (TAC), increases by 4 ADD. The use of M_HYO vaccination alone (N=221) or together with PCV2 vaccination (N=507) was associated with a statistical higher TAC than group 0 representing use of none of the three vaccines (N=380): TAC was 19.9 and 31.8 ADD higher per weaner compared with group 0, respectively. The use of only PCV2 vaccination (N=290) was associated with 15.3 ADD per weaner more when compared to group 0. The rest of the groups, reflecting the remaining vaccine combinations, were not associated with statistically different levels of TAC (p>0.05).

Regarding ACGI, PCV2 vaccination was the only significant variable and explained 0.4% of the variance in the ACRI (R^2=0.004, F=6.1, p=0.01). Herds using PCV2 vaccination (N=830) were associated with a statistically higher (7.6 ADD) ACGI, than herds not using this vaccination (N=585).

In relation to the ACRI, the results indicated that the variable representing different combinations of vaccines explained 2% of the variance in the ACRI (R^2= 0.02, F=3.5, p=0.001). This final model suggested that use of M_HYO vaccination alone (N=134) or in combination with PCV2 vaccination (N=349) was associated with a higher ACRI, compared to group 0. M_HYO vaccine alone was associated with 24.7 ADD more per weaner compared to group 0; and M_HYO+PCV2 vaccination with 17.3 ADD more (ACRI) compared to group 0. The changes in ACRI associated with the remaining vaccine combinations were not statistically significant (p>0.05).

Discussion

LAW vaccination is used to prevent proliferative enteropathy (Stege *et al*., 2000). Thus, it would have been expected that ACGI would have been reduced in herds using the vaccine instead of the ACRI, as shown in our results. This could suggest that – too some extent – AM are being used for other disease categories than those officially prescribed by the veterinarians. Certain AM, such as some doxycyclines, are not registered for treating infections with gastrointestinal indication in Denmark, although they are known to be effective in everyday practice (contact to Danish pig veterinarians). These findings are in agreement with previous studies and suggest that using this vaccine could reduce the amount of AMs used in pig herds – see e.g. Bak and Rathkje (2009) and Thaker and Bilkei (2006).

However, the results of the multivariable analyses revealed that when taking into account all three vaccines simultaneously, the differences in AC between the groups that used LAW vaccination and those that did not use it, were no longer statistically significant. This probably because only 62 herds (7.4%) used this vaccine – hence the effect was "diluted" in the large analysis.

Herds using M_HYO vaccination had a higher ACRI than herds not using it. It is likely that these herds had previously detected M_HYO and, subsequently, had had a higher ACRI because of need to control M_HYO infection. Thacker and Minion (2012) point to the predisposition of M_HYO–infected animals to secondary invaders, especially other pulmonary pathogens. Another explanation is that these herds could have been using AM with the aim of preventing respiratory disorders and, after detecting M_HYO, started the vaccination as a complementary tool. This approach makes sense, since pigs treated with AM such as tetracyclines and macrolides have less clinical signs and a lower number of secondary infections (Vicca *et al*., 2004; Ciprián *et al*., 2012). Moreover, vaccination is associated with reduced clinical signs, diminished lung lesions and fewer treatment costs (Maes *et al*., 1999).

Herds using PCV2 vaccination had a higher ACGI than those not using it. PCV2 is associated with enteritis, which may explain the higher ACGI for vaccination and, at the same time higher ACGI weaners. Above all, the weaners as an age group are associated with gastrointestinal inflammation, increased permeability of the gut (Moeser & Blikslager, 2007) and high occurrence of diarrhoea that may lead to high AM use (Oostindjer *et al*., 2014). These herds could also have initiated routine to PCV2 vaccination with the aim of preventing various other clinical syndromes which PCV2 can be linked to (Tomás *et al*., 2008). Moreover this vaccination has been associated with decreased mortality rate and increased average daily weight gain (Kristensen *et al*., 2011).

Herds that used both M_HYO and PCV2 vaccines were associated with a higher ACRI when compared to those not using any of the three vaccines. However, when M_HYO and PCV2 were used together, ACRI were lower than the ones just using M_HYO. This suggests that the use of both vaccines might provide a better immune protection to the animals than the use of M_HYO on its own and therefore, reducing the need for AM. In fact, interaction between both of these agents has been described previously (Harms *et al*., 2002; J. Kim *et al*., 2003). Herds that used both M_HYO and PCV2 vaccines were associated with increased TAC compared to the ones not using any of the three vaccines. In this situation, is possible that presence of M_HYO amplified the persistence of PCV2, that is in agreement with previous studies (Opriessnig *et al*., 2004). Thus, being the ones with a higher TAC. Another possibility is that these herds have been using AM routinely with the aim of preventing/treating early health problems and, after detection, started vaccination against both of these agents. Results also showed a lower TAC in herds which used PCV2 vaccination alone compared to herds that used both PCV2 and M_HYO vaccines. These findings are in agreement with an experimental study undertaken by Seo et.al (2014), which aimed at determining the effects of both vaccines in disease severity. On the other hand, PCV2 is linked with several disease syndromes. Thus, protection by vaccination may be justified to use less AM for various indications.

The F-statistics for the final models suggest a strong association between the AC and the use of vaccines. However, the adjusted squared correlation coefficient (R^2) for all three final models was low, which means that other unknown factors are important to describe the variation in AC. Some of the possible factors are: (1) vaccination protocols, (2) time of vaccination, (3) use of other vaccines, (4) herd health status, (5) internal and external biosecurity, (6) management practices, (7) turnover of animals in each herd and (8) requirement from buyers of weaners. At the time of this study, this information was not available limiting the ability to assess causality.

Conclusion

On average, herds using the different combinations of vaccines had higher use of antimicrobials than herds not using the vaccines – probably as a result of health problems in the herds existing prior to the use of vaccination.

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To have access to a full reference list, please contact the first author.

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