UNIVERSIDADE DE LISBOA

FACULDADE DE MEDICINA VETERINÁRIA

BIOSECURITY IN DAIRY CATTLE FARMS IN THE NORTH AND CENTRE OF PORTUGAL

MAFALDA PEDRO MIL-HOMENS

ORIENTADORA:
Doutora Yolanda Maria Vaz

TUTOR:
Mestre Telmo Renato Landeiro Raposo Pina Nunes

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Dedicado à Professora Yolanda Vaz e ao Professor Telmo Nunes, duas pessoas que me ensinaram muito, sem as quais, realizar este projeto nunca teria sido possível.

"The Artist always has the masters in his eyes."
— Ralph Waldo Emerson

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Resumo

Biossegurança em explorações de bovinos leiteiros no Norte e Centro de Portugal

Existe um entendimento crescente de que a falta de biossegurança e a resistência aos antimicrobianos são pontos cruciais na produção animal e a Lei de Saúde Animal enfatiza esses dois aspetos. Ao implementar um questionário baseado na avaliação de biossegurança do Biocheck da universidade de Ghent, este projeto teve como objetivos caracterizar a biossegurança em explorações leiteiras no Norte e Centro de Portugal, criar um índice de biossegurança, avaliar possíveis fatores de risco, caracterizar o uso de antibióticos e criar uma ferramenta que permitisse aos veterinários ajudar e aconselhar os produtores.

Foram amostradas 151 explorações leiteiras, implementando o questionário através de entrevista pessoal e *online* através do Googleforms, foi criado um relatório em *html*, o qual foi enviado aos veterinários que participaram no projeto. Os aspetos de biossegurança contidos no questionário e no relatório estão relacionados com compra de animais, maneio reprodutivo, higiene e desinfeção, movimento de pessoas e animais na exploração, controlo de pragas e contacto com outros animais, maneio sanitário, maneio de partos, de vitelos e de animais adultos, maneio de alimentação e abeberamento, tendo-se adicionado também um ponto referente à utilização de antibióticos e vacinas. Observou-se que havia grandes variações nas pontuações de biossegurança total, que iam do mínimo de 42 até máximo de 80,4 pontos (em 100 pontos), com pontuações médias de biossegurança externa e interna de 71,3 e 67,7 (em 100 pontos) respetivamente.

As variáveis com maior influência nas pontuações de biossegurança foram as relacionadas com o uso de equipamentos de proteção individual, higiene das mãos, limpeza de maternidades e enfermarias e colocação de animais em quarentena. Em relação ao uso de antibióticos, apesar de os mesmos serem administrados em todas as explorações, apenas 47,4% possuíam um protocolo de utilização de antibióticos.

Isto significa que ainda há muito a melhorar em relação à biossegurança e na sensibilização em relação ao uso de antibióticos, no entanto para obter uma caracterização mais confiável e representativa da realidade, uma amostra maior deveria ser coletada.

Palavras-chave: Gado leiteiro; Biossegurança; Prevenção

Abstract

Biosecurity in dairy cattle farms in the North and Centre of Portugal

There is an increasing understanding that lack of biosecurity and antimicrobial resistance are crucial points in animal production and the Animal Health Law emphasizes these two aspects. By implementing a questionnaire based on Biocheck from Ghent University's biosecurity assessment, this project aimed to make a characterization of dairy farms' biosecurity in North and Centre of Portugal, it aimed to create a biosecurity index, evaluate possible risk factors, characterize antibiotics use and create a tool that allows veterinarians to help and advise farmers.

The questionnaire was implemented in 151 dairy farms through personal interview and through Googleforms; a report was built and sent to the veterinarians that participated in the project. The biosecurity aspects present in both questionnaire and final report are related to animal purchase, reproduction management, hygiene and disinfection, people and animal movements, health, calving, calf, adult animals, dairy and feed and drinking water management, with one group also added for antibiotic and vaccine use.

A difference was observed between total biosecurity scores that went from 42 to 80.4 (in 100 points) and external and internal biosecurity mean scores of 71.3 and 67.7 (in 100 points) respectively. Variables with bigger influence in biosecurity scores were the ones related to the use of individual protective equipment, hands hygiene, cleaning of calving and sick pens and putting animals in quarantine. Regarding antibiotics' use, every farm treated animals with antibiotics, but only 47.4% had an antibiotic protocol for its responsible use.

This means that there is still a lot to improve regarding biosecurity and awareness regarding the use of antibiotics, although a bigger sample should be taken to make a more reliable and significant characterization.

Keywords: Dairy cattle; Biosecurity; Prevention

Index

List of Figures

List of Tables

List of acronyms and abbreviations

ABLN	Associação de apoio à Bovinicultura Leiteira do Norte/ Northern Dairy Association
AHL	Animal Health Law
DGAV	Direção Geral de Alimentação e Veterinária/ Portuguese General Directorate of Food and Veterinary
FAO	Food and Agriculture Organization
HACCP	Hazard Analysis Critical Control Point
IPE	Individual Protection Equipment
OPP	Organização de Produtores Pecuários/Livestock Production organization
SNIRA	Sistema Nacional de Informação e Registo Animal/ National Animal Information and Registration System
UCADESA	União de Cooperativas de Agrupamentos de Defesa Sanitária de Entre Douro e Minho/Union of Cooperatives and Sanitary Defense Groups from Entre Douro e Minho
USDA	United States Department of Agriculture
WHO	World Health Organization

1. Chapter I – Internship report and project introduction

1.1. Internship report

This dissertation field work started on May of 2019. There were meetings with veterinarians and staff from Agros, Northern Dairy Association (ABLN) and Union of Cooperatives and Sanitary Defense Groups from Entre Douro e Minho (UCADESA) during June and July. A project presentation was made in August in AGRO-week for both veterinarians and farmers. In October a meeting with the General Director and with the Subdirector of Portuguese General Directorate of Food and Veterinary (DGAV) was taken to present the project.

During September and October, the student made an extracurricular internship in a small animal clinic on weekends, and during the week, competences regarding the use of R programming, Microsoft Office Excel, and Quantum Geographic Information System were developed, under Professor Telmo Nunes supervision. The development of these skills was essential to manage the data, to create the Biosecurity index and to build the final report for the veterinarians. A conference from the Federation Européenne pours la Santé Animale et la Securité Sanitaire – FESASS in Elvas, and the IX Congress of the Swine Scientific Society in Santarém were attended, both conferences focused on biosecurity issues and its importance nowadays.

The student stayed in Póvoa de Varzim from November 20th, to December 20th. On November 20th, the Biosecurity project was presented, inserted in a day of workshops for veterinarians and farmers, and on December 4th, and February 5th, presentations for Proleite's veterinarians, took place. The visits to the dairy farms were monitored by veterinarians, two veterinarians from Agros, one from ABLN, and two from the Livestock Production organization (OPP). During the visits to the farms, the Biosecurity questionnaire was implemented; issues regarding biosecurity and animal welfare were discussed with veterinarians and farmers, and participation in clinic and surgery procedures was also possible.

On January 5th, 2020, the collected data analysis and the final report building procedure began. On February 21st and February 24th, 2020, presentations focused on results regarding the interviews implemented on the farms took place. The first presentation was taken at the Faculty of Veterinary Medicine in Lisbon, through Webinar for professors, veterinarians, and students, and the second one took place in Agros headquarters through an oral presentation for both veterinarians and farmers.

On May 23rd, June 27th, and July 4th, the student participated in an epidemiological study focused on COVID-19 seroprevalence in Almeirim. From August

3rd to August 21st, the student made an extracurricular internship at Hospital Escolar Veterinário (HEV).

1.2. Project introduction

The present dissertation main goals were to make a biosecurity characterization on dairy farms at the north and centre regions of Portugal, to raise awareness of its importance to farmers and veterinarians, to build a biosecurity index, to identify risk indicators and to make a characterization of the antibiotics' use and convey the importance of its responsible use.

Biosecurity is defined by the Food and Agriculture Organization as:

> (...) a strategic and integrated approach that encompasses the policy and regulatory frameworks (including instruments and activities) that analyze and manage risks in the sectors of food safety, animal life and health, and plant life and health, including associated environmental risk (Food and Agriculture Organization FAO 2003).

To accomplish the proposed objectives, several steps were taken. Firstly, a literature review was carried out to identify dairy farms risk factors and their importance for animal and human health and for the environment, as well as the biosecurity measures that should be taken to prevent those risks.

Secondly, the Biocheck questionnaire for dairy cattle from Ghent University was translated and adapted to our country's reality using experts' opinions to evaluate the biosecurity measures, and adapt them to real farm problems and risks. After this procedure, a protocol focusing on corrective measures for biosecurity flaws was also built.

The process of preparing and developing the final questionnaire and the relative weight of each parameter related to the biosecurity aspects, took place during a period from May 2019 to November 2019.

The final model was therefore adapted to local practices and at the same time allowed to make a comparison with Biocheck from Ghent University. This was important to understand existing differences between countries and in which aspects our biosecurity can be improved.

This dissertation was divided in 5 parts. Starting with a literary review in Chapter 2 to introduce the theme, focusing on biosecurity importance, association between infectious diseases and biosecurity, biosecurity risk assessment, preventive biosecurity measures, responsible use of antibiotics and some examples of biosecurity protocols implemented in several countries.

In Chapter 3, there is a detailed explanation about the methodology used to develop the project, followed by presentation of results and discussion of the same results in Chapters 4 and 5 respectively.

2. Chapter II – Literature review

2.1. The importance of Biosecurity

According to FAO (2003), biosecurity is a holistic concept that covers environmental protection, food safety and agriculture sustainability, by implementing disease and pest prevention and control, and by managing invasive genotypes and species, among others. It has an important role nowadays due to globalization. Globalization is a process of economic integration at a global level, which is facilitated by lower transaction costs and less barriers in capital and goods movements. There are some aspects that define the globalization process, such as an outward-oriented policy and freer trade, the emergence of transnational companies and freer movement of capital, innovation in technology and international flow of knowledge and an increase in people's mobility within and across borders. Globalization also had an important role on food consumption patterns resulting in a convergence of these patterns (FAO 2015; FAO 2019).

Biosecurity in agriculture is a concept that covers multiple areas; therefore, it requires the interaction between farmers and their suppliers and also the competent authorities of agriculture, environment and public health. It can also involve the work of non-governmental organizations, interest groups, media, research institutes, universities, industry, public opinion and government administrative bodies responsible for imports, exports and legislation (FAO 2007).

In the Animal Health Law (Regulation (EU) 2016/429, Article 4), 'biosecurity' means:

> (…) the sum of management and physical measures designed to reduce the risk of the introduction, development and spread of diseases to, from and within:
>
> (a) an animal population, or
>
> (b) an establishment, zone, compartment, means of transport or any other facilities, premises or location.

Biosecurity can also be divided in external and internal. External biosecurity focuses on interactions between the farm and the exterior, and its goal is to prevent pathogens entrance and exit. Internal biosecurity focuses on preventive measures taken inside the farm, with the goal of preventing pathogen's dissemination (Biocheck UGent c2019).

There are several factors that can influence the preventive practices such as: the agent's biologic features, like pathogenicity, contagiousness and the capacity to persist in the environment, the population's characteristics and the knowledge, and perception of risk that farmers, veterinarians and technicians have (Morley PS 2002).

Failing to implement biosecurity can lead to disease spread and relevant losses to the farms. These losses can be due to a decrease in production and number of animals (direct losses) or to economic costs and blocked access to markets (indirect losses) (Knight-Jones and Rushton 2013).

Is extremely important to be aware of biosecurity flaws, to take action as soon as possible, and to use prevention tools in the main areas that can affect the farm (Brennan and Christley 2012). According to the Animal Health Law (Regulation (EU) 2016/429, Article 11) all the people that have contact with the animals, such as farmers, veterinarians, transporters, etc., must have knowledge in biosecurity procedures, animal welfare and responsible use of antibiotics.

Brennan and Christley (2012) showed on a study that the majority of farmers did not isolate animals when they returned to the farm after going to sales or markets, there were more farmers treating animals than making health checks, 28% of farms said that external vehicles entered in areas where animals were kept, only 4% referred that dead stock collectors took cleaning and disinfection procedures and only 7% of the 56 farms reported taking biosecurity measures between animal groups.

These are examples of biosecurity flaws that can lead, according to this study in North-West England, to 57% of Bovine Viral Diarrhea prevalence, 52% of Bovine Tuberculosis prevalence, 43% of Leptospirosis prevalence and 26% of Infectious Bovine Rhinotracheitis prevalence, decreasing health and increasing production losses on farms (Brennan and Christley 2012).

Foot and Mouth Disease is a good example of the impact a disease can have in a population. This is a viral disease caused by an *Aphtovirus* from the *Picornaviridae* family which affects cloven-hoofed animals, with vesicles appearing on hoofs and mucosal tissues (Jamal and Belsham 2013). In 1839 a dairy herd owner in Islington, United Kingdom reported to the vet that his cows were limping and drooling. The disease was not known and it started to spread quickly (Woods A 2004*)*. Despite the measures taken to control the disease, there were several outbreaks along the years until the eradication in the 1980's. In 2001 a major outbreak took place and the United Kingdom was declared disease-free on January 2002 (Department of Environment, Food and Rural affairs c2008). Thompson D *et al.* (2002) published a study that evaluated the amount of losses due to the 2001 outbreak, focusing mainly on economic losses. Starting with agriculture and the food chain, the estimated amount of losses rounded the 3.1 billion pounds, focusing on tourism, the total amount of losses rounded 3.3 billion pounds achieving 2.5 billion in rural areas and 1.2 billion in urban areas (Thompson D *et al.* 2002).

Fasina FO *et al.* (2011) published a study focusing on economic benefits of disease prevention by implementing biosecurity measures regarding African swine fever. In this study specific points were defined, in which biosecurity should be applied. Maintaining closed herds, implementing farm operations that boosted workers' pride in what they were doing, ensuring animals were free from infection while entering and leaving the farm, preventing direct and indirect sources of infection, and promoting biocontainment to avoid disease's dispersion to other farms. All these measures focused on three pillars: segregation, cleaning and disinfection and according to the study, biosecurity measures were essential to avoid disease outbreaks and the loss in profit was practically irrelevant, because outbreaks can lead to extreme damage on farms especially in diseases such as Foot-and-Mouth-Disease or African Swine Fever, that will certainly end in massive sanitary slaughters. Therefore, implementing a biosecurity plan is extremely important to decrease the negative effects on production, health, economy and even politics.

2.2. Biosecurity Plan

According to the World Organization for Animal Health (OIE 2017), a Biosecurity plan must identify significant potential pathways for disease's introduction and spread, and must describe the measures that should be applied to decrease disease's risk of introduction and spread. The plan must ensure that these measures are reassessed periodically, altered if necessary and audited.

Some steps were defined and must be followed to accomplish a biosecurity plan. Firstly, hazard identification and prioritization take place, secondly, a risk impact assessment is done to evaluate disease impacts, after this, the Critical Control Points are defined and possible corrective measures are identified. There must be mitigation, management and correction of critical control points to prevent disease entry or escape. If the disease enters the farm, a contingency plan must be implemented for biocontainment, diagnostic tests should be implemented, animal clinical evaluation should be done and the disease's status must be monitored. All these parameters are audited by a veterinarian and the veterinary authorities should verify and endorse all the previous steps (Dewulf and Immerseel 2018).

The relationship between Biosecurity and risk must be taken into account in agricultural and farming management due to the changing risk environment these systems present (Meuwissen MPM *et al.* 2001; Donaldson A 2008). By evaluating, and communicating risk, it can be defined which factors are going to increase disease's dispersion, impact and risk in a population. Risk analysis enables the possibility to minimize negative impacts, to apply preventive measures and to act quickly when necessary (FAO 2007).

To analyze risk, some steps should be followed. Firstly, a risk assessment must be done to divide risks into several levels. To achieve this, relevant hazards should be identified, the negative impact that hazards could have on farm production must be defined, and the frequency of disease's occurrence must be determined, to identify which hazards represent the greatest risk. It is beneficial to define production targets that can be affected, such as: economic losses, market losses due to public and animal health risks, transmission to people who have contact with the farm animals, such as employees, family members, or farm visitors (Wells SJ 2000).

An exposure assessment must be made to evaluate disease transmission. This procedure starts by implementing risk factor analyses, followed by herd testing, through the estimation of the percentage of herds that are experiencing hazard, or through the estimation of the population attributable fraction (percentage of a population's disease that can be prevented through risk factors removal) and finally, a risk characterization should be made to determine disease's virulence, prevalence within herds and prevalence between herds (Wells SJ 2000).

2.2.1. Risk assessment in Biosecurity plans

Risk factor is, according to Burt B (2001, p.1007-1008):

> (…) an aspect of personal behavior or lifestyle, an environmental exposure, or an inborn or inherited characteristic which, on the basis of epidemiological evidence, is known to be associated with health-related condition(s) considered important to prevent.

According to Adams LB, Gray GD, Murray G (2012), risk factors contributing to disease's emergence and amplification can be the proximity between animals, humans and wildlife, urbanization, animal movement, trade, climate change, population's growth, intensive animal production and environmental degradation.

Several studies aim to determine risk factors according to different age groups and different locations inside the farm to define the main areas where the risk of disease's introduction is bigger and what can be done to prevent that.

Starting with calves' management, it was found that respiratory diseases and diarrhoea were the main causes that lead to calves' death (Gulliksen SM *et al.* 2009). This could have happened because calves were put on collective pens at 2 weeks of age (Svensson and Liberg 2006; Gulliksen SM *et al.* 2009). Another study showed that putting calves allocated next to an outer wall could lead to more diarrhoeas and that an increase in ammonia concentration could lead to respiratory disease cases (Lundborg GK, Svensson EC, Oltenacu PA 2005).

Calves only achieve an immune maturity at 4-5 months of age, which increases colostrum intake importance, mainly to avoid the peak of pneumonia that occurs after 2 weeks of age, when calves have the lowest levels of IgG1, IgG2, and IgA (Corbeil LB *et al.* 1984; Waldner and Rosengren 2009), something that can explain animals' high susceptibility of contracting diseases at this age (Valarcher and Hagglund 2006).

According to Lorenz, I *et al.* (2011) overpopulation, sharing airspace with older animals, inadequate ventilation, collective pens, groups with wider age differences and washing pens with calves inside, are risk factors that can lead to respiratory disease, mainly in calves, due to the increase of humidity, noxious gases, dust levels, and bacteria content in pens.

Some factors lead to immunosuppression for both adult and young animals, due to stress, which can increase the probability of getting a disease. These factors can be lack of nutritional intake, early weaning, transport, grouping animals in parks with high animal density, mixing different ages and mixing animals from different sources (Toews D, Martin SD, Meek AH, 1986; Svensson and Liberg 2006; Valarcher and Hagglund 2006).

According to Unnerstad HE *et al.* (2009), there are different risk factors associated with specific pathogens that can be found in mastitis. *Streptococcus uberis* appeared more if the bedding was made with straw or peat, *Klebsiella* spp. was isolated more frequently when sawdust was used, *Escherichia coli* was identified with higher milk yield and loose housing systems, contrary to *Staphylococcus aureus* that was isolated more in tie stalls. *Trueperella pyogenes* appeared more with low milk yield and in cows that suffered from teat lesions, just like *Streptococcus dysgalactiae* that was isolated in cases where teat lesions were found.

Fasina FO *et al.* (2011) showed in a study focusing on African swine fever, a group of risk factors that were mainly biosecurity flaws. Some of the factors can be found in dairy cattle farms, such as the presence of infected animals inside the farm, contacting with infected animals that were introduced in the farm without quarantine, using infected animals for natural mating, blending animals from different origins and exposures, feeding animals contaminated food, changing feed bags at the feed mills, workers having other farm animals at home, workers visiting other farms, workers visiting abattoirs, veterinarians and input suppliers visiting several farms a day, cattle buyers going to markets and farms, contaminated vehicles transporting feed and animals, contaminated farm equipment being used in multiple farms, animal´s waste, dead animals and body fluids being disposed of improperly, lack of veterinary counseling and services, lack of measures regarding cleaning, disinfection, and decontamination.

In conclusion, implementing measures to decrease the occurrence of risk factors is extremely important to maintain animal health and to obtain safe animal products.

2.3. Dairy cattle infectious diseases

To implement a biosecurity program it is essential to collect information on specific parameters related to infectious diseases, such as the reservoir, ways of transmission, agent characteristics, incubation period, and the period of active transmission, to reduce the probability of disease's introduction and transmission on-farm (Barrington G *et al.* 2002).

Infection can have several pathways. If based on contact, it can be direct contact with infected animals or body secretions and excretions, contact with carriers (animals that do not develop clinical signs or are not demonstrating clinical signs but can spread diseases), and through fomites. Infection can also happen due to spores present in the environment, due to contaminated food and water ingestion, aerosols, and due to arthropod bites. Other kinds of infections are iatrogenic (infection induced by medical procedures), nosocomial (infection contracted in a hospital environment), and endogenous (these are usually due to opportunistic agents that are part of animal's non-pathogenic microflora). These agents take advantage of immunosuppression or lack of other microorganism's competition and multiply (Timoney J *et al.* 1988).

Therefore, it is important to determine critical points where there can be immunosuppression and a higher probability of contracting infectious diseases as is revealed by a Wisconsin University study (2017). These points were: the first sixty days of life, in which the prevalent infections were caused by pathogens that lead to pneumonia, omphalitis and diarrhoea; the first mating, in which the prevalent infections were related to uterus, udder and hooves, and the transition to dry period, in which the prevalent infections were related to udder diseases (Ruegg P 2017).

Starting with diseases related to calves, one of the most common is neonatal diarrhoea that leads to big economic losses and is the cause of 5.5% of death in calves from birth to 21 days of age. Being a multifactorial disease, neonatal diarrhoea depends on environmental conditions, management practices, nutritional and immune status, pathogen exposure, and respective strain variation (Barrington G *et al*. 2002). Respiratory disease is another major cause of death in approximately a quarter of pre-weaned calves and half of weaned calves. The main pathogens causing respiratory disease according to Lorenz I *et al.* (2011) are bovine herpesvirus 1, bovine respiratory syncytial virus, parainfluenza 3 virus, *Mycoplasma bovis*, *Pasteurella multocida*, *Mannheimia haemolytica* and *Histophilus somni*.

Focusing on adult cows, according to Mekibib B *et al*. (2010), that developed a study in 107 cows, mastitis occurrence was higher in cases where udder hygiene was discarded and when teat lesions were present. The most prevalent pathogen in this study was *Staphylococcus aureus* with a prevalence of 47.1% followed by Coagulase Negative *Staphylococcus* with a prevalence of 30.1%. Other microorganisms isolated in this study were *Streptococcus* spp, *Micrococcus* spp, *Klebsiella pneumoneae*, *Escherichia coli, Corynebacterium* spp*, Enterobacter aerogenes* and *Bacillus* spp. According to Stankovic B *et al*. (2012), farm's biosecurity can be related to cattle's reproductive status, due to indicators related to cows health that are influenced by biosecurity procedures, such as farm's isolation, animals quarantine, equipment and tools management, vehicles control, visitors policy, and efficacy of sanitary programs implementation. Flaws in these procedures revealed the vulnerability of reproduction success, threatening dairy production.

2.4. Antimicrobial resistance

Antimicrobial resistance is the microorganism's capacity to contradict the inhibitory or killing function of an antimicrobial (Verraes C *et al.* 2013), is therefore related to animal, human and environmental health, and is considered a public health threat. Biosecurity measures are essential to decrease antibiotics use and to prevent the development and proliferation of infectious diseases. These measures include vaccination, access to clean water, better hygiene, and improvement of diagnostic means (WHO 2014).

The antimicrobial resistance can be intrinsic or acquired. In intrinsic resistance, the treatment with antibiotics won't be successful, because the bacterium is genetically predisposed to resist, and treating these cases could trigger secondary infections. Acquired resistance is related to the capacity certain bacterial strains have to mutate and evolve (Verraes C *et al.* 2013).

Antimicrobial resistance leads to flaws in medical treatment, reduced antimicrobial choices, secondary infections due to opportunistic bacteria, and an increase in virulence due to the selection of more virulent bacteria (Verraes C *et al.* 2013).

The process of predicting exact values regarding antimicrobial use on dairy farms is difficult and has some obstacles. The lack of veterinary counseling, lack of treatment records and treatment guidelines, antibiotics administration by untrained staff, and implementation of different protocol procedures from farm to farm are some of them. These parameters lead to the necessity of implementing evaluation procedures related to antimicrobial use since there isn't a unique standard procedure that can cover every farm (Sharma C *et al.* 2018).

In a study taken by the Teagasc Glanbia Monitor Farm Programme the association between biosecurity measures and antibiotic use on dairy farms was evaluated. For this study tails were clipped and udders were trimmed in advance, the udder was cleaned, and the person that administered the teat sealant wore gloves, after that, the dried off cows were separated from other cows and their beds were limed two times a day for a week. Treatments with antibiotics were applied if the Somatic Cell Count was higher than 100 000 cells/mL (Teagasc c2017).

This study showed that through proper biosecurity measures implementation, a decrease in the antibiotics use without compromising animals' production can be achieved.

2.5. Dairy cattle biosecurity - current overview and biosecurity measures

Consumers and the food industry's expectations related to food safety can be achieved through the use of good practices in milking hygiene, nutrition, animal welfare, animal health, environment, and socio-economic management (FAO 2011).

A study developed in Ireland revealed that 83% of farmers would implement biosecurity if it prevented disease introduction or if it resulted in an improvement for both cattle's health and welfare (Sayers RG *et al*. 2013). But biosecurity has an impact on much more than animal health, it is also important to guarantee environmental and human health (FAO 2011). And this message must be transmitted to farmers, so they can feel the responsibility of applying biosecurity on their farms because, according to Sayers RG *et al.* (2013), 27% of the farmers would only implement biosecurity measures if they received some kind of economic benefit.

Farm's biosecurity is focused on a series of parameters and protocols that rely on management practices (Stanković B *et al.* 2016), which lay, according to Sayers RG *et al*. (2013), on three parameters: closed herd, quarantine, and testing of purchased animals. According to Anderson D (2010), there must be a focus on personal biosecurity practices, such as the use of boots, head covering and coverall use, equipment biosecurity, such as truck maintenance, needle use, instrument maintenance and farm biosecurity practices, such as separation of animals, and decrease the movement of possible fomites inside the farm. According to Stanković B *et al.* (2016), visitors' policy, attitude towards equipment use, pest control, and farm impact on the environment are other parameters that should be evaluated when biosecurity is implemented. In the Biocheck system from Ghent University (c2019), as previously mentioned, biosecurity management is divided in external and internal and the following parameters are included on the assessment: external biosecurity focuses on purchase and reproduction, transport and carcass removal, feed and water, the entrance of visitors and employees, vermin control and contact with other animals or presence of other animals on the farm; internal biosecurity focuses on health management, calving management, calf management, dairy management, adult animals management, work and materials' organization (Biocheck UGent c2019).

Biosecurity procedures and good management practices should be implemented to limit the spread of diseases between and within herds. It is important to determine pathogen load on farms, verify the colostrum-intake, nutrition (Yates WD 1982; Barrington G *et al.* 2002), stocking density, ventilation, hygiene, and animal movement within the farm. Restraining animals' introduction, trying to introduce animals during summer months (when virus infections have lower incidence), applying quarantine measures, personnel and parks' hygiene, and "all-in-all-out" procedures in herd's different sections, were shown to be preventive measures for infectious disease's introduction (Wathes CM *et al.* 1983; Barrington G *et al.* 2002; Callan and Garry 2002; Svensson and Liberg 2006; Valarcher and Hagglund 2006).

Establishing the habit of hand hygiene and disinfection, wearing protection clothes, boots and gloves, especially if the person is a visitor, can lead to a decrease in disease's introduction and spread. Providing animal treatment, rapid detection of diseases, and isolation of sick animals can reduce both health and economic losses (Valarcher and Hagglund 2006).

According to FAO's dairy farming good practice guide (2011), there are several areas where biosecurity must be established. Regarding animal health and animal welfare, there must be management programs, protocols for medicine and chemicals' use, decrease in disease entry and dispersion, and the “five freedoms” should be implemented and accomplished, to achieve animal welfare standards.

Regarding water, feed, and milking procedures. Controlling water and feed quality, controlling feed storage, and guaranteeing hygiene during milk harvesting are biosecurity aspects important for the animal’s milk production and health (Adams LB, Gray GD, Murray G 2012).

One example of a biosecurity protocol is the one implemented by the National Mastitis Council, of the United States of America. This protocol focuses on management practices, management of clinical mastitis, udder health, equipment maintenance, therapy during the dry period, hygiene, and records keeping (Garcia S, Osburn B, Cullor J 2019).

The checklist follows several steps regarding biosecurity procedures, and to achieve proper udder health, there must be somatic cell count monitoring, microbiological culture on milk with high somatic cell counts, determination of mastitis rates and distributions, and establish and update udder health protocols. Dry cow management must be provided through feeding reduction, before dry off, through hygienic procedures implementation, through vaccination, and through body hair excess removal on flank and udder (Garcia S, Osburn B, Cullor J 2019).

Another parameter focused on the National Mastitis Council protocol is the milking procedure. Is an important aspect of dairy farms’ biosecurity and there are two parameters where biosecurity measures should be taken: the milking itself and the milking equipment. During the milking period, protocols regarding udder’s cleaning and disinfection, like Pre-dip and Post-dip should be implemented, and the milking park must have hygiene protocols. The milking equipment must have regular maintenance and liners, seals, valves, and teat liners must be replaced (Garcia S, Osburn B, Cullor J 2019).

Another protocol’s aspect is the records keeping because maintaining an animal health database leads the farmer to a better understanding of the problems the dairy farm is having and why those problems are happening. A good animal’s health record should have cow’s identification, days in milk, cases of mastitis, treatments received, treatment outcomes, and milk culture results. Records about the cause of mastitis and about somatic cell counts on the bulk tank should be kept too. A program’s and record’s review should be made by a veterinarian, extension specialist, or product technician (Garcia S, Osburn B, Cullor J 2019).

Regarding the farm’s personnel biosecurity measures, these are mainly focused on hygiene procedures. The hygiene processes can start with the simple act of handwashing and disinfection with alcohol-based sanitizers, followed by cleaning and disinfection of rubber boots and clothing. A specific area must be settled to personnel meals, there must be a separate place from animal housing, and animals should not be handled during worker's meal times, to reduce the risk of zoonotic infections (Morley PS 2002).

Applying cleaning protocols to surfaces, instruments, feeders, water throughs, and changing beds frequently are procedures with extreme importance since a clean environment diminishes the probability of disease introduction. Dumpsters and cleaning tools must have a specific identification according to their purpose (Morley PS 2002).

Another way to guarantee biosecurity is through the implementation of a Hazard Analysis and Critical Control Points (HACCP) protocol, as it was mentioned previously. HACCP was developed to identify and quantify risk magnitude, and to establish and implement corrective measures, in a way that eliminates or diminishes risk (Gardner IA 1997; Morley PS 2002).

There are several steps in the HACCP approach. Starting with hazards identification and definition of respective preventive measures, followed by critical control point's identification (a point where the control is applied and hazard is prevented or diminished). Limits are established to each control point and monitoring and adjustments must be made to each defined limit. When a critical limit is exceeded, a correction is made and verifications of these corrections must be recurrent. Recordkeeping, as previously mentioned, is important because allows observing issues that occurred along the process, and which solutions were taken to correct those issues (Morley P 2002).

The dairy industry HACCP system has the following measures established: diagnostic tests development and implementation on milk tank, collecting individual samples, and collecting environmental samples so that chemical and medical residues and microorganisms can be detected (Gardner IA 1997).

One point that was defined by the HACCP system as critical for foodborne diseases, was the cow's teat condition, something that can be improved through the implementation of hygiene procedures, proper management, and health treatment, leading to improvements in both animal welfare and health, but also in milk quality and quantity (Garcia S, Osburn B, Cullor J 2019).

2.6. Biosecurity evaluation procedures

Score systems must be defined to allow biosecurity evaluation, and to facilitate guidelines implementation regarding biosecurity measures. Around the world, score systems and guidelines were developed to evaluate biosecurity; a few examples are referred to next.

Biocheck from Ghent University is an online risk-based scoring system to evaluate the quality of farms biosecurity and it can be filled at www. Biocheck.UGent.be. This tool is composed by all relevant components of biosecurity on pig, cattle, and poultry farms and is subdivided into external and internal biosecurity. The scoring system takes the relative importance of the different biosecurity aspects into account, resulting in a risk-based weighted score, which was constructed based on experts' factor weighting. Biosecurity scores are provided immediately after the questionnaire completion, and the scores for each subcategory can be compared with national averages (Gelaude P *et al.* 2014).

In Biocheck.UGent the biosecurity score is calculated following these steps:

-each answer receives a score from 0 (total absence of preventive measures or presence of risk) to 1 (presence of preventive measures or total absence of risk);

-the score is then multiplied by each question's weight (given by experts);

-the questions' results inside a subcategory are summed and divided by the highest score possible for that subcategory;

-each subcategory result is then multiplied by the respective subcategory's weight (given by experts);

-the final score of internal and external biosecurity is the sum of the different subcategory scores;

-the sum of internal and external biosecurity scores is the overall biosecurity score (Gelaude P *et al.* 2014).

In Ireland, another dairy farm scoring system was built. The system had a questionnaire consisting of 120 questions, each one of the questions was evaluated by experts and, according to their opinion, questions they considered relevant were added. The questionnaire was pre-tested by dairy researchers at Teagasc and was piloted on 7 commercial dairy farms. The survey's purpose was to document and characterize the implementation of Biosecurity practices on dairy farms (Sayers RG *et al.* 2013).

In Germany, the program PraeRi (c2020) is being implemented in 750 farms across Northern, Eastern, and Southern Germany. The project is funded by the Federal Ministry of Food and Agriculture and has the participation of three universities from Berlin, Hannover, and Munich.

A group of veterinarians visits the farms and evaluates biosecurity procedures, animals' health, and nutrition. A report with corrective measures is sent to the farmers after the evaluation, and an award of 50 euros is attributed (PraeRi c2020).

These programs were based on a scoring system; contrarily to what happens in the National Animal Health Monitoring System in the United States (NAHMS), which is an educational program. This system was initiated in 1983 to implement data collection, respective analyses, and communication regarding animal health, management, and productivity. A multidisciplinary group composed by veterinarians, livestock commodity specialists, statisticians, trade economists, technical communicators, and technical support staff was organized to make studies related to one animal commodity or production type, through samples' collection in, at least 70 percent of the targeted animal population, and at least 70 percent of operations with the targeted animal in the United States. Swine, dairy, beef, and poultry commodities' studies are taken about every 5 years, while other commodities are studied at longer intervals. Epidemiologists, statisticians, and commodity specialists work as a team and generate summaries that reflect both national and regional animal populations and interpret the results. Epidemiologic models were developed to simulate disease spread and control. Some parameters were defined and values were attributed according to literary review, available data, and subject matter elicitation. The parameters were animal population, disease's dynamic, and disease's transmission, detection, surveillance and control (United States Department of Agriculture 2019).

The USDA has control, eradication, and biosecurity programs. One of the biosecurity programs is focused on poultry, and it is called "Defend the Flock". This is an educational program that encourages poultry owners to learn more and to apply biosecurity measures, by providing information, tools, and resources. Participants have access to checklists, videos, webinars, and educational resources available for download or print (United States Department of Agriculture 2019).

Another program regards sheep and goat biosecurity. This is also educational and provides information focusing on biosecurity principles, emergency management, zoonotic diseases, and information for veterinarians such as: which individual protection equipment they should wear and what they can do when an outbreak occurs (United States Department of Agriculture 2019).

3. Chapter III – Dairy cattle biosecurity in Portugal

3.1. Materials and Methods

The project consisted of a cross-sectional study focusing on biosecurity practices in Portuguese dairy farms in two different regions (North and Centre). As previously mentioned, the main goals were (1) to make a biosecurity characterization, (2) to increase awareness of farmers about biosecurity's importance, (3) to create a biosecurity index, (4) to identify lack of safety risk indicators, and (5) to create a tool to help veterinarians providing biosecurity counselling to farmers.

3.1.1. Project awareness and preparation of field work

Two awareness-raising actions on biosecurity were carried out, one at Agro-week on August 30th, 2019, and a second presentation on November 20th, 2019, within the framework of workshops and lectures organized by Agros and UCADESA for dairy cattle producers. In the Agro-week session, inserted in a series of lectures, the Dairy Cattle Biosecurity project was presented focusing on biosecurity importance and principles, and antibiotics' responsible use significance in animal production, the international patterns regarding these issues were also presented. In the workshop for dairy cattle producers, framed in a set of lectures with themes related to animal welfare, biosecurity, and water quality, the Dairy Cattle Biosecurity project was presented and discussed with the audience, focusing the same issues reported above.

On December 4th and on February 5th two meetings were carried out with Proleite's (Portugal's centre region agricultural cooperative for dairy cattle) veterinarians to present the Biosecurity project, to raise awareness on biosecurity's importance, to explain how the project was going to be implemented and to present the *html* report format.

3.1.2. Building and implementing the questionnaire

3.1.2.1. Eligible holdings and sampling

All dairy cattle holdings belonging to the region covered by UCADESA and Proleite with more than 20 cows over 2 years old were considered eligible for this project. The farms where the questionnaire was carried out were selected by convenience according to the farmer's availability to answer the questionnaire.

The results presented in this dissertation were obtained firstly through interview taken by the author during the period from November 20th to December 20th, 2019 (making a total of 87 dairy cattle farms sampled), and secondly through an online Googleforms ™ questionnaire taken by the veterinarians from UCADESA and Proleite during the period from January 1st to May 31st, 2020, making a total amount of 64 dairy cattle farms sampled through Googleforms™.

3.1.2.2. Questionnaire building

The biosecurity's assessment was carried out through the application of a questionnaire, built and tested by the trainee, its mentor, and tutor, by the veterinarians working for the livestock producers' organization (OPP), and by veterinarians working in the Portuguese General Directorate of Food and Veterinary (DGAV). The parameters included in the questionnaire were based on the Biocheck.UGent scoring system, developed by the University of Ghent, and were reviewed by the project partners.

To build the questionnaire 8 meetings were held, on 16-05-2019, 31-05-2019, 06-06-2019, 05-07-2019, 30-08-2019, 20-09-2019, 21-10-2019, and 06-11-2019, where the questionnaire was reviewed, some questions and answers were changed, and where the scores attributed to the different questionnaire indicators were defined.

The final questionnaire was composed of 157 questions inserted in 13 biosecurity aspects, classified as External (representing 65% of total score) and Internal (representing 35% of total score). The number of requisites in each biosecurity aspect and the respective score weighting is shown in Table 1.

Table 1: External and Internal Biosecurity indicators and respective scores

Biosecurity indicators	Number of requisites	Score
Farm characterization	8	2
Purchase of animals and reproduction	23	20
Transport and carcass removal	11	10
Feed and water	4	3
Personnel and visitors	19	20
Vermin control and other animals	11	10
External Biosecurity total		**65**
Health management	13	10
Calving management	10	6
Calf management	16	3
Dairy management	17	3
Adult animals management	4	4
Work organization and equipment	7	6
Use of antibiotics and vaccines	14	3
Internal Biosecurity total		**35**

The questionnaire was inserted in Googleforms™ and was tested before being implemented on farms and online. The test was taken by the trainee, its mentor, its tutor, private veterinarians, veterinarians from the official services, and it was tested on a farm in the Lisbon and Vale do Tejo area.

3.1.2.3. Implementing the questionnaire

The invitation to participate in the project was made through its dissemination, in the Agro-week on August 30th, 2019, a second presentation was made on November 20th, 2019, within the scope of workshops and lectures organized by Agros and UCADESA for the training of dairy cattle farmers, as previously mentioned, and a third presentation was made for Proleite's veterinarians on December 4th. The online questionnaire was available from 20-11-2019 until 31-05-2020 at:

→https://docs.google.com/forms/d/e/1FAIpQLSenav1kc4wGL_YSR64BHua2qp5M1879u-WOQAvvHtl_KcLDVA/viewform;

→https://docs.google.com/forms/d/e/1FAIpQLSe_MQfdAlFj1Bjv7ldB_JbRIveUGLCzjKkfPYhzZdM75hDpXA/closedform.

The surveys were firstly carried out through interviews, as previously mentioned. During these interviews, the trainee was supported by two veterinarians responsible for the animal welfare audits implemented by Agros, by a veterinarian and a zootechnical engineer who worked in the ABLN, and two veterinarians who worked for the OPP. During this period, 87 surveys were made through an interview, as previously mentioned. From January 1st to the end of May 31st, questionnaires were implemented on the farms by veterinarians from UCADESA's OPP and by veterinarians who worked for Proleite, making a total of 64 surveys (57 from Proleite farms and 7 from UCADESA farms). The final amount of sampled farms from November 20th to May 31th was 151.

3.1.3. Individual report

Individual reports were created and sent by e-mail in a dynamic *html* file to the veterinarians. The reports were built using RStudio™ 3.6.1 (packages used in RStudio™ 3.6.1 can be consulted in Appendix II), a free software environment for statistical computing. These reports had two different formats and were built for each milk company. Firstly, a report was developed for Agros' farms, with farms' biosecurity assessment indexes related to total, internal and external biosecurity, with indexes related to each biosecurity aspect, focused on the questionnaire, with a list of biosecurity best practices (corrective measures) according to the responses given to the questionnaire, a benchmarking page where the values obtained by each farm were compared to the values obtained by other farms, the farm's geographical risk factors, and a page for material and methods explaining the report. Secondly, a report was developed for farms belonging to UCADESA but that were not integrated into Agros, with indexes calculated for total, internal and external biosecurity, and for each one of the biosecurity aspects referred in the questionnaire, with a list of biosecurity best practices, and a page for material and methods explaining the report. Two other reports were built for Proleite with the same parameters as the ones mentioned for Agros' farms.

3.1.3.1. Biosecurity evaluation

To achieve the final biosecurity scores, the value obtained in each answer was divided by 100 and was multiplied by the score attributed to each question, to obtain the question's score. For each one of the biosecurity aspects, scores obtained for questions were summed, divided by the sum of scores attributed to each question by the experts, and multiplied by the scores attributed to each biosecurity aspect by the experts. A sum of the biosecurity aspects' final scores was made and the percentage calculated taking into account the maximum value that could be reached if all biosecurity practices were applied with the highest score. The results were presented in dashboard graphs, color-coded according to 3 percentage thresholds: Green>= 75%; Orange>= 40% - < 75%, and Red<40% of the maximum score (Figure 1).

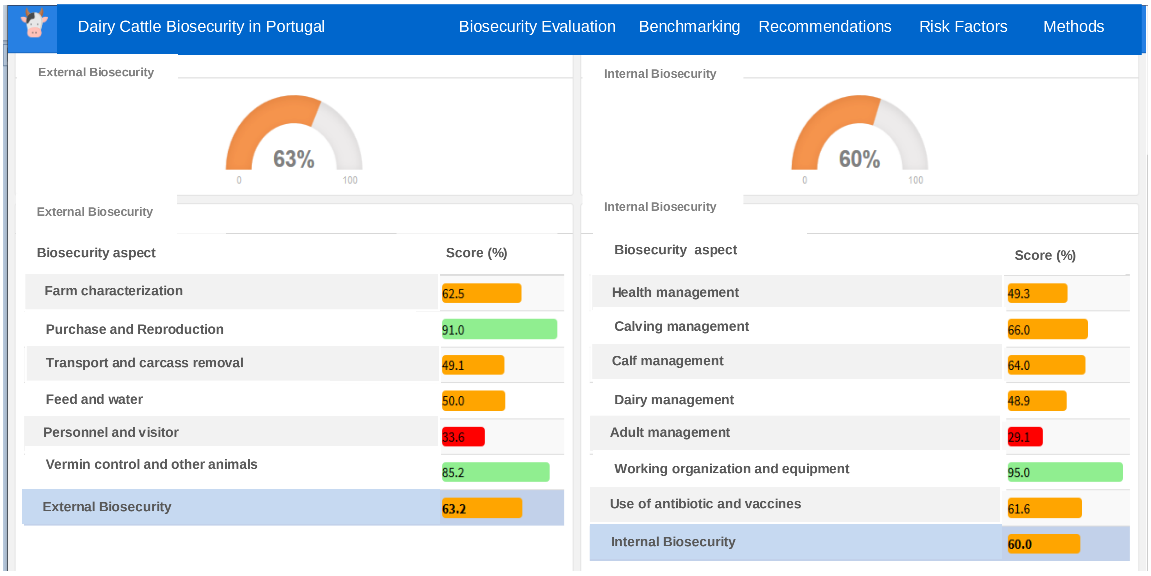

Figure 1: Biosecurity dashboard graphs. The scores calculated and observed in the figure represent each biosecurity parameter weight relative to the maximum value that could be achieved in each biosecurity aspect. Green> = 75%; Orange> = 40% and <75% and Red if <40% of the maximum score.

3.1.3.2. Biosecurity best practices

Depending on the answers given to the survey, a dynamic list of biosecurity measures was developed with an explanation of the importance that these measures have on the dairy farm. This list was created with input from previous projects and discussed with partners.

3.1.3.3. Geographical risk factors

A set of risk factors based on farms' geographic distribution was defined: farms' density in the neighbouring area (3 km radius), animals' density (3 km radius), animals' movement on farms (entrances and exits, in the last 2 years), and movement from vehicles that made dead stock transportation (in the last 2 years). National Animal Information and Registration System's (SNIRA) data, provided by DGAV, was used to calculate these factors' spatial distribution and, for each one of the eligible farms, the percentile in which each farm was found for each risk factor was also calculated.

For example, a density of cattle with a 90th percentile meant that 90% of eligible farms were located in areas with a lower animal density than the farm in question. For each risk factor's group, a global geographical risk index was calculated.

These indexes were relative and were calculated as follows:

- ➔ Low risk = less than 50th percentile;
- ➔ Medium Risk = percentile greater than or equal to 50 and less than 75;
- ➔ High Risk = percentile greater than or equal to 75 and less than 90;
- ➔ Very High Risk = percentile greater than or equal to 90.

This index should be interpreted as a relative value and not as an absolute value; an example of how the geographical risks were presented on the report can be seen in Figure 2.

In the individual report, the indexes were tabulated with the individual risk's percentage, the farm's relative position (benchmarking), and coloured lines, which represented the risk's degree. Low risk was represented by a green line, the medium risk was represented by a yellow line, high risk was represented by an orange line, and very high risk was represented by a red line.

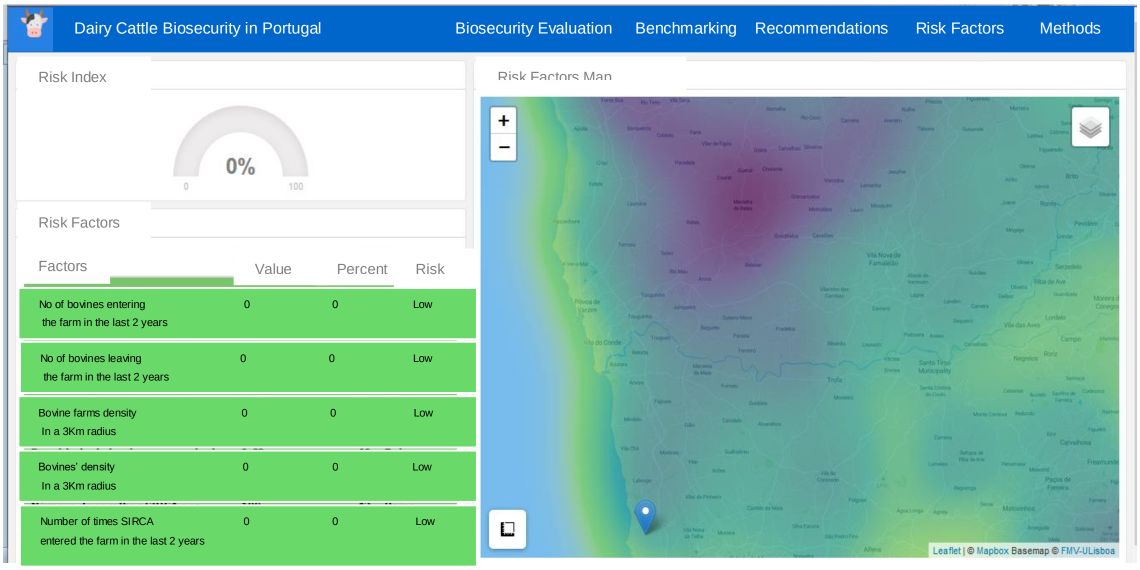

Figure 2: Risk factors based on geographic distribution. Farms in the neighbouring area (3 km radius) and animal density (3 km radius) can be observed in the map and in the board, animal movement on farms (entrances and exits) and movement from vehicles that make dead stock transportation can be observed in the board (both representing the last 2 years).

3.1.3.4. Benchmarking

Benchmarking gives each farm's relative position compared to other farms regarding the 13 biosecurity aspects present in the survey. Figure 3 represents the graph from the individual report sent to the veterinarians, explaining the parameters for benchmarking.

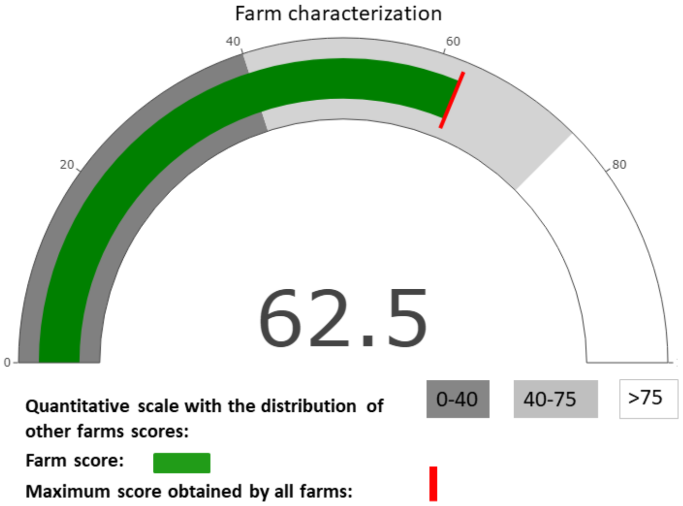

Figure 3: Benchmarking graph. Compares each aspect referred in the questionnaire for each farm with the general pattern for the totality of farms. The grey scale of colours addresses to a quantitative scale with the other farms' scores distribution. The scale is divided in three levels: from 0-40%, 40-75% and >75%. The green line is related to the farm score and the red vertical line is the maximum score obtained taking into account all farms.

3.1.4. Questionnaire data analysis

Data handling was divided into descriptive and quantitative statistical analyses and was made in RStudio™ 3.6.1 and Microsoft Excel™ 2010. The way the analysis was conducted, is going to be explained in the following points related to data handling, starting with descriptive analysis.

3.1.4.1. Descriptive Analysis

The data were extracted from the database created in Googleforms™ and analysed using descriptive statistical methods. Each answer's percentage was calculated for categorical variables, mean, median, minimum, and maximum values were calculated for numerical variables. The results are presented in histograms, bar graphs, boxplots, and tables. Data regarding the answer's frequency to each question is presented in Appendix III.

3.1.4.2. Normality test

Lilliefors' test and Shapiro-Wilk's test were applied to check data's distribution normality. If the p-value was over 0.05 the distribution was considered to be approximately normal.

3.1.4.3. Association between variables and biosecurity scores and between internal and external biosecurity scores

Pearson and Spearman's correlations were applied to check the association between variables and between variables and the biosecurity scores. Pearson's correlation was applied when the Lilliefors' test and Shapiro-Wilk's test p-values were over 0.05 and Spearman's correlation was applied when Lilliefors' test and Shapiro-Wilk's test p-values were under 0.05. If the correlation coefficient was between 0.6 and 1 or between -1 and -0.6, the variables would have a moderate to strong correlation with each other, or with the biosecurity scores (Akoglu H 2018).

Linear regression computes the output variables as linear arrangements of the input variables. This kind of regression was applied between internal and external biosecurity scores to evaluate how these two variables influenced each other, and was also applied to compare Biocheck scores with the scores obtained in this project, as it is going to be mentioned on materials and methods' point 3.1.5.

Logistic regressions were used in three dichotomous variables related to the questionnaire's "Use of antibiotics and vaccines" biosecurity aspect, to evaluate a possible relationship between these variables and the final biosecurity scores.

Chi-square tests were used between every variable belonging to the questionnaire's "Use of antibiotics and vaccines" group and the final biosecurity scores. These tests were applied to check if there was a significant association between the different categories of each variable with the biosecurity scores.

3.1.4.4. Evaluate patterns between variables

Multiple correspondence analysis (MCA) handles data with a group of nominal variables that describes a set of observations. This analysis decomposes the chi-square statistics into two groups of orthogonal observations that define a pattern of relations between variables (Abdi and Béra 2017). These relationships are shown as points in a multidimensional space and the proximity between points reveals a pattern where these variables are predominantly used together by a certain group of individuals, contrarily to points that are separated from each other. Another aspect that should be taken into account, is the proximity to the x and y axes because if points lie far from the axes, this means that those variables do not belong to the pattern of variables that appear most frequently (Ribbens S *et al.* 2008). Firstly, all categorical variables present in the questionnaire were put under an MCA and secondly, after evaluating correlation coefficients between biosecurity aspects and total biosecurity scores, another MCA was applied using variables present in the three biosecurity aspects that had correlation coefficients superior to 0.6. Only the second MCA will be presented in the results.

3.1.4.5. Evaluate variable's influence on biosecurity scores

The Principal Component Analysis (PCA) aims to remove the most significant information from a data set, compressing the data, and examining both individuals and variables. The Principal Components are variables that are attained as linear groupings of the original variables. The first Component has the wider possible variance, the second is calculated, being orthogonal to the first Component, and the rest of the Components are computed the same way (Abdi and Williams 2010). To apply the PCA all scores obtained in each answer of every biosecurity aspect present in the questionnaire were used and were related to the final biosecurity scores. Three clusters were created to evaluate total, internal and external biosecurity scores' multidimensional distribution. After that, the answer's pattern to the questionnaire, which contributed the most to the creation of the clusters, was constructed with the top 20 answers that had the highest contribution to each dimension. The PCA interpretation should take into account the 4 graphs created, to evaluate the spatial distribution of higher or lower biosecurity scores, the multidimensional distribution of each individual and of each answer with respective influence in graph's dimension 1 or 2.

3.1.4.6. Differences between groups

To evaluate existing differences between groups, Kruskal-Wallis, and Analysis of variance (ANOVA) tests were made. The first one was applied between variables that did not have a normal distribution, and the second one was applied between variables that had a normal distribution. After observing the Kruskal-Wallis and the ANOVA tests' significance, Tukey's test and Dunn's test were taken to evaluate which groups had a significant influence on biosecurity scores.

3.1.5. Comparing scores with Biocheck

As previously mentioned, the questionnaire used in this project adapted from Biocheck from Ghent University. The 151 questionnaires were inserted in Biocheck online dairy cattle survey to check how much the scores obtained in Biocheck differed from the ones obtained in this project. The questionnaire used in Portugal was not the same as the one used in Biocheck, because questions were added, answers were changed and a group for antibiotics and vaccines was also added. Scores were calculated differently, another reason that can explain the big differences between these two scores' systems. To compare the scores, linear regressions were taken between total, internal, and external biosecurity scores, to see if values from each scores' system evolved in the same direction, a t-test to compare means between total biosecurity scores was taken and differences between internal and external biosecurity scores were checked through Mann Whitney U tests.

3.1.6. Presentation of results to the community

A results' presentation regarding the farms sampled through an interview (n=87) was made on February 21st and February 24th, 2020. These presentations were mainly focused on the descriptive analyses and were made at the Faculty of Veterinary Medicine in Lisbon, through Webinar for professors, veterinarians, and students and in Agros' headquarters through an oral presentation for both veterinarians, and farmers. A written report was published on Agros' magazine, and the article can be read in Appendix I.

4. Chapter IV – Results

4.1. Sample

A total of 151 farms were sampled in a universe of 1287 farms (301 farms from Proleite and 986 from Agros), which corresponds to 11.73%. The results will be presented altogether and not for each cooperative union separately. The farms' geographic distribution can be observed in Figure 4.

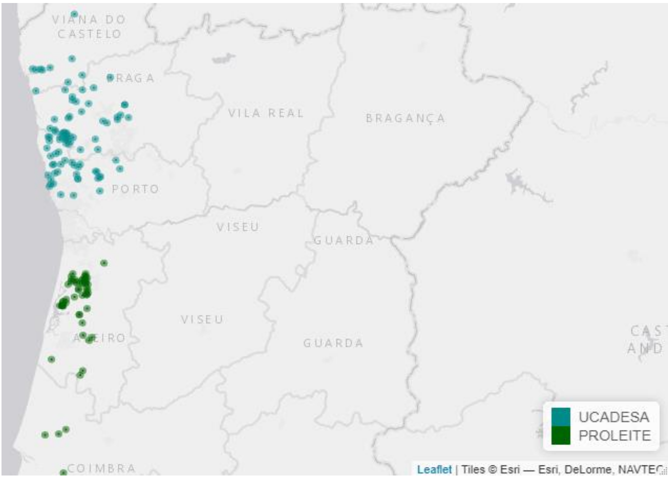

Figure 4: Geographic distribution of sampled farms. The number of farms belonging to UCADESA was 94 and the number of farms belonging to Proleite was 57, in a total of 151 farms.

Some farms did not report the exact number of animals, being the minimum herd size of 23, the maximum was 1574 and the average was 156 animals per holding.

4.2. Biosecurity scores

Biosecurity scores are presented in Table 2, which contains the mean, median, minimum and maximum values for total, internal and external biosecurity scores. Biosecurity scores distribution can be observed in the histograms present in Figure 5.

Table 2: Minimum, maximum and mean scores for total, external and internal biosecurity

	Minimum	Maximum	Mean	Median
Total Biosecurity	42.0	80.4	65.5	65.9
External Biosecurity	44.1	85.1	71.3	73.7
Internal Biosecurity	37.8	80.3	67.7	69.6

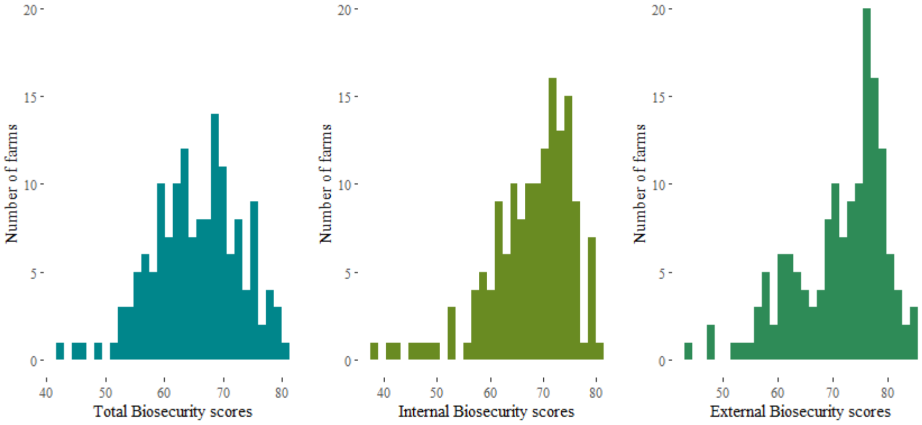

Figure 5: Biosecurity scores distribution. Histograms with distribution of Total, Internal and External biosecurity scores.

Spearman's correlation results showed that there was a strong positive correlation of 0.87 between internal and external biosecurity, and the linear regression (Figure 6) resulted in around 52% of the variability in Internal Biosecurity being explained by the scores obtained in External biosecurity (Adjusted $R^2=0.52$, p-value<0.05).

$$Internal\ Biosecurity = 16.81 + 0.71 \times External\ Biosecurity$$

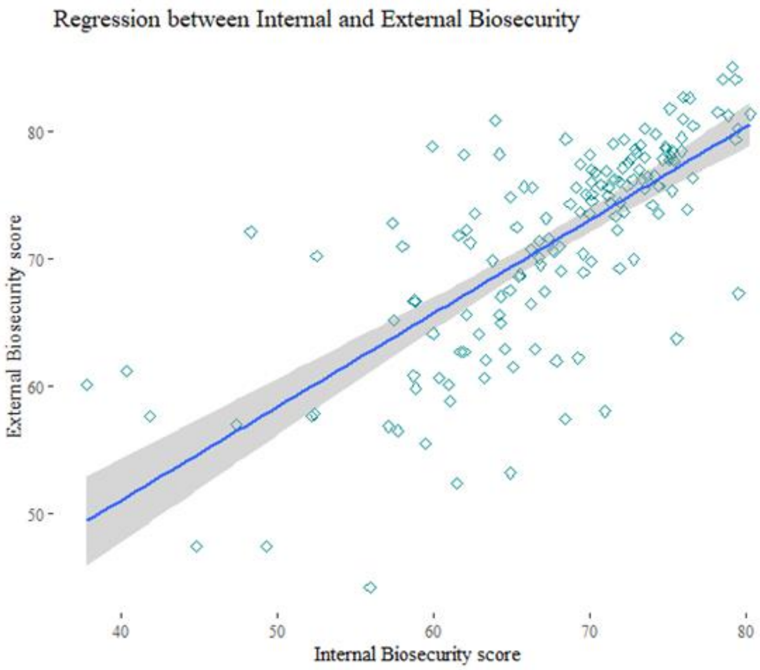

Figure 6: Linear regression between Internal and External Biosecurity. In the x-axis and y-axis, Internal and External biosecurity scores are respectively presented.

4.3. Scores obtained in each biosecurity aspect

Biosecurity aspect's minimum, maximum and mean values are presented in Table 3. Each score can vary between 0 and the score already presented in Table 1.

Table 3: Minimum, maximum and mean scores obtained in each biosecurity aspect

Biosecurity Aspect	Minimum (%)	Maximum (%)	Mean (%)
Farm characterization	0.0	65.0	45.0
Purchase of animals and reproduction	36.5	100.0	81.5
Transport and carcass removal	41.0	94.0	71.0
Feed and water	10.0	100.0	76.7
Personnel and visitors	20.5	87.5	56.0
Vermin control and other animals	39.0	100.0	86.0
Health management	26.0	98.0	64.0
Calving management	46.7	83.3	66.7
Calf management	33.3	90.0	73.3
Dairy management	26.7	80.0	53.3
Adult animals management	2.5	57.5	42.5
Work organization and equipment	5.0	100.0	86.7
Use of antibiotics and vaccines	3.3	60.0	46.7

The group with lower mean value was "Adult animals management", the group that scored the highest was "Work organization and equipment", followed by "Vermin control and other animals", "Purchase of animals and reproduction" and "Feed and water", all of them, above 75%.

4.4. Biosecurity measures - Descriptive analysis

A descriptive analysis, focused on the most relevant answers of each of the thirteen biosecurity aspects, is presented in this point. The complete descriptive analysis is annexed in Appendix III.

4.4.1. Farm characterization

The survey's results show that in 67.8% (n=103) of farms, its management was carried out by the farmer and by farmer's family members and that 90.6% (n=135) of farmers had a professional qualification in farm management. Farmer's age classes and minimum, maximum, and mean values of years of farming experience are presented in Table 4. The biosecurity scores' distribution according to the different age classes can be observed in Figure 7.

Table 4: Number of farmers by each group of age and years of field experience in farming

Age	Number of people	
18-30	21	
31-40	26	
41-50	31	
51-60	47	
61-70	13	
>71	7	
Missing values	6	
Years of farming experience		
Mean	**Minimum**	**Maximum**
28,3	2	65

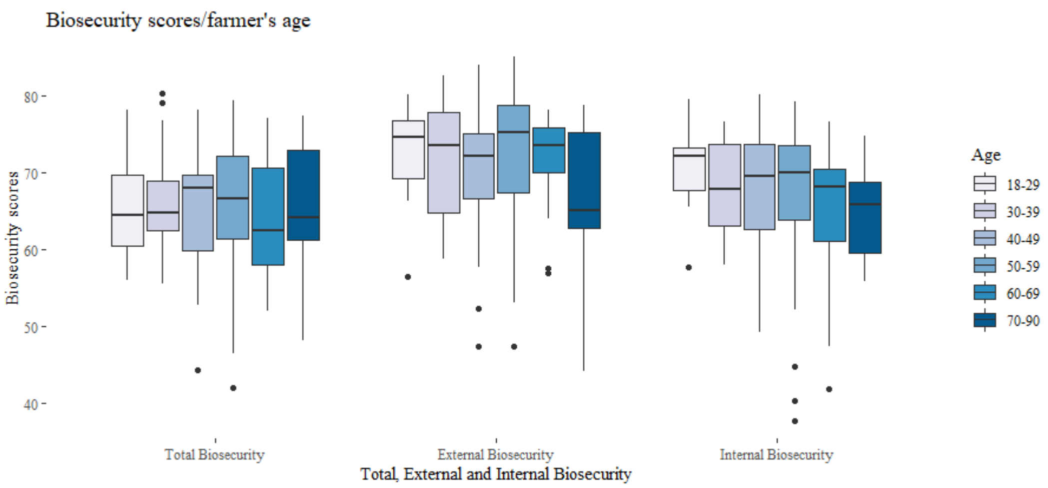

Figure 7: Distribution of biosecurity scores per farmer's age class. Farmer's ages were divided in six classes. From 18 to 29 years old, from 30 to 39 years old, from 40 to 49 years old, from 50 to 59 years old, from 60 to 69 years old, from 70 to 90 years old. It can be observed the distribution of total, internal and external biosecurity scores per age class.

4.4.2. Purchase of animals and reproduction

It was found that 73% (n=111) of farms did not buy animals, 50% (n=20) of the 27% (n=40) of farmers who bought animals in the last two years, reported that the animals purchased did not have direct or indirect contact with animals from other farms before being unloaded from the truck. The animals' purchase in 46.2% (n=18) of farms (of the 27% who bought), was carried out by commercial exchanges through different stakeholders or in markets, and 52.5% (n=21) of respondents admitted they did not ask for proof of health status, of diseases not mandatory by law, and 65% (n=26) referred that animals entering the farm, were never tested for diseases. Of the 27% farmers who purchased animals, 76.9% (n=30) said they did not use quarantine, and 20.5% (n=8) of farmers revealed to always use quarantine after an animal acquisition. In 95.2% (n=139) of farms, the animals left the farm and went to other farms or the slaughterhouse, and did not return to the farm of origin. Regarding reproduction management, 90.7% (n=135) of respondents said they used artificial insemination.

4.4.3. Transport and carcass removal

It was said by 97.3% (n=146) of the farmers that vehicles entering the farm did not undergo a wash at the farm's entrance, 46.3% (n=69) admitted that external vehicles had access to areas where animals were kept. Regarding carcass removal, 53% (n=80) of farms reported that the vehicle for deadstock transportation entered the farm to collect dead animals, and in 70.2% (n=106) of farms there was no storage for the animals' carcasses.

4.4.4. Feed and water

In 97.3% (n=146) of the farms, the water was tested annually, however, 92% (n=139) had never submitted the water in the watering areas to bacteriological analyses. Regarding feed administration, 86.8% (n=131) of farms used specific utensils to feed the animals.

4.4.5. Personnel and visitors

In 43.3% (n=65) of farms, visitors asked for farmer's permission to enter the farm, in 72% (n=108) of farms, visitors did not have direct contact with animals and 82.4% (n=122) of farmers said they had an appropriate place where employees and visitors could change clothes and wash their hands. The veterinarian, the inseminator, and the hoof trimmer, always used individual protective equipment, such as boots, clothes, and gloves in the majority of times they went to farms. As for the cattle trader, 98% (n=149) referred the cattle trader entered the farm, 85.1% (n=126) admitted the trader never wore appropriate clothes, 76.7% (n=115) referred the trader never wore appropriate boots and never wore gloves or washed hands in 72.6% (n=106) of farms. In 88% (n=132) of farms, that farmers or employees worked only on their farm and did not attend other dairy farms, and 71.1% (n=86) of farmers said they or their employees had other farm animals at home.

4.4.6. Vermin control and other animals

It was observed that 80.1% (n=121) and 88.8% (n=135) of farms had insects and rodent control plans respectively, however, 96.7% (n=147) of farms did not have a bird control plan. Regarding possible contact with other animals, 96.5% (n=136) of farms claimed the dairy cattle did not have contact with animals from other farms and 48% (n=72) of farms had other production animals in addition to the dairy cattle, mostly poultry, but also rabbits, sheep, goats, and swine. Regarding domestic animals, 70.2% (n=106) of farms had dogs and cats loose on the farm, with the possibility to have direct contact with the dairy cattle. In 71.7% (n=109) of farms, other farms' manure, or slurry, was spread within 500 meters of the assessed farm.

4.4.7. Health management

In 62.9% (n=95) of farms, sick animals were always physically separated from healthy animals, which means in 37.1% (n=56) farms, sick animals could have contact with healthy animals. Regarding biosecurity procedures taken inside the sick animals pen, 79% (n=94) of farmers had specific material to deal with sick animals, which was cleaned and disinfected in 50.5% (n=50) of farms and 73.7% (n=56) of respondents who had sick animals pen said they used gloves or washed their hands when dealing with sick animals. After the animal had left the pen, the material was removed in 84.4%

(n=92), being the pen always cleaned according to 94.8% (n=91) of farmers and always disinfected according to 60.9% (n=42). Different needles were used in 76.8% (n=116) depending on the animal's age or treatment, and 98.7% (n=149) kept medication and treatment records.

4.4.8. Calving management

The calving pen was present in 59.9% (n=91) and was located in the same place as the sick animals pen in 58.1% (n=54) of farms. Respondents entered the calving pen, using gloves or after washing their hands in 80.8% (n=42), and when assisting calvings, 83.5% (n=127) said they washed and disinfected their hands or used gloves and cleaned and disinfected the utensils used during calving. In 61.8% (n=94) of farms, farmers did not clean or disinfect the cow's hind limbs after calving or an abortion. The calf was separated from the cow in the first hour in 78.3% (n=119) of farms, 48.7% (n=74) of farmers never tested for possible causes of miscarriage, 50.7% (n=77) said that foetal membranes were placed in the manure and 48% (n=73) said that foetal membranes went to the slurry pit.

4.4.9. Calf management

Regarding calves' management, 47% (n=70) of farmers administered an average of 4 litres of colostrum in the first 6 hours, this coming from the mother in 96.7% (n=145) of cases. The colostrum could be stored in the refrigerator 41.8% (n=61) of farms, 50.7% (n=76) had frozen colostrum stored and 78% (n=117) of farmers did not test the colostrum quality. Regarding cleanliness and disinfection procedures applied in the material used to administer the colostrum, these procedures were applied in 77.8% (n=116) of the farms.

Calves were put in individual pens in 86.7% (n=130) of farms, the pens' cleaning was implemented after the calf's removal, in 94.6% (n=122) of farms, buckets used for feeding were cleaned after each use in 95.1% (n=136) of farms and 54.7% (n=81) of farmers never fed the calves discarded milk. Regarding collective pens, the average amount of calves per pen was 7.3 and the pen's bed was removed after its use in 91.3% (n=136) of farms, being always cleaned in 89.4% (n=126) and always disinfected in 48.7% (n=55) of farms.

4.4.10. Dairy management

Regarding the milking equipment maintenance, this was done once a year according to 62.3% (n=91) of farmers, and the milking machine disinfection was done through the application of hot water after the milking process in 84.6% (n=126) of farms, the teat liners were changed after 12 months in 28% (n=38) and after 6 months in 37.2% (n=56) of farms. Teat liner disinfection was done after milking mastitic cows in 19.1% (n=29) of farms and was done between every cow, in 49.3% (n=75). According to 77.6% (n=118) of respondents, mastitic cows were milked after all the healthy cows, and in 57.2% (n=87) of farms, cows had the opportunity to stand between half an hour and one hour, without laying down, after the milking process had finished.

The teats were cleaned and dried before milking, in 60.5% (n=92) of farms, and were disinfected by immersion after milking in 88.2% (n=134). As for the fore stripping, they were inspected in 77% (n=117) of farms, before the milking process had started.

4.4.11. Adult animals management

In the 151 farms sampled, on average, there was a general cleaning of the facilities 231 times a year (4-5 times per week), with a minimum of 0 times a year (n=3) and a maximum of 1095 times (3 times a day) (n=1). On average, disinfection using lime or other products was performed 86 times a year (1-2 times per week), with a minimum of 0 times (n=3) and a maximum of 1095 times (n=1). Spearman's correlations were made between each biosecurity score, and cleaning and disinfection frequency, but statistically significant results were not observed. A Spearman's correlation was also taken between cleaning and disinfection procedures, revealing a moderate correlation of 0.64, meaning that farmers who clean more frequently also apply disinfection procedures more often. Frequently, 72.9% (n=110) of farms performed footbaths, 70.1% (n=89) of which had their outflow to discard the footbath solution.

4.4.12. Work organization and equipment

In 94.7% (n=144) of farms, animals were grouped by age, with a total separation of age classes, according to 79.9% (n=115) of respondents, and total separation of equipment between age classes in 84.1% (n=122) of farms. The material used in 96.7% (n=147) of the farms belonged only to the farm, with no exchange of equipment with other farms.

When dealing with animals, 96.1% (n=124) of producers did not change boots between age classes, 97.7% (n=126) never changed clothes, and 86.1% (n=111) never washed their hands or wore gloves. In 52.8% (n=76) of farms, there was no specific order for the animals' management, and in 31.2% (n=45) farmers worked firstly with the oldest animals and secondly with the youngest ones.

4.4.13. Use of antibiotics and vaccines

In 47.4% (n=72) of farms, an antibiotic protocol advised by the veterinarian was being implemented, with 94.4% (n=68) directing the protocol towards specific diseases, being mastitis the most common disease referred to in the protocols. Regarding antibiotic sensitivity tests, 43% (n=64) of respondents said they applied these tests sometimes throughout the year, and 55.3% (n=84) did selective treatment during cows' dry period, with the majority applying antibiotics in cows that had clinical signs of mastitis, or that had mastitis during the lactation period. Regarding vaccines, 78.8% (n=119) of respondents said they had vaccination protocols, which included the following diseases: Bovine Viral Diarrhoea, Infectious Bovine Rinotraqueitis, clostridiosis, neonatal diarrhoea and Bovine Respiratory Disease.

4.5. Biosecurity scores

Pearson's correlations were made between biosecurity aspects and total biosecurity scores. Coefficients superior to 0.6 revealed a positive, moderate to perfect association between variables and are presented in Table 5.

Table 5: Correlation coefficient between biosecurity scores and biosecurity aspects

Biosecurity aspect	Correlation coefficient
Purchase of animals and reproduction	0.60
Personnel and visitors	0.75
Calving management	0.65

The correlation coefficients identify that biosecurity aspects with higher influence in the final biosecurity scores are "Personnel and visitors", "Calving management", and "Purchase of animals and reproduction".

4.6. Farm size influence on biosecurity scores

To evaluate the influence of farm size in the final biosecurity scores, an ANOVA test followed by a Tukey's post-hoc test was applied, comparing total biosecurity scores by farm size classes; a Kruskal-Wallis' test followed by a Dunn's test was also applied between external, internal biosecurity scores and the farm size classes. The ANOVA and Kruskal-Wallis test results showed that the farm's size had influence in total (p-value=0.0005) and internal biosecurity scores (p-value=0.018), in both cases with larger holdings presenting higher scores. Tukey's and Dunn's tests revealed that the main differences in both total and internal biosecurity scores were found between small and medium-sized farms. In Figure 8, the biosecurity scores' distribution per farm size class can be observed.

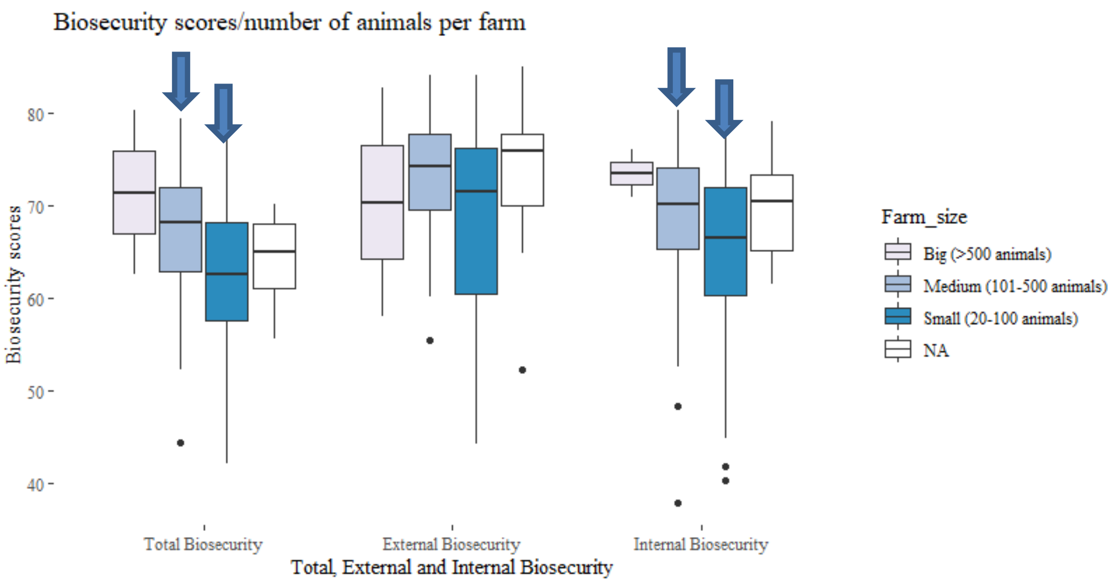

Figure 8: Biosecurity scores distribution per farm size. The farm sizes were divided in three classes, the smallest ones from 20 to 100 animals, medium size farms from 101 to 500 and bigger farms are the ones with more than 500 animals, the ones with NA were farms that did not have complete data regarding the number of animals. It can be observed the distribution of total, internal and external biosecurity scores per farm size. The blue arrows indicate the classes where significant results were obtained in Tukey's and Dunn's test.

4.7. Biocheck biosecurity scores

The results regarding Biocheck biosecurity scores are presented in Table 6 and the ones regarding Biocheck scores for biosecurity aspects can be observed in Table 7. Linear regressions (Figure 9), t-tests and Mann Whitney U tests were taken between Biocheck scores and the ones obtained in this project. A comparison between maximum, minimum, and mean values regarding both Biocheck scores and the ones obtained in this project is shown in Figure 10.

Table 6: Biocheck - minimum, maximum and mean scores for total, external and internal biosecurity, and worldwide mean scores

	Minimum	Maximum	Mean	Worldwide mean
Total Biosecurity	26	84	61.8	57.0
External Biosecurity	18	82	69.6	46.0
Internal Biosecurity	22	92	53.4	66.0

Table 7: Biocheck - minimum, maximum and mean scores obtained in each biosecurity aspect

	Minimum	Maximum	Mean
Purchase of animals and reproduction	10	100	83.0
Transport and carcass removal	12	94	48.6
Feed and water	14	100	61.8
Personnel and visitors	8	98	62.0
Vermin control and other animals	5	96	74.0
Health management	8	93	52.0
Calving management	6	94	40.8
Calf management	15	91	56.8
Dairy management	29	93	59.3
Adult animals management	0	100	63.0
Work organization and equipment	0	90	60.2

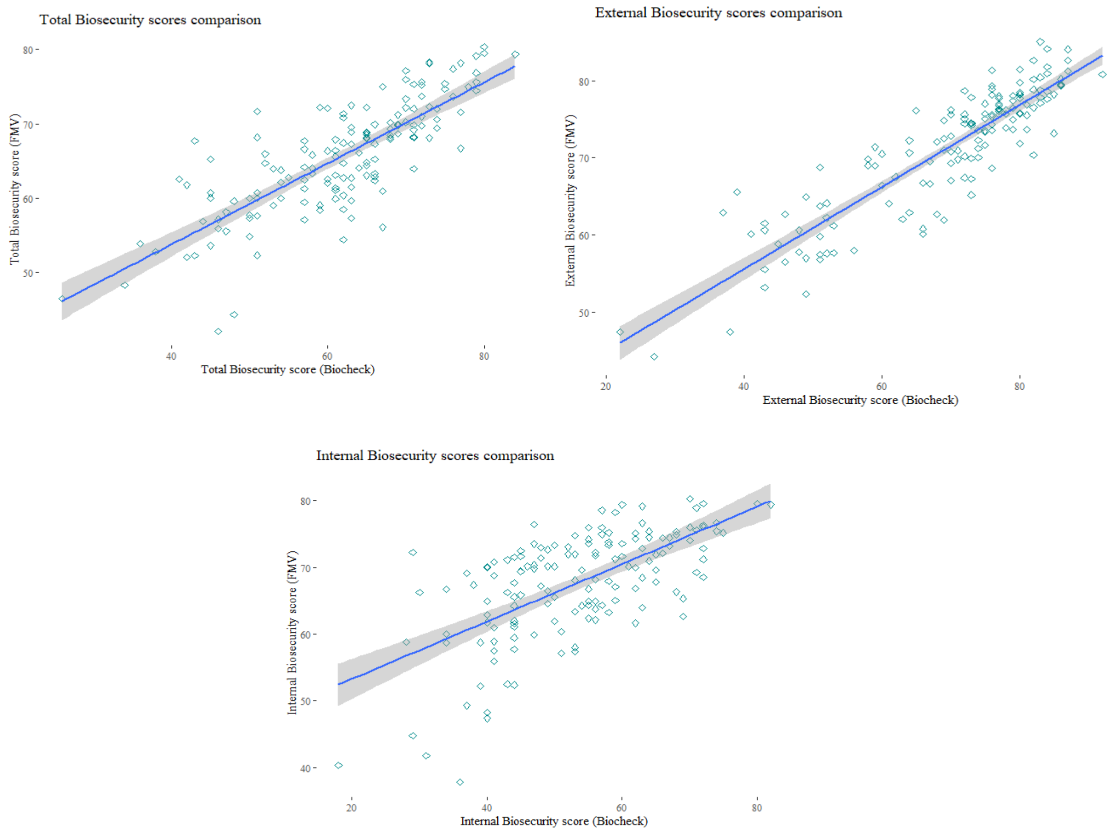

Figure 9: Linear regressions between biosecurity scores obtained in Biocheck and biosecurity scores obtained in this project. In the top left image, it can be observed a linear regression regarding total biosecurity scores, in the top right image it can be observed a linear regression regarding external biosecurity scores and in the centred image it can be observed a linear regression regarding internal biosecurity scores. The x-axis represents Biocheck biosecurity scores and the y-axis represents biosecurity scores obtained in this project.

The graphs showed above enable the conclusion that both scores had the same behaviour, which means; when for example an internal biosecurity score was higher in one score system it was higher in the other one too. The t-test showed that there were significant differences between total biosecurity scores from Biocheck and total biosecurity scores obtained in this master dissertation project (p-value < 0.05). The Mann Whitney U tests showed significant differences between internal biosecurity scores (p-value < 0.05) but not between external biosecurity scores. As previously mentioned in materials and methods, it should be taken into account that these score systems were calculated differently and that the questionnaires weren’t equal.

Figure 10: Minimum, maximum and mean biosecurity scores - Biocheck vs Biosecurity project. The bar graphs represent the biosecurity scores' mean, minimum and maximum for external, internal and total biosecurity, obtained in Biocheck and in the Biosecurity project. The labels on the right indicate which biosecurity score and which score system are represented in each bar.
"EB_Biocheck" and "EB_Project" represent the external biosecurity scores for Biocheck and the Biosecurity project respectively, "IB_Biocheck" and "IB_Project" represent the internal biosecurity scores for Biocheck and the Biosecurity project respectively, "TB_Biocheck" and "TB_Project" represent the total biosecurity scores for Biocheck and the Biosecurity project respectively.

4.8. Multiple correspondence analysis

As previously mentioned in "Material and Methods", a multiple correspondence analysis was taken between the three biosecurity aspects that had higher correlation coefficient ("Purchase of animals and Reproduction", "Personnel and Visitors" and "Calving management") and total biosecurity scores. In this case, 7.1% of final results' variance is explained by dimension 1, which means that the answer's pattern between the individuals in "Purchase of animals and Reproduction" and the association between variables in this biosecurity aspect, influence the final results in 7.1%. Regarding dimension 2, 5.7% of final results' variance is explained by this dimension, which means that the answer's pattern between the individuals in "Personnel and visitors" and "Calving management" and the association between variables in these biosecurity aspects, influence the final results in 5.7%. This influence is low, but the response pattern is interesting and should be observed, mainly in dimension 1. The variables that affect predominantly dimension 1 are presented in Table 8, with the respective contribution values, and the ones that affect predominantly dimension 2 are presented in Table 9. These two groups of variables weren't spatially related to each other.

Table 8: Variables with the highest contribution to dimension 1 in the multiple correspondence analysis

Question	Answer	Contribution (%)
15. Is quarantine used after an animal acquisition?	Always	4.80
17. Do workers use specific boots only for the quarantine pen?	Never	5.10
17. Do workers use specific clothes only for the quarantine pen?	Never	5.10
17. Do workers wash hands or change gloves after working in the quarantine pen?	Never	3.83
18. Is all the material from the quarantine pen removed after the animal leaves?	Always	4.80
19. Is the quarantine pen cleaned before the arriving of other animals?	Always	5.40
20.1. Do animals in the quarantine pen have contact with other animals from the farm?	No	3.48

Table 9: Variables with the highest contribution to dimension 2 in the multiple correspondence analysis

Question	Answer	Contribution (%)
26. The semen used is from farms with higher or equal health status?	Always	2.54
45. Do workers wear specific clothes?	Always	1.42
46. Does the veterinarian use specific boots?	Never	2.90
52. Does the hoof trimmer use specific boots?	Never	1.62
52. Does the hoof trimmer wear specific clothes?	Never	1.92
81. Is there a complete separation between animals in the maternity pen and other animals?	Yes	2.99
82. Do workers use specific boots inside the maternity pen?	Never	3.05

From the analysis it can be concluded that in farms where animals were put in quarantine the following characteristics are also observed:

- workers never used specific boots and specific clothes, or wore gloves or washed hands when they were dealing with animals that were in quarantine, and
- the pen where animals were put was completely separated from other animals and was always cleaned and all the material was removed before other animal had to enter the pen for the quarantine period.

It was also observed that farms where animals present in maternity pens were completely separated from other animals, are also those where:

- workers did not use specific boots to enter the maternity pen, but used specific clothes to work on the farm,
- veterinarian and the hoof trimmer did not use individual protective equipment properly, and

- semen used in reproduction management was always from farms with higher or equal health status.

4.9. Principal component analysis

The principal component analysis for every variable, of the top 20 variables with higher contribution to dimension 1 and 2, is summarized in Table 10. It can be observed the variable's name (R followed by a number or by a number and a letter) with the respective question, and the contribution for dimension 1 or 2.

Table 10: Variables present in the principal component analysis

Variables	Contribution (%)	Dimension	Question
R_38	2.6	2	38. Are food storage facilities protected from insects and animals?
R_45_a	8.4	1	45. Do the workers wear specific boots?
R_45_b	9.0	1	45. Do the workers wear specific clothes?
R_45_c	4.3	2	45. Do the workers wear gloves or wash hands?
R_46_a	8.8	1	46. Does the veterinarian wear specific boots?
R_46_b	2.9	1	46. Does the veterinarian wear specific clothes?
R_48_a	7.3	1	48. Does the inseminator wear specific boots?
R_48_b	7.1	1	48. Does the inseminator wear specific clothes?
R_52_a	8.9	1	52. Does the hoof trimmer wear specific boots?
R_52_b	2.7	1	52. Does the hoof trimmer wear specific clothes?
R_70_c	3.8	2	70. Do workers use new gloves or wash hands before entering the infirmary
R_72_a	4.1	2	72. The infirmary is always disinfected before other animal enters the pen?
R_72_b	2.9	2	72. The infirmary is sometimes disinfected before other animal enters pen?
R_72_c	7.5	2	72. The infirmary is never disinfected before other animal enters the pen?
R_77_a	7.3	1	77. What happens to animals infected with a disease - Euthanized?
R_77_b	2.2	1	77. What happens to animals infected with a disease - Segregation?
R_79	9.9	2	79. Does the farm have maternity pens?
R_97_b	5.1	2	97. The individual pen is disinfected before other calf enters the pen?
R_104_b	2.7	2	104. Are the collective pens cleaned before other animals enter the pens?
R_117	2.2	1	117. After the milking process the cows stay up for how long?

The Principal Component Analyses with hierarchical clusters analysis' results can be observed in Figures 11, 12 and 13.

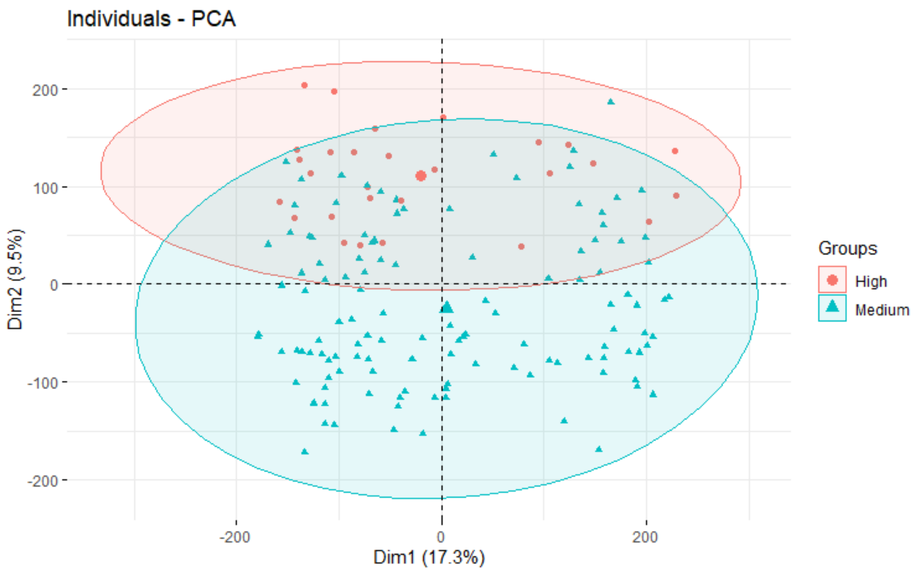

Figure 11: Principal component analysis cluster with total biosecurity scores. Two clusters were obtained, in pink there is a cluster regarding farms with higher total biosecurity scores and in blue there is a cluster regarding farms with medium total biosecurity scores. As it was mentioned on materials and methods, a higher score has values >75, a medium score has values between 40 and 75 and a lower score has values <40.

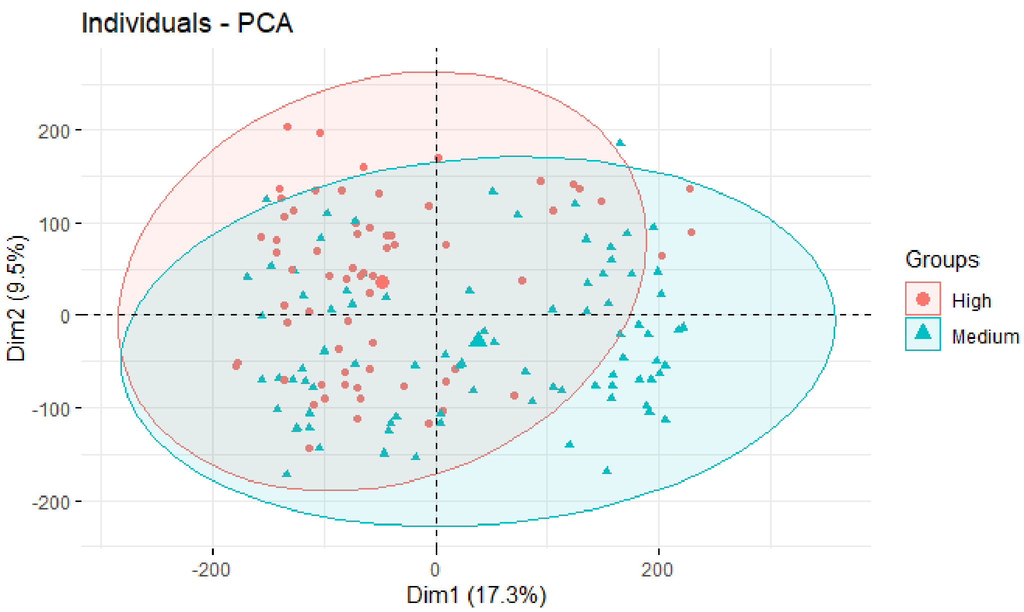

Figure 12: Principal component analysis cluster with external biosecurity scores. Two clusters were obtained, in pink there is a cluster regarding farms with higher external biosecurity scores and in blue there is a cluster regarding farms with medium external biosecurity scores. As it was mentioned on materials and methods, a higher score has values >75, a medium score has values between 40 and 75 and a lower score has values <40.

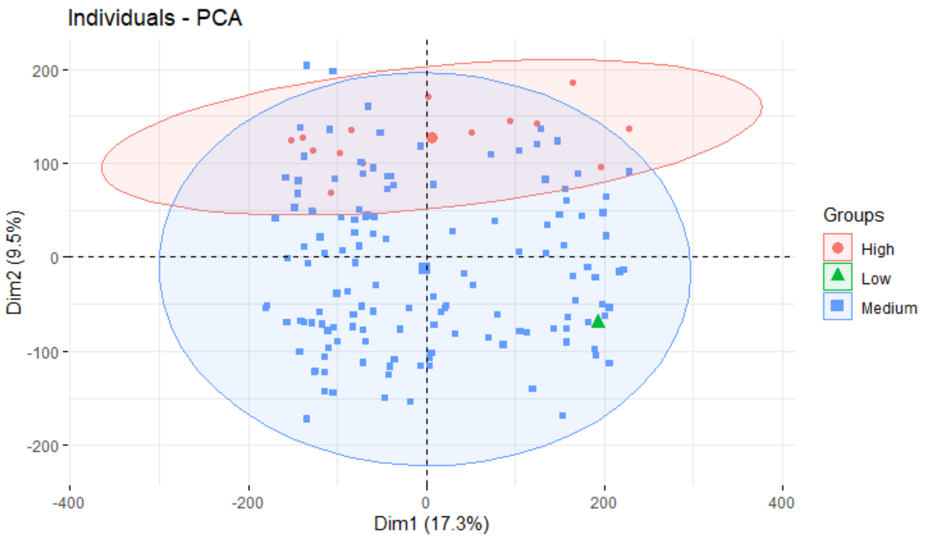

Figure 13: Principal component analysis cluster with internal biosecurity scores. Two clusters were obtained, in pink there is a cluster regarding farms with higher internal biosecurity scores and in blue there is a cluster regarding farms with medium internal biosecurity scores. One farm had internal biosecurity score under 40 and it is represented by the green triangle. As it was mentioned on materials and methods, a higher score has values >75, a medium score has values between 40 and 75 and a lower score has values <40.

Firstly, the influence each dimension had on the final results' variance was observed, being dimension 1 responsible for 17.3%. This dimension is mainly composed by biosecurity measures regarding the use of individual protective equipment, with the use of specific clothes by the workers contributing 9% to this dimension. Dimension 2 influences the final results in 9.5%. This dimension is mainly composed by biosecurity measures regarding the infirmary and maternity pens, and cleaning and disinfection of calves' pens, with the presence of maternity pens contributing 9.9% to the second dimension.

Secondly, the influence each biosecurity score had on the PCA dimensions was evaluated, showing an influence of 69% and 65% by total and external biosecurity scores respectively on dimension 1 and an influence of 53% by internal biosecurity scores on dimension 2. Figure 14 shows variables' and individuals' multidimensional distribution.

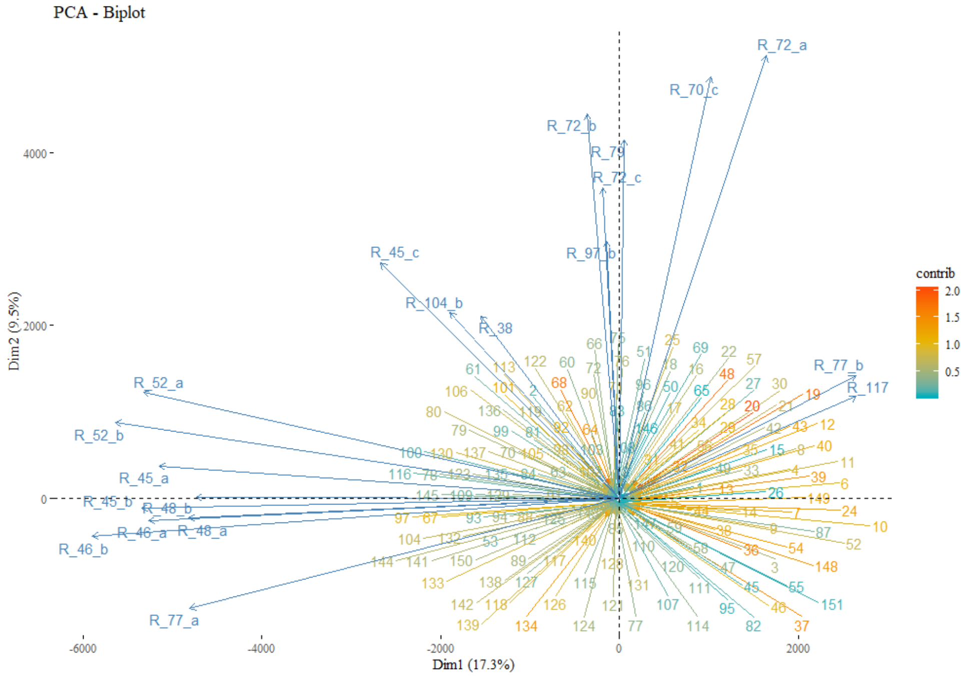

Figure 14: Principal component analysis with every individual and top 20 variables. The blue arrows point to the dimension where each answer (variable) is located and the numbers represent each farm (individual) with respective contribution to the PCA dimensions.

It can be observed that farms with higher total, internal and external biosecurity scores were farms where proper individual protective equipment use and hygiene procedures were applied by workers, hoof trimmers and veterinarians, were also farms that applied proper food storage, that had disinfection procedures in infirmary, individual pens and collective pens, and were farms where calving occurred in maternity pens.

4.10. Use of antibiotics and vaccines – Logistic regressions

The dichotomous variables used in logistic regression analyses were the implementation of vaccination and antibiotherapy protocols and the implementation of selective treatment during the dry period (Table 11). It was observed that an increase in one value on internal biosecurity scores was related to an increase in the log odds by 0.13 on vaccination protocols implementation, an increase in one value in total and internal biosecurity scores, was related to an increase in the log odds by 0.07 and 0.11 respectively on antibiotherapy protocols implementation. An increase in one value in total, internal, and external biosecurity scores was related to an increase in the log odds by 0.07, 0.09, and 0.04 respectively in selective treatment implementation. In conclusion, farms where vaccination protocols, antibiotherapy protocols, and selective treatment during the dry period were applied had higher biosecurity scores. The other variables present in this biosecurity aspect of the questionnaire were put under a chi-square test and did not have statistically significant results. In Figure 15 it can be observed the use of antibiotic sensitivity tests' frequency and its relation to the different biosecurity scores.

Table 11: Logistic regression between vaccination protocols, antibiotherapy protocols and selective treatment on dry period, with biosecurity scores

	Estimate		
	Total Biosecurity	**Internal Biosecurity**	**External Biosecurity**
Vaccination protocols	-	0.13	-
Antibiotherapy protocols	0.07	0.11	-
Selective treatment	0.07	0.09	0.04

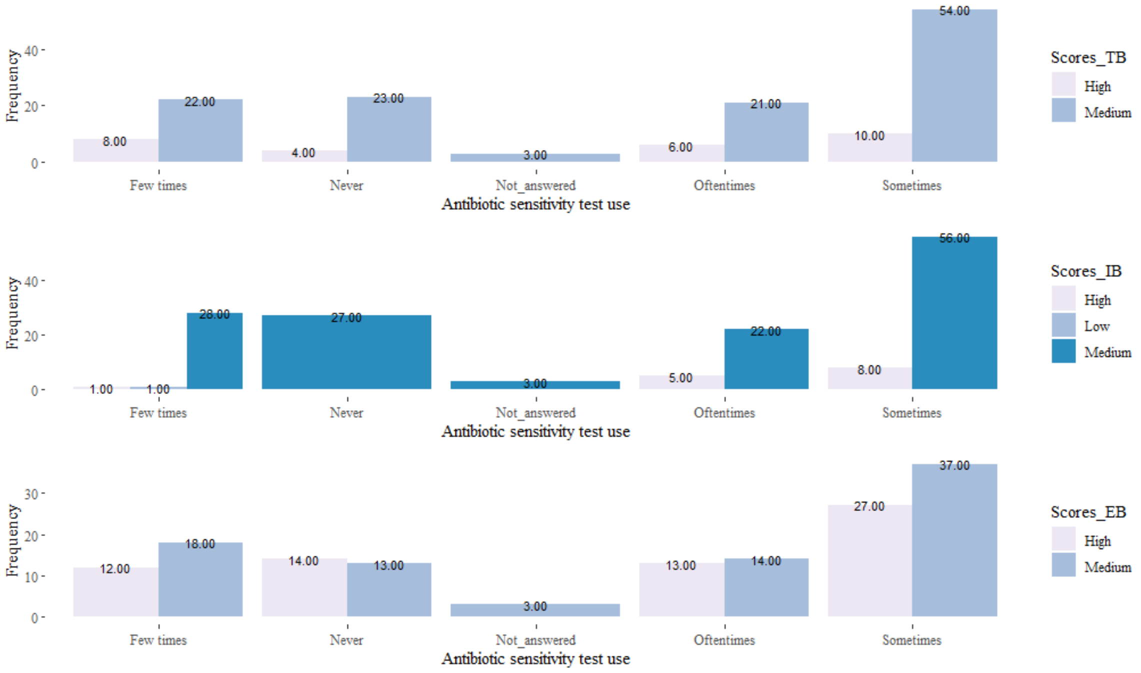

Figure 15: Association between antibiotic sensitivity test use and biosecurity scores. The bar graphs show the frequency of antibiotic sensitivity tests' use (numbers present on the top of each bar) related to each biosecurity score. On the top it can be seen the association with total biosecurity scores, in the middle there is the association with internal biosecurity scores and in the bottom there is the association with external biosecurity scores. There are four classes of answers to the tests' use: never, few times, sometimes and oftentimes. The ones that did not answered are marked as "Not answered". The biosecurity classes were defined according to what was already mentioned on material and methods: Low scores < 40, medium scores >=40 and <=75 and high scores =>75.

It can be observed that the majority of farmers apply this kind of test sometimes and that even farms that obtained higher biosecurity scores may never apply these tests to treat animals with antibiotics.

The association between the frequency in which antibiotics sensitivity tests were used, with the final biosecurity scores, did not differ much between total, internal, and external biosecurity. An association between the antibiotics' sensitivity test frequency and the biosecurity scores was not found when the chi-square test was applied.

5. Chapter V – Discussion and Conclusion

5.1. Discussion

One of this project's main goals was to make a biosecurity characterization in dairy farms of the centre and northern regions of Portugal, where the higher concentration of these farms is located. Most interviews were done in farms where animal welfare inquiries were taking place at the same time, so in some cases, the farmers were already going through a process to improve their farms in some biosecurity aspects, and in other cases, even though the trainee and the veterinarians had explained the study was not going to be part of the welfare inquiries, the farmers showed some stress and reluctance in answering the questionnaire. Therefore the study was applied in a convenience sample, which can bias the results, having this sample a higher biosecurity score than the practiced by dairy farmers in the region.

The practices connected to good biosecurity maintenance in the farm included keeping a closed herd, testing animals, applying quarantine protocols, applying hygiene and disinfection measures and guaranteeing the use of specific equipment to deal with the animals as it was mentioned by Sayers RG *et al*. (2013), and by the Animal Health Law (article 10th, nº4, b). Some measures must be applied to guarantee a safe animal entrance inside the farm, regarding biosecurity procedures for vehicles', people's and animals' movements and the adoption of special measures according to the risk involved in the transportation.

The majority of farmers did not buy any animals (73%, n=111) and animals did not leave and return to the farm in 95.2% (n=139) of farms, making it easier to guarantee a closed herd and decrease external pathogens' impact, as it was observed in Belgian farms, according to Damiaans B *et al.* (2020), and contrarily to what happened in Canadian farms, according to Robichaud J *et al.* (2020), where only 41% (n=150) of farms had a closed herd.

This study revealed that in 97.3% (n=146) of farms, external vehicles did not have to pass through a wash or disinfection when they entered the farm, and in 46.3%

(n=69), those vehicles had access to areas where animals were kept; regarding vehicles that made animal loading, they already had animals from other farms in 56.6% (n=86) of farms, but 31.9% (n=23) referred that vehicles were cleaned and disinfected, and 20.8% (n=15) referred that vehicles were always cleaned. This is similar to that observed by Sarrazin S *et al.* (2014), in Belgian farms, where most farmers affirmed that trucks were clean, when they were already loaded with animals.

Regarding animal unloading, at the majority of farms that bought animals, it was observed that trucks were sometimes with other animals and not only the ones that were purchased by the farm. So, although there weren't that many new animals coming to the farm, there were a lot of vehicles entering the farm, which can be a way of diseases' dispersion from farm to farm, increasing the importance of maintain vehicles outside the farm.

The Animal Health Law (article 10th, nº1, b) refers the necessity to apply a period of quarantine when animals are bought and it was shown that of the 40 who bought animals, 30 never put new animals in quarantine, something that was also observed by Sarrazin S *et al.* (2014), where Belgian farms got lower scores on the application of quarantine measures. Regarding health status proof (for those diseases not mandatory by law), 21 did not ask for this kind of tests, and 26 never tested new animals for diseases upon arrival, contrarily to what happened in Canadian farms, according to Robichaud J *et al.* (2020), where 39% (n=143) of respondents tested animals before and after they enter the farm. The multiple correspondence analysis taken in the present study, revealed that the use of specific individual protective equipment and the implementation of hygiene procedures, like washing hands, to deal with animals that were bought and put in quarantine weren't being taken properly.

Taking into account what was previously mentioned, it can be said that farmers who usually bought animals failed in 4 crucial points to maintain biosecurity according to Wathes CM *et al.* (1983); Barrington G *et al.* (2002); Callan and Garry (2002); Svensson and Liberg (2006) and Valarcher and Hagglund (2006), which are:

- keep a closed herd,
- test new animals for specific diseases,
- put animals in quarantine, and
- workers must use specific individual protection equipment and wash hands when dealing with animals in quarantine.

Another aspect that should be pointed here, regarding the Animal Health Law article 10th, nº4, b), is the entrance of veterinarians, inseminators, hoof trimmers, and cattle traders inside the farm. The results showed that veterinarians, inseminators, and hoof trimmers usually used appropriate clothing, boots, gloves, and washed their hands when dealing with animals, but despite using the right equipment, most of the times the same equipment was used in more than one farm, and regarding the cattle trader and visitors, they entered the farm without any equipment to deal with the animals and did not wash hands, the majority of times. According to Anderson D (2010), the use of protective individual equipment to deal with the animals is essential to maintain biosecurity.

Similarly to what happened in Portuguese farms, also Belgian and Canadian studies, observed that visitors did not use specific clothes or boots in the majority of cases, despite having the means to do that (Sarrazin S *et al.* 2014), visitors could enter the farm without implementing any biosecurity procedure in 2/3 of farms, according to Damiaans B *et al.* (2020), and never were asked to wear clean, disposable or farm-provided clothes and footwear inside the farm, or to change them between groups of animals in the majority of farms according to Robichaud J *et al.* (2019).

Another important point that is mentioned in the Animal Health Law (article 10th, nº4, b) is the appropriate management and elimination of dead animals and animal byproducts. It was shown that in 53% (n=80) of farms, the vehicle that transported deadstock had to enter the farm to pick the animal's carcasses, as it was also observed in Belgian farms, where less than half of farms provided a place to collect carcasses at the farm's entrance (Sarrazin S *et al*. 2014), and in 70.2% (n=106) of farms there was not a dead animals' storage. Deadstock trucks represent a higher risk of disease introduction on farms because they go from farm to farm picking carcasses and animal sub-products, so these vehicles' entrance inside the farm should be avoided (Biocheck UGent c2019). Regarding animal carcasses, these should be put in a closed place, near the farm's entrance, because sometimes animals die from infectious diseases or unconfirmed diagnosis and the carcass can become a source of infection to other animals (Biocheck UGent c2019).

Pest control is a biosecurity measure (physical protection) being mentioned on the Animal Health Law (article 10th, nº4, a) and the results showed that the majority of farmers applied preventive measures against insects and rodents, which is a positive point regarding biosecurity since these animals can be reservoirs or act as mechanical transport of pathogens (Biocheck UGent c2019). The efficacy of the implemented measures, however, was not evaluated. Other animals can be as important in disease transmission as insects or rodents, like dogs, cats or even other farm animals, and in this case, farmers weren't applying the appropriate biosecurity measures, because as it was shown in the results, 48% (n=72) of farms had other production animals, such as poultry, pigs, goats, rabbits and 70.2% (n=106) had cats and dogs with access to the park where the dairy cattle was present as it was also observed by Damiaans B *et al.*

(2020), and Robichaud J *et al.* (2019), in Belgian and Canadian farms respectively. These animals can contribute to environmental contamination with pathogens such as *E. coli* or *Salmonella* spp. and can become a direct reservoir of infection and spread of *Neospora* or *Leptospira* (Biocheck UGent c2019). Regarding feeders and water throughs, these are points of animal gathering, so it is important to keep feed well preserved and closed to avoid contamination from other animals (such as cats, dogs, rodents), and to decrease bacterial and fungal proliferation. Water from the water throughs should be tested to check for microbiological pathogens and chemicals, and its cleanliness should be maintained (Biocheck UGent c2019). This study showed that the majority of farms kept feed well preserved but never tested water in the water throughs.

During the visits, it was observed that in some cases, calves were housed with or next to poultry and rabbit cages. Calves' pens were being contaminated with feed, faeces, and bed material from those animals. Since calves do not have a strong immune response developed yet, these practices should be avoided.

The contact with animals from neighbouring farms is a biosecurity flaw, this did not happen in 96.5% of farms in Portugal, which is a positive point, contrarily to what happened in Belgian farms where, in half of the farms direct contact between animals in neighbouring farms was possible, increasing the risk of disease dispersion (Damiaans, B *et al.* 2020).

Another aspect was related to the fact that 71.7% (n=109) of respondents had reported that manure from other farms was being spread within 500 meters of the respondent's farm, something that was also observed in 91% (n=30) of Belgian farms according to Sarrazin S *et al.* (2014), which could lead to pasture and crops' contamination (Biocheck UGent c2019).

According to the Animal Health Law (article 12th, nº1, c), the veterinarian has the responsibility to prevent diseases and the article 10th, nº4, b), refers that there are measures that must be applied to manage diseased animals to prevent disease spread, being identification, and separation of sick animals two parameters of the utmost importance.

Results showed that 62.9% (n=95) of farms had an infirmary, meaning that, in 37.1% (n=56), sick animals still had contact with healthy animals, making it hard to control diseases, which leads to health and economic losses (Valarcher and Hagglund 2006). According to Damiaans B *et al.* (2020), in half of the 120 Belgian farms tested, hospital pens weren't present, and even when farms had hospital pens, animals weren't completely isolated, and in the majority of farms the "Health management" biosecurity aspect was the one with lower biosecurity scores in internal biosecurity. In the present biosecurity project it was the third aspect with lower minimum biosecurity scores in internal biosecurity, being “Adult animals management” and “Work organization and equipment” the ones with lower minimum scores.

It was also observed in several farms, that dead and sick animals were placed inside or next to calves’ pens, or in calving pens, and regarding what was mentioned before about calves’ immune response, advice should be given to farmers not to act this way. This was also reported by Sarrazin S *et al.* (2014), in Belgian farms, where sick animals were put in calving pens and by Robichaud J *et al.* (2019), in Canadian farms, where 73% (n=268) housed sick animals inside the calving pen.

The Animal Health Law, nº 81 also reinforces, the importance of vaccines as an instrument for disease prevention, control, and eradication, something that 78.8% (n=119) of respondents put into practice, similarly to what Robichaud J *et al.* (2019), observed in Canadian farms, where written vaccination protocols were applied in 70% (n=257) of farms.

Regarding another aspect that can influence disease introduction and spread, and that is related to a longer treatment period, animal’s death, reinfections and lower immune response, antibiotic resistance. In Animal Health Law recital 32, it is referred as very important to act against it, in a preventive way, and article 11th, nº1, e) refers to the importance of providing training to farmworkers about these resistances and its implications. By applying biosecurity measures and acting in prevention, fewer animals get sick, and therefore fewer antibiotic treatments are needed (WHO 2014). What can be seen in the results is that 52.3% (n=79) did not apply protocols to use antibiotics responsibly, only 43% (n=64) of respondents said they applied antibiotic sensitivity tests sometimes and only 18.8% (n=28) applied these tests often throughout the year. This question should be directed to the specific lactation phase where the test was taken. Regarding selective treatment it was not applied in 45% (n=68) during cows’ dry period. The results also revealed that farms in which antibiotic and vaccination protocols and selective treatment were implemented had higher biosecurity scores.

Some farmers are truly trying to achieve ideal goals to improve biosecurity and overcome antibiotic resistance, but there is still a lot to improve in this area and farmers should be advised properly regarding these two issues and should be enlightened for the future impacts that can happen in the trade market. Countries worldwide are working to implement antimicrobial stewardship, enhance monitoring in animals’ antimicrobial use, address inappropriate use of antimicrobials and ban the use of antimicrobials as growth promoters (Food and Drug Administration’s Centre for Veterinary Medicine, 2018). Restrictions in imports already exist in some countries, according to Johnson R (2011), so it is important to guarantee that Portuguese farmers are on the right track to implement the increasing demands regarding antibiotic use.

According to the Animal Health Law (article 10th, nº4, b), biosecurity management procedures to use equipment, should be applied, and a positive point regarding this aspect was the fact that 96.7% (n=147) of the respondents had their equipment and did not make any exchange with other farms, something that could lead to disease introduction inside the farm as fomites or through workers, contrarily to what happened in Canadian farms where 33% (n=121) of respondents, admitted they shared equipment or vehicles with neighbouring farms, and only a few had measures to control the access to the farm (Robichaud J *et al.* 2019). But it should be taken into account that the farms sampled for this biosecurity project do not always respect the 200 meters distance from other farms according to Portaria 42/2015, article 4, b). So, despite the fact that there were no equipment exchanges or visits from workers belonging to another farm, the proximity between farms can be an issue regarding disease dispersion.

Preventing the entrance of diseases inside the farm is a significant measure, but when that prevention fails, it comes to farmers and workers to develop the capacity to decrease the probability of disease spread inside the farm. Separating animals and using different equipment between groups of animals is something that can decrease this spreading (Stanković B *et al.* 2016). Results showed total separation of animals per age class, in 79.9% (n=115) of farms, and total separation of equipment between age classes, in 84.1% (n=122), which are good biosecurity procedures implementation. The fact that workers did not change clothes or boots and were not applying something as simple as washing hands, or wearing other gloves between groups, in 86.1% (n=111) of farms, or having a specific work order to deal with animals, something that happened in 52.8% (n=76) of farms, makes it difficult to prevent disease dispersion inside the farm.

These issues were also reported by Belgian farms where, according to Damiaans, B *et al.* (2020), animals from different age groups were not physically separated and only 20% (n=24) of farms applied a working procedure from young to older animals. Something similar was observed in another study by Sarrazin S *et al.* (2014), where it was also reported that workers did not change clothes or washed and disinfected their hands between animal groups. Changing boots and clothes between each group is difficult, but washing boots, washing hands and changing gloves are simple things that can be done and that decrease disease dispersion.

To prevent disease spread, it is important to guarantee good cleaning and disinfection procedures according to Morley PS (2002), and also the Animal Health

Law (article 10th, nº4, a) and Portaria 42/2015 article 6th, c). This study showed that the differences between farms regarding these procedures were big, with a minimum of 0 times per year and a maximum of 1095 times per year in both procedures. As previously mentioned, some respondents weren't completely honest in some answers due to the pressure of being evaluated in animal welfare at the same time the biosecurity interview was being taken. However, it was observed during the interviews, farms where animals had their pens, feeders and water throughs in perfect cleaning and disinfection conditions, and other farms where animals, mostly calves, were in really bad hygiene conditions. So, the contrast between farms isn't probably as big as from 0 to 1095 per year (3 times a day), but there are farms that really try to accomplish good cleaning and disinfection procedures, and there are others that underestimate these procedures' significance. Studies in Belgian farms and Canadian farms focused the hygiene procedures on calves' pens and calving pens, with individual pens being cleaned and disinfected the majority of times, according to Damiaans B *et al.* (2020), but calving pens being used to house sick animals without cleaning and disinfecting, and without applying these procedures after an abortion or calving had occurred, in the majority of farms, according to Sarrazin S *et al.* (2014), something that was also reported by Robichaud J *et al.* (2019), in Canadian farms, where the majority of farmers did not have cows and calving pens cleaned.

In dairy farms, the milking process must undergo biosecurity measures to guarantee milk's quality and security, and to prevent the appearance of mastitis in cows due to teat lesions or pathogens (Mekibib B *et al*. 2010). Regarding equipment maintenance, it was observed that the majority of farmers made an equipment review once a year, which was put under disinfection procedures after the milking process and teat liners were changed frequently (after 6 months). Regarding the milking itself, the cleaning and disinfection procedures were applied before and after the milking process and mastitic cows were milked after the others in the majority of farms too, procedures that were also applied in Belgian farms according to Damiaans B *et al.* (2020). This revealed that in dairy management, farmers applied the recommended procedures to maintain biosecurity (Garcia S, Osburn B, Cullor J 2019). Some points that were not on the questionnaire were the milking room's hygiene and disinfection, which is an important aspect to prevent mastitis due to the existence of environmental pathogens that can contaminate the udder (Mekibib B *et al*. 2010), the milk's cooling temperature in the milk tank, that should be around 4º C (Upton *et al.* 2013), and the colostrum and milk pasteurization, which is important to prevent diseases and antibiotics transmission to calves (European Food Safety Authority 2017).

Another aspect that can influence biosecurity scores is the herd size, as it can be observed by the ANOVA and Kruskal-Wallis analyses. In a study taken by Robichaud J *et al.* (2019), in Canadian dairy farms, it was observed that farms with higher production level were farms where biosecurity measures were applied more often than in farms with lower production. In this biosecurity project, that could be also one point that explains the differences between biosecurity scores because, with higher milk production and bigger herds, there is more investment in disease prevention, to maintain the production levels and to decrease the probability of having both animal and economical losses. But two other reasons were observed during the visits, which can explain these differences between biosecurity scores in farms. Most of the farms belonged to a family and in some cases, the sons or daughters did not want to keep the farm, so, the farmers stopped investing on biosecurity measures, the second one is related to the fact that in some small farms, farmers could not compete with bigger farms in both milk production and animal welfare, so they began the process to end the business.

5.2. Limitations and future perspectives

It would be interesting to apply the same questionnaire forward in time and compare the results to see the biosecurity scores' evolution, mainly on some biosecurity parameters that farmers should improve as soon as possible, and apply the same study to a greater sample of dairy farms and in other regions, to have a better characterization of Portugal's biosecurity level. Although the questionnaire used for this study was built using Biocheck questionnaire, a reference used by several countries, it should be revised and changed to be more accurate for smaller farms and familiar types of animal production, and for other areas of animal production (beef production, small ruminants' production).

Another aspect that was thought for this project was to relate biosecurity scores with data about dairy productivity, cases of mastitis, days in milk, somatic cell counts, Californian mastitis test results, diseases present on farms, but those data weren't available. It would be interesting to see which biosecurity aspects could influence increase or decrease in certain diseases' prevalence, to see which biosecurity aspects could influence the animals' productivity itself, it would be also interesting to relate biosecurity scores and disease prevalence with vehicles' movements from farm to farm.

Biosecurity and animal welfare are two concepts that are becoming more and more relevant to animal production, due to the "prevention is better than cure" approach of the European Commission, and having veterinarians in the field just advising farmers regarding biosecurity procedures would allow for a great improvement in this area like Agros is doing by having a team of veterinarians just to evaluate animal welfare. This kind of approach takes time, effort, and work, and sometimes, veterinarians want to help in this area, but cannot do it properly because they have to provide medical care in many farms at the same time. Veterinarians working for the official authorities with the help of veterinarians that work in the field could be the ones making the bridge between farmers and the government, advising, evaluating biosecurity and antibiotics use on farms, and giving a voice to farmers, showing the government the main barriers that block biosecurity implementation, guaranteeing that possible future biosecurity measures are realistic and applied for Portuguese farms, based on proper information collected with tools, such as the one that was created for this project, and not only by taking measures based on other countries' biosecurity parameters.

5.3. Conclusion

This study allowed the characterization of the biosecurity level in dairy farms were in the north and centre of Portugal using direct interview in 151 holdings, with a questionnaire based in Biocheck.Ugent and further developed and scored with a participatory approach, creating a biosecurity index. This work had also the objective of providing a counselling tool to raise farmers and veterinarians' awareness on biosecurity and responsible use of antibiotics and allowed to understand which biosecurity aspects are being properly applied and which should be improved to decrease the risk of pathogenic agents introduction and spread.

It can be concluded that total biosecurity in this sample of dairy farms have room for improvement because scores revealed 65.5% of compliance (minimum – 42.0 and maximum 80.4%) and only 61.8% of compliance if Biocheck score is used. External and internal biosecurity are highly correlated (r^2=0.87), and global values were 71.3% (minimum 44.1 and maximum 85.1%) for external and 67.7% (minimum 37.8 and maximum 80.3%) for internal biosecurity. This means that some farmers were taking biosecurity seriously but other farmers lack of information or of economic capacity to apply the necessary measures.

In the descriptive analysis, the aspects of biosecurity not followed by more than 50% of holdings were, the lack of cleaning and disinfection of vehicles and the entry of traders without appropriate IPE, the lack of bacteriological control of water at the drinking points, the lack of hygiene procedures (for example washing hands/boots) when handling different groups of animals inside the holding and the contact with other animals outside the holding.

Biosecurity results are also correlated with the size of holding, as identified by variance analysis, where medium-size holdings had higher scores in total and internal biosecurity, comparing to small holdings. Multiple correspondence analysis revealed that farmers who usually apply quarantine, despite taking the hygiene of these premises seriously, never use IPE and never wash hands to deal with animals inside the quarantine pen. These were farms that had higher scores in the following biosecurity aspects: “Purchase of animals and reproduction”, “Personnel and visitors” and “Calving management”.

It can also be concluded, by the Principal Components Analysis, that farms where biosecurity scores were above average, were farms that applied proper use of individual protective equipment and hand hygiene, that stored animals feed adequately, that disinfected the sick animals and calves pens frequently and that had calving occurring in calving pens.

In relation to antibiotic usage, logistic regression identifies the lack of vaccination protocols and antibiotic use protocols and the lack of selective treatment as correlated with lower scores of biosecurity.

As observed every farm is unique, has its own problems, barriers and solutions, and veterinarians play an important role in motivating and advising the farmers for biosecurity improvement. This project developed a tool that allows the individual diagnosis of the holding with feedback on geographical risk, benchmarking and advising tips to help farmers and veterinarians and promote dialogs for biosecurity improvement.

Through this study it can be concluded that procedures which would result in biosecurity improvement in this production system are:

- Vehicles: do not allow entry into animal premises and inspect and require cleaning and disinfection;
- Entry of replacement animals: require pretesting (or post testing) of main endemic diseases (except those already required officially) and carry out quarantine for a sufficient period, in disinfected premises and in total separation from the herd;
- Diagnosis of disease: investigate abortion or sick animals and separate these animals for treatment;
- Personnel: do not allow contact with ruminants outside the holding and systematic use of protective equipment, with special care of washing hands and boots between different groups of animals;

- Cleaning and disinfection of premises: carried out with appropriate methods especially in maternity, calves' premises, milking parlour and infirmaries;
- Antibiotics use: implementation of antibiotherapy protocols and application of selective treatment.

Farmers may have to work harder to improve their biosecurity, but they should not be doing that alone, veterinarians have the knowledge to help and they should use it. Veterinarians in fact have a crucial role in biosecurity, and should be the voice that advises, that assures biosecurity measures are being taken properly, that explains why these measures should be taken in the first place, that explains what antimicrobial resistance is and why farmers should work to prevent it. Veterinarians should also be proactive in developing protocols for the use of antibiotics or vaccines

In conclusion, Portugal dairy farms should continue to implement biosecurity protocols, not only because of the Animal Health Law, to be implemented from April 2021, but mainly because they need to increase public health protection, prevent antimicrobial resistance and maximise animal productivity, being therefore competitive within the Common European Market. Prevention is the key.

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Appendix I – Report from Agros magazine

BIOSSEGURANÇA EM EXPLORAÇÕES LEITEIRAS DO ENTRE DOURO E MINHO – APRESENTAÇÃO DO RELATÓRIO DA 1ª FASE.

TEXTO UCADESA

No passado dia 24 de fevereiro, decorreu um seminário sobre o projeto de biossegurança em Explorações leiteiras, organizado pela UCADESA, e que contou com a colaboração da equipa responsável da Faculdade de Medicina Veterinária da Universidade de Lisboa (FMV-UL), liderada pelo Professor Telmo Nunes, em conjunto com Mafalda Mil-Homens e Jéssica Vicente, e com a participação da Diretora de Serviços de Alimentação e Veterinária do Norte, Dr.ª Elsa Machado.

O Seminário, dirigido aos Médicos Veterinários das OPP de Entre Douro e Minho e Produtores envolvidos na primeira fase do inquérito, contou com duas apresentações, uma dedicada à apresentação dos resultados do questionário às Explorações sobre indicadores de biossegurança e outra sobre o tema do efeito da temperatura nas taxas de mortalidade em Explorações leiteiras.

Partindo para a apresentação do relatório de biossegurança, a oradora foi a Mafalda Mil Homens que fez também a recolha dos dados desta 1.ª fase. O projeto consistiu na sensibilização dos Produtores e Médicos Veterinários para os aspetos de biossegurança das Explorações leiteiras e na preparação, testagem e implementação de um questionário sobre biossegurança, pela mestranda da FMV-UL Mafalda Mil-Homens, com o apoio de Médicos Veterinários das OPP (Organizações de Produtores Pecuários).

INTRODUÇÃO E OBJETIVOS.

A biossegurança é um dos pilares da sanidade animal, permitindo prevenir a introdução e a propagação de doenças transmissíveis e consequentemente os seus impactos negativos na produção pecuária. A biossegurança diz respeito a um conjunto de medidas físicas e de gestão que devem ser aplicadas diariamente pelos produtores, sob a orientação do seu médico veterinário.

Deste modo torna-se imperativo o desenvolvimento a curto prazo de um sistema de avaliação e classificação oficial da biossegurança das explorações pecuárias, pelo que a Direção Geral de Alimentação e Veterinária (DGAV), conjuntamente com a União das Cooperativas Agrícolas de Defesa Sanitária de Entre Douro, Minho e Trás-os-Montes (UCADESA), Laboratório de Sanidade Animal e Segurança Alimentar (SEGALAB) e a Faculdade de Medicina Veterinária da Universidade de Lisboa (FMV-UL), elaboraram um projeto de avaliação voluntária de biossegurança, com os seguintes objetivos:

- Permitir o diagnóstico inicial do estado de biossegurança do setor de bovinos leiteiros na área de influência da UCADESA;
- Dotar os produtores e seus médicos veterinários de uma ferramenta de autoavaliação de biossegurança e partilhar as melhores práticas a implementar relativamente a este assunto;
- Permitir a adequação dos futuros modelos oficiais de caraterização e classificação de biossegurança às especificidades nacionais, no âmbito da legislação referida;

RESULTADOS OBTIDOS

FIGURA 1 – EXEMPLO DE UM RELATÓRIO DE UMA EXPLORAÇÃO ADERENTE AO INQUÉRITO.

CARATERIZAÇÃO DAS EXPLORAÇÕES

O histograma (figura 3) representa uma distribuição do número de animais por exploração no total das 88 explorações. O mapa da figura 4 apresenta a distribuição geográfica das explorações que integraram a primeira fase do projeto.

RESULTADOS DA PONTUAÇÃO DE BIOSSEGURANÇA

Numa escala de até 100 pontos, a biossegurança (BS) Total resultou em 70,9 pontos, no caso da BS Externa a média foi 74,1pontos e no caso da Interna 65 pontos (Tabela 2).

TABELA 2 – RESULTADOS DA PONTUAÇÃO MÉDIA DE BIOSSEGURANÇA

	MÉDIA	DESVIO-PADRÃO
Biossegurança Total	70,9	5,6
Biossegurança Externa	74,1	6,6
Biossegurança Interna	65	6,7

Na Figura 5 pode observar-se a distribuição dos diferentes valores obtidos na análise efetuada aos dados obtidos nos inquéritos.

Através desta caracterização preliminar dos níveis de biossegurança, identificaram-se pontos fulcrais para a aplicação de medidas preventivas nas explorações que devem ser tidos em conta pelos produtores e pelos médicos veterinários.

Os principais riscos identificados foram as trocas comerciais com negociantes, a falta de procedimentos de quarentena, entrada de veículos externos, frequência de limpeza e desinfeção, que variava bastante entre explorações, proximidade de explorações, presença de outros animais de produção e de animais domésticos, partilha de espaços entre enfermaria e maternidade, eliminação de membranas fetais de forma incorreta e entreajuda na altura da colheita de silagens, que leva a grande movimentação entre explorações.

A outra apresentação do seminário, a cargo de Jéssica Vicente, teve como objetivo perceber de que forma as temperaturas influenciam os padrões de mortalidade em bovinos em Portugal, a partir de informação tratada cientificamente e face ao peso desse efeito negativo como adaptarmos os nossos sistemas de produção animal, de forma a dar resposta aos desafios dos climas atuais e futuros. Os dados reportaram-se a 4 anos entre 2015 e 2019, e basearam-se na base de dados do SNIRA. A discussão dos resultados tinha como objectivo encontrar formas de reduzir o efeito do stress térmico através de duas medidas principais, o ajustamento do arraçoamento e a alteração das condições ambientais. As conclusões do trabalho apontam para 3 aspetos a destacar:

- Demonstra-se claramente os impactos que a temperatura tem na mortalidade dos bovinos em Portugal;
- Existem perdas efetivas na produção pecuária que podem ser minimizadas com acções diárias de maneio;
- Este problema com o avançar das alterações climáticas e do aquecimento global, vai ter cada vez mais impacto na produção.

A encerrar a sessão, a Dr.ª Elsa Machado, Diretora dos Serviços de Alimentação e Veterinária do Norte, referiu que a biossegurança é fundamental para o trabalho sanitário que todos os agentes do setor se têm de comprometer, para erradicarmos as principais doenças sanitárias que assolam o País e a região. Deu ainda alguns exemplos de casos recentes que ocorreram na região precisamente por não se cumprirem e negligenciarem alguns aspectos ligados às questões de biossegurança.

Appendix II – RStudio Packages

Package
dplyr
ggplot2
ggthemes
ggpubr
GGally
googledrive
googlesheets
rpart
flexdashboard
FactoMineR
factoextra
leaflet
data.table
viridisLite
RColorBrewer
nortest
Hmisc
summarytools
knitr
reshape2

Appendix III – Descriptive analyses: answers' frequency

G1

Variable	Statistic / Values	Freqs (% Valid)	Missing
X2.Who manages the animals?	1. Full-time employees 2. Part-time employees 3. Producer 4. Producer's family members 5. Producer's family members, Part-time employees 6. Producer, Full time employees 7. Producer, Part-time employees 8. Producer, Producer's family members 9. Producer, Producer's family members, Full-time employees 10. Producer, Producer's family members, Full-time employees, Part-time employees 11. Producer, Producer's family members, Part-time employees	1 (0.7%) 1 (0.7%) 3 (2.0%) 2 (1.3%) 1 (0.7%) 7 (4.6%) 1 (0.7%) 103 (67.8%) 25 (16.4%) 1 (0.7%) 7 (4.6%)	0 (0%)
X2.1. Do workers have professional training in farming?	1. No 2. Yes	14 (9.4%) 135 (90.6%)	3 (1.97%)
X3. Number of bulls	Mean (sd) : 0.5 (2.6) min < median < max: 0 < 0 < 31 IQE (CV) : 0 (5.9)	0 : 130 (86.1%) 1 : 11 (7.3%) 2 : 6 (4.0%) 3 : 2 (1.3%) 8 : 1 (0.7%) 31 : 1 (0.7%)	1 (0.66%)
X4. Number of adult cows (2 years)	Mean (sd) : 86.9 (89.8) min < median < max: 14 < 65 < 911 IQE (CV) : 54 (1)	71 values	1 (0.66%)
X5. Number of heifers	Mean (sd) : 38.4 (47.2) min < median < max: 0 < 25 < 430 IQE (CV) : 35 (1.2)	56 values	7 (4.61%)
X6. Number of calves from 2 to 8 months	Mean (sd) : 30.9 (117.5) min < median < max: 0 < 15 < 1430 IQE (CV) : 18 (3.8)	46 values	4 (2.63%)
X7. Number of calves less than 2 months old	Mean (sd) : 10.2 (10.4) min < median < max: 0 < 7 < 70 IQE (CV) : 8 (1)	29 values	3 (1.97%)

G2

Variable	Statistic / Values	Freqs (% Valid)	Missing
X8. Does the farmer buy animals? (take into account the last two years)	1. No 2. Yes, bulls for breeding 3. Yes, bulls for breeding; Yes, pregnant females 4. Yes, calves, breeds and non-pregnant heifers; Yes, pregnant females 5. Yes, calves, reared and non-pregnant heifers 6. Yes, calves, reared and non-pregnant heifers; Yes, lactating females 7. Yes, calves, reared and non-pregnant heifers; Yes, pregnant females; Yes, lactating females 8. Yes, lactating females 9. Yes, pregnant females 10. Yes, pregnant females; Yes, lactating females	111 (73.0%) 2 (1.3%) 1 (0.7%) 1 (0.7%) 17 (11.2%) 2 (1.3%) 1 (0.7%) 5 (3.3%) 8 (5.3%) 4 (2.6%)	0 (0%)
X9.1. How often do you buy pregnant females per year?	1. 0 2. 1 3. 1X YEAR 4. 2 5. 2 times 6. 3 7. 3 x year 8. 4 9. as needed	3 (15.8%) 6 (31.6%) 1 (5.3%) 4 (21.1%) 1 (5.3%) 1 (5.3%) 1 (5.3%) 1 (5.3%) 1 (5.3%)	133 (87.5%)
X9.2. How often do you buy lactating females per year?	1. 0 2. 1 3. 11 4. 2 5. I do not buy	6 (35.3%) 7 (41.2%) 1 (5.9%) 2 (11.8%) 1 (5.9%)	135 (88.82%)
X9.3. How often do you buy non pregnant calves or heifers per year?	1. > 2 2. 0 3. 1 4. 2 5. 2x month 6. 3 7. 6 8. I do not buy	2 (8.7%) 4 (17.4%) 10 (43.5%) 2 (8.7%) 1 (4.3%) 2 (8.7%) 1 (4.3%) 1 (4.3%)	129 (84.87%)
X9.4. How often do you buy bulls for breeding per year?	1. 0 2. 2 3. 2X year 4. I do not buy	7 (70.0%) 1 (10.0%) 1 (10.0%) 1 (10.0%)	142 (93.42%)
X10.Do you always buy animals from the same place of origin?	1. No, I buy from multiple sources 2. No, I turn to stakeholders or the livestock market 3. Yes	15 (38.5%) 18 (46.2%) 6 (15.4%)	113 (74.34%)
X11. Is it possible, for animals bought, to have direct or indirect contact with animals from different farms before arriving at the farm?	1. I do not know 2. No 3. Yes	8 (20.0%) 20 (50.0%) 12 (30.0%)	112 (73.68%)

X12. When you buy animals from another farm, do you ask for health status proof guaranteeing that the farm you are buying from has health status equal to or higher than the one of your farm? (excluding brucellosis, tuberculosis or leucosis)	1. Always 2. Never 3. Sometimes	17 (42.5%) 21 (52.5%) 2 (5.0%)	112 (73.68%)
X13. Do you check the maternal immunity status when you buy calves?	1. Always 2. Never	1 (8.3%) 11 (91.7%)	140 (92.11%)
X14. Are animals tested for specific diseases when they enter the farm?	1. Always 2. Never 3. Sometimes	10 (25.0%) 26 (65.0%) 4 (10.0%)	112 (73.68%)
X15. Is quarantine used after an animal acquisition?	1. Always 2. Never 3. Sometimes	8 (20.5%) 30 (76.9%) 1 (2.6%)	113 (74.34%)
X16.What is the minimum quarantine period in days?	Mean (sd) : 22.1 (18.3) min < median < max: 1 < 15 < 60 IQE (CV) : 15 (0.8)	1 : 1 (11.1%) 8 : 1 (11.1%) 15 : 4 (44.4%) 30 : 1 (11.1%) 40 : 1 (11.1%) 60 : 1 (11.1%)	143 (94.08%)
X17. Do workers use specific boots only for the quarantine pen?	1. Always 2. Never	1 (11.1%) 8 (88.9%)	143 (94.08%)
X17. Do workers use specific clothes only for the quarantine pen?	1. Never	8 (100.0%)	144 (94.74%)
X17. Do workers wash hands or change gloves after working in the quarantine pen?	1. Always 2. Never	3 (33.3%) 6 (66.7%)	143 (94.08%)
X18. Is all the material from the quarantine pen removed after the animal leaves?	1. Always 2. Never	8 (88.9%) 1 (11.1%)	143 (94.08%)
X19. Is the quarantine pen cleaned before the introduction of a new animal?	1. Always	9 (100.0%)	143 (94.08%)
X19. Is the quarantine pen disinfected before the introduction of a new animal?	1. Always 2. Sometimes	5 (71.4%) 2 (28.6%)	145 (95.39%)
X19. Is the quarantine pen dried before the introduction of a new animal?	1. Always	5 (100.0%)	147 (96.71%)
X20. During the quarantine period, are newly introduced animals milked?	1. No 2. Not applicable 3. Yes, last	2 (14.3%) 7 (50.0%) 5 (35.7%)	138 (90.79%)

X20.1. During the quarantine period do animals in the quarantine pen have contact with the other animals?	1. No 2. Yes	7 (43.8%) 9 (56.2%)	136 (89.47%)
X21. Do you test new animals' milk for microbiological pathogens before introducing them in the farm or at the beginning of lactation in the quarantine period?	1. Always 2. Never 3. Sometimes	7 (41.2%) 8 (47.1%) 2 (11.8%)	135 (88.82%)
X22. Do animals leave the farm and go back in?	1. No 2. Yes	139 (95.2%) 7 (4.8%)	6 (3.95%)
X23. Are animals returning to the farm put into quarantine?	1. Never	10 (100.0%)	142 (93.42%)
X24. Is there reproductive management on your farm? If so how is it made?	1. Both 2. Yes, artificial insemination / Embryo Transplant 3. Yes, natural breeding with bull	11 (7.3%) 137 (90.7%) 3 (2.0%)	1 (0.66%)
X25. Is fresh bull semen tested for sexually transmitted diseases?	1. Always 2. Never	15 (53.6%) 13 (46.4%)	124 (81.58%)
X26. Does fresh semen come from a farm or institution with a health status greater than or equal to the one of your farm?	1. Always 2. Never	31 (81.6%) 7 (18.4%)	114 (75%)

G3

Variable	Statistic / Values	Freqs (% Valid)	Missing
X27. Do all vehicles have to be washed before entering the farm?	1. Always 2. Never 3. Sometimes	1 (0.7%) 146 (97.3%) 3 (2.0%)	2 (1.32%)
X28. Do external vehicles and transporters have access to areas where animals are kept?	1. No 2. Yes	80 (53.7%) 69 (46.3%)	3 (1.97%)
X29. When purchased animals are unloaded on the farm are there other animals present inside the transport truck?	1. Always 2. Never 3. Sometimes	33 (71.7%) 9 (19.6%) 4 (8.7%)	106 (69.74%)
X30. Are the vehicles that will load animals from the farm always empty before they enter the farm?	1. Always 2. Never 3. Sometimes	39 (25.7%) 27 (17.8%) 86 (56.6%)	0 (0%)
X31. Is truck that transports animals always cleaned and disinfected before entering the farm?	1. Clean and disinfected 2. Do not know 3. Just clean 4. No	23 (31.9%) 19 (26.4%) 15 (20.8%) 15 (20.8%)	80 (52.63%)
X32. Is there a dead stock storage	1. No	106 (70.2%)	1

with a hard floor?	3. Yes	45 (29.8%)	(0.66%)
X33. Is the dead stock storage cleaned and disinfected after each use?	1. No 2. Yes	9 (18.8%) 39 (81.2%)	104 (68.42%)
X34. Is the dead stock storage protected from the action of insects, rodents, dogs and cats?	1. No 2. Yes	20 (40.8%) 29 (59.2%)	103 (67.76%)
X35. Does the dead stock truck enter the farm to remove animals' carcasses?	1. No 2. Yes	71 (47.0%) 80 (53.0%)	1 (0.66%)
X36. Is dead stock handled with gloves or are the hands cleaned and disinfected after each manipulation?	1. Always 2. Never 3. Sometimes	133 (88.1%) 9 (6.0%) 9 (6.0%)	1 (0.66%)
X37. Is all material used in dead stock's handling cleaned and disinfected?	1. Always 2. Never 3. Sometimes	125 (82.2%) 13 (8.6%) 14 (9.2%)	0 (0%)

G4

Variable	Statistic / Values	Freqs (% Valid)	Missing
X38. Are food storage facilities protected from insects and animals?	1. No 2. Yes, animals only 3. Yes, from animals and insects	45 (29.6%) 52 (34.2%) 55 (36.2%)	0 (0%)
X39. Are feeding utensils used only for food?	1. No 2. Sometimes 3. Yes	5 (3.3%) 15 (9.9%) 131 (86.8%)	1 (0.66%)
X40. Is drinking water subjected to bacteriological analysis annually at the source or in the storage tank?	1. No 2. No, but I'm using company water 3. Yes	2 (1.3%) 2 (1.3%) 146 (97.3%)	2 (1.32%)
X41. Is drinking water annually subjected to bacteriological analysis at watering sites?	1. No 2. Yes	139 (92.0%) 12 (8.0%)	1 (0.66%)

G5

Variable	Statistic / Values	Freqs (% Valid)	Missing
X42. Are visitors required to notify their presence before entering the farm?	1. Always 2. Never 3. Sometimes	65 (43.3%) 37 (24.7%) 48 (32.0%)	2 (1.32%)
X43. Is there a separate place to change boots, clothes and gloves or to wash hands?	1. No 2. Yes	26 (17.6%) 122 (82.4%)	4 (2.63%)
X44. Is it customary to attend or go to	1. No	132 (88.0%)	2

work on other farm? (Farmer/Employees)	2. Yes	18 (12.0%)	(1.32%)
X44.1. Do employees have farm animals at home?	1. No 2. Yes	35 (28.9%) 86 (71.1%)	31 (20.39%)
X45. Do workers wear specific boots to be on the farm?	1. Always 2. Never 3. Sometimes	106 (89.1%) 8 (6.7%) 5 (4.2%)	33 (21.71%)
X45. Do workers wear specific clothes to be on the farm?	1. Always 2. Never 3. Sometimes	99 (83.9%) 11 (9.3%) 8 (6.8%)	34 (22.37%)
X45. Do workers wear gloves or wash hands?	1. Always 2. Never 3. Sometimes	81 (68.1%) 11 (9.2%) 27 (22.7%)	33 (21.71%)
X46.1. Frequency of veterinarian visit/ year	1. 10 2. 12 3. 15 4. 15 in 15 days 5. 17 6. 18 7. 20 8. 30 9. 4 10. 8 11. 3x week	2 (2.5%) 12 (14.8%) 1 (1.2%) 1 (1.2%) 1 (1.2%) 2 (2.5%) 1 (1.2%) 1 (1.2%) 1 (1.2%) 1 (1.2%) 111 (75%)	
X46. Does the veterinarian wears specific boots?	1. Sometimes 2. Never 3. Always	18(11.9%) 21(13.9%) 19(12.6%)	
X46. Does the veterinarian wears specific clothes?	1. Always 2. Never 3. Sometimes	103 (68.7%) 34 (22.7%) 13 (8.7%)	2 (1.32%)
X46. Does the veterinarian wear gloves or wash hands?	1. Always 2. Never 3. Sometimes	143 (94.7%) 6 (4.0%) 2 (1.3%)	1 (0.66%)
X47. Does the artificial insemination technician go to the farm?	1. No (go to 49) 2. Yes	70 (46.1%) 82 (53.9%)	0 (0%)
X47.1. Frequency of visits by the artificial insemination technician/year	1. 0 2. 100 3. 12 4. 180 5. 20 6. 22 7. 25-30x 8. 30 9. 36 10. 365 DAYS 11. 40 12. 50 13. 52 14. 55 15. 60	1 (2.9%) 2 (5.9%) 2 (5.9%) 1 (2.9%) 1 (2.9%) 1 (2.9%) 1 (2.9%) 5 (14.7%) 1 (2.9%) 2 (5.9%) 6 (3.9%) 1(2.9%) 1(2.9%) 1(2.9%) 2(5.9%)	

	16. 72 17. 75 18. 80 19. 2x week 20. When needed	1(2.9%) 1(2.9%) 2(5.9%) 1(2.9%) 1(2.9%)	
X48.Does the artificial insemination technician wear specific boots?	1. Always 2. Never 3. Sometimes	61 (74.4%) 16 (19.5%) 5 (6.1%)	70 (46.05%)
X48. Does the artificial insemination technician wear specific clothes?	1. Always 2. Never 3. Sometimes	60 (74.1%) 16 (19.8%) 5 (6.2%)	71 (46.71%)
X48. Does the artificial insemination technician wear gloves or wash hands?	1. Always 2. Sometimes	79 (97.5%) 2 (2.5%)	71 (46.71%)
X49. Does the stakeholder go to the farm?	1. No 2. Yes	3 (2.0%) 149 (98.0%)	0 (0%)
X49.1. Frequency of visits by the stakeholder/year	1. > 10x year 2. 10 3. 12 4. 15 5. 16 6. 20 7. 24 8. 25 9. 26 10. 30 11. 6 12. 8 13. 45 14. 52 15. When needed 16. Weekly 17. Variable 18. 2 times a month	1 (1.6%) 5 (8.1%) 24 (38.7%) 1 (1.6%) 1 (1.6%) 2 (3.2%) 9 (14.5%) 1 (1.6%) 1 (1.6%) 4 (6.5%) 1 (1.6%) 3 (4.9%) 1 (1.6%) 3 (4.9%) 1 (1.6%) 1 (1.6%) 2 (3.2%) 1 (1.6%)	90 (59.21%)
X50. Does the stakeholder wear specific boots?	1. Always 2. Never 3. Sometimes	25 (16.7%) 115 (76.7%) 10 (6.7%)	2 (1.32%)
X50. Does the stakeholder wear specific clothes?	1. Always 2. Never 3. Sometimes	16 (10.8%) 126 (85.1%) 6 (4.0%)	4 (2.63%)
X50. Does the stakeholder wear gloves or wash hands?	1. Always 2. Never 3. Sometimes	31 (21.2%) 106 (72.6%) 9 (6.2%)	6 (3.95%)
X51. Does the hoof trimmer go to the farm?	1. No 2. Yes	25 (16.6%) 126 (83.4%)	1 (0.66%)

X51.1. Frequency of hoof trimmer visits/year	1. 0 2. 1 3. 10 4. 12 5. 12x 6. 15 7. 2 8. 2 or 3 times a year 9. 20 10. 25 11. 3 12. 4 13. 5 14. 6 15. 8 16. 26 17. 30 18. 40 19. 2x month 20. Weekly	1 (1.9%) 2 (3.8%) 1 (1.9%) 11 (20.8%) 1 (1.9%) 2 (3.8%) 5 (9.4%) 1 (1.9%) 1 (1.9%) 1 (1.9%) 4 (7.7%) 8 (15.3%) 1 (1.9%) 6 (11.5%) 1 (1.9%) 2 (3.8%) 1 (1.9%) 1 (1.9%) 2 (3.8%) 1 (1.9%)	99 (65.13%)
X52. Does the hoof trimmer wear specific boots?	1. Always 2. Never 3. Sometimes	92 (73.0%) 32 (25.4%) 2 (1.6%)	26 (17.11%)
X52. Does the hoof trimmer wears specific clothes?	1. Always 2. Never 3. Sometimes	88 (69.8%) 33 (26.2%) 5 (4.0%)	26 (17.11%)
X52. Does the hoof trimmer wears gloves or wash hands?	1. Always 2. Never 3. Sometimes	118 (93.7%) 6 (4.8%) 2 (1.6%)	26 (17.11%)
X53. Do visitors have contact with the animals?	1. No 2. Yes	108 (72.0%) 42 (28.0%)	2 (1.32%)
X53.1. Frequency of visitor visits/year	1. 0 2. 1 3. 100 4. 12 5. 15 6. 18 7. 2 8. 20 9. 3 10. 30 11.5 12.8 13. 36 14. 48 15. Sometimes 16. Doesn't know 17. Variable 18. Monthly	19 (40.4%) 3 (6.4%) 1 (2.1%) 1 (2.1%) 2 (4.3%) 1 (2.1%) 7 (14.9%) 1 (2.1%) 1 (2.1%) 1 (2.1%) 2(4.3%) 1 (2.1%) 1 (2.1%) 1 (2.1%) 1 (2.1%) 2 (4.3%) 1 (2.1%) 1 (2.1%)	105 (69.08%)
X54. Do visitors wear specific boots?	1. Always 2. Never 3. Sometimes	9 (17.6%) 38 (74.5%) 4 (7.8%)	101 (66.45%)
X54. Do visitors wear specific clothes?	1. Always 2. Never	9 (17.6%) 39 (76.5%)	101 (66.45%)

	3. Sometimes	3 (5.9%)	
X54. Do visitors wear gloves or wash hands?	1. Always 2. Never 3. Sometimes	14 (28.0%) 33 (66.0%) 3 (6.0%)	102 (67.11%)

G6

Variable	Statistic / Values	Freqs (% Valid)	Missing
X55. Is there an insect control plan on the farm?	1. No 2. Yes	30 (19.9%) 121 (80.1%)	1 (0.66%)
X56. Is there a rodent control plan on the farm? (other than the use of cats)	1. No 2. Yes	17 (11.2%) 135 (88.8%)	0 (0%)
X57. Is there a bird control plan on the farm?	1. No 2. Yes	147 (96.7%) 5 (3.3%)	0 (0%)
X58. Do animals go to pasture? (Including young animals)	1. No 2. Yes	125 (82.8%) 26 (17.2%)	1 (0.66%)
X59. Do animals have access to natural water sources, ponds, lakes on pastures?	1. No 2. Yes	31 (73.8%) 11 (26.2%)	110 (72.37%)
X60. Is there contact with animals from other farms?	1. No 2. Yes	136 (96.5%) 5 (3.5%)	11 (7.24%)
X61. Are there other cattle animals on the farm for other commercial purposes?	1. No 2. Yes	130 (89.7%) 15 (10.3%)	7 (4.61%)
X62. Can these animals come into contact with dairy cattle?	1. No 2. Yes	16 (64.0%) 9 (36.0%)	127 (83.55%)
X63. Do you keep other farm animals on the farm?	1. Not answered 2. Birds 3. Goats, Birds 4. None 5. Pigs 6. Pigs, Poultry 7. Sheep, Birds	1 (0.7%) 47 (30.9%) 1 (0.7%) 79 (52.0%) 7 (4.6%) 15 (9.9%) 2 (1.3%)	0 (0%)
X64. Do domestic animals have access to the stables?	1. Cats 2. Dogs and cats 3. None	31 (20.5%) 106 (70.2%) 14 (9.3%)	1 (0.66%)
X65. Are manure and slurry from other farms spread less than 500m from your farm and or pastures?	1. Do not know 2. No 3. Yes	5 (3.3%) 38 (25.0%) 109 (71.7%)	0 (0%)

G7

Variable	Statistic / Values	Freqs (% Valid)	Missing
X66. Are sick animals physically separated from healthy animals?	1. Always 2. Never 3. Sometimes	95 (62.9%) 33 (21.9%) 23 (15.2%)	1 (0.66%)
X67. Are there materials and equipment for sick animals in the infirmary? (Thermometer, gastric tube, etc)	1. No 2. Yes	25 (21.0%) 94 (79.0%)	33 (21.71%)
X68. Is the equipment cleaned and disinfected before a new animal enters the infirmary?	1. Clean 2. Clean and disinfected 3. No	40 (40.4%) 50 (50.5%) 9 (9.1%)	53 (34.87%)
X69. Is there total separation between animals in the infirmary?	1. No 2. Yes	58 (50.4%) 57 (49.6%)	37 (24.34%)
X70. Do workers put other boots before entering the infirmary?	1. Always 2. Never 3. Sometimes	13 (18.6%) 54 (77.1%) 3 (4.3%)	82 (53.95%)
X70. Do workers wear other clothes before entering the infirmary?	1. Always 2. Never 3. Sometimes	10 (14.5%) 57 (82.6%) 2 (2.9%)	83 (54.61%)
X70. Do workers wear gloves or wash hands after leaving the infirmary?	1. Always 2. Never 3. Sometimes	56 (73.7%) 19 (25.0%) 1 (1.3%)	76 (50%)
X71. Is the infirmary's material (bed) removed after an animal leaves?	1. No 2. Yes	17 (15.6%) 92 (84.4%)	43 (28.29%)
X72. Is the infirmary cleaned before a new animal is introduced?	1. Always 2. Never 3. Sometimes	91 (94.8%) 3 (3.1%) 2 (2.1%)	56 (36.84%)
X72. Is the infirmary disinfected before a new animal is introduced?	1. Always 2. Never 3. Sometimes	42 (60.9%) 15 (21.7%) 12 (17.4%)	83 (54.61%)
X72. Is the infirmary dried before a new animal is introduced?	1. Always 2. Never 3. Sometimes	43 (61.4%) 18 (25.7%) 9 (12.9%)	82 (53.95%)
X73. Do you deal with sick animals before or after dealing with healthy animals?	1. After 2. Before 3. In no specific order	90 (62.1%) 1 (0.7%) 54 (37.2%)	7 (4.61%)
X74. In case of a disease outbreak sick animals can be completely isolated from healthy animals?	1. Always 2. Never 3. Sometimes	80 (53.0%) 56 (37.1%) 15 (9.9%)	1 (0.66%)
X75. Do you keep an animal health record?	1. No 2. Yes	2 (1.3%) 149 (98.7%)	1 (0.66%)
X76. Do you have vaccination, treatment and hygiene protocols?	1. No 2. Yes	30 (19.9%) 121 (80.1%)	1 (0.66%)
X77. What happens to animals with disease? Slaughter.	1. Always 2. Sometimes	85 (88.5%) 11 (11.5%)	56 (36.84%)
X77. . What happens to animals with	1. Always	101 (91.8%)	42

disease? Segregation.	2. Sometimes	9 (8.2%)	(27.63%)
X77. What happens to animals with disease? Nothing.	1. Always 2. Sometimes	8 (66.7%) 4 (33.3%)	140 (92.11%)
X77. What happens to animals with disease? Late slaughter	1. Always 2. Sometimes	4 (36.4%) 7 (63.6%)	141 (92.76%)
X78. Are different needles used according to treatment or age group?	1. No 2. Yes	35 (23.2%) 116 (76.8%)	1 (0.66%)

G8

Variable	Statistic / Values	Freqs (% Valid)	Missing
X79. Are there maternity pens on the farm?	1. No 2. Yes	61 (40.1%) 91 (59.9%)	0 (0%)
X80. Is the maternity hospital used for sick animals or is it next to the sick animal park?	1. Always 2. Never 3. Sometimes	54 (58.1%) 32 (34.4%) 7 (7.5%)	59 (38.82%)
X81. Is there a complete separation of animals inside the maternity pen?	1. No 2. Yes	41 (51.9%) 38 (48.1%)	73 (48.03%)
X82. Before entering the maternity pen do workers put other boots?	1. Always 2. Never 3. Sometimes	9 (17.3%) 39 (75.0%) 4 (7.7%)	100 (65.79%)
X82. Before entering the maternity pen do workers put other clothes?	1. Always 2. Never 3. Sometimes	11 (21.6%) 36 (70.6%) 4 (7.8%)	101 (66.45%)
X82. Before entering the maternity pen do workers put gloves or wash hands?	1. Always 2. Never 3. Sometimes	42 (80.8%) 8 (15.4%) 2 (3.8%)	100 (65.79%)
X83. Is the maternity pen cleaned before other animal's introduction?	1. Always 2. Never 3. Sometimes	50 (92.6%) 2 (3.7%) 2 (3.7%)	98 (64.47%)
X83. Is the maternity pen disinfected before other animal's introduction?	1. Always 2. Never 3. Sometimes	26 (59.1%) 9 (20.4%) 9 (20.4%)	108 (71.05%)
X83. Is the maternity pen dried before other animal's introduction?	1. Always 2. Never 3. Sometimes	22 (59.5%) 10 (27.0%) 5 (13.5%)	115 (75.66%)
X84. When assisting calving are hands and material always cleaned and disinfected before and after delivery or miscarriage?	1. Clean 2. Clean and disinfected 3. No	20 (13.2%) 127 (83.5%) 5 (3.3%)	0 (0%)
X85. Are the cow's hind limbs always cleaned and disinfected after each birth or miscarriage?	1. Clean 2. Clean and disinfected 3. No	45 (29.6%) 13 (8.6%) 94 (61.8%)	0 (0%)
X86. When is the calf separated from its progenitor?	1. Another 2. As soon as it is seen	1 (0.7%) 26 (17.1%)	0 (0%)

	3. Continues with the parent to be breastfed 4. In the first hour after birth	6 (4.0%) 119 (78.3%)	
X87. After a miscarriage has occurred is the cow tested to detect the cause?	1. Always 2. Never 3. Sometimes	27 (17.8%) 74 (48.7%) 51 (33.6%)	0 (0%)
X88. After a delivery or a miscarriage where are the fetal membranes and tissues placed?	1. Another 2. Ingested by the cow / dog … 3. Placed on manure	73 (48.0%) 2 (1.3%) 77 (50.7%)	0 (0%)

G9

Variable	Statistic / Values	Freqs (% Valid)	Missing
X89. How many liters of colostrum are administered in the first 6 hours after delivery?	1. 0 2. 1 3. 1.5 4. 2 5. 2.5 6. 3 7. 4 8. 5 9. 6	1 (0.7%) 2 (1.3%) 4 (2.7%) 28 (18.8%) 1 (0.7%) 40 (26.9%) 70 (47.0%) 1 (0.7%) 2 (1.3%)	
X90. Does the colostrum come from the mother? (If she has enough colostrum)	1. No 2. Yes	5 (3.3%) 145 (96.7%)	2 (1.32%)
X91. Is the colostrum quality tested?	1. No 2. Yes	117 (78.0%) 33 (22.0%)	2 (1.32%)
X92. Is there frozen or artificial colostrum storage? (If the mother doesn't have enough colostrum or the colostrum's quality isn't enough)	1. Artificial 2. No 3. Yes, from other cows that belong to the farm	3 (2.0%) 71 (47.3%) 76 (50.7%)	2 (1.32%)
X93. Is colostrum left in the refrigerator between milking and its administration to the calf?	1. No 2. Yes	85 (58.2%) 61 (41.8%)	6 (3.95%)
X94.Is material used to administer colostrum cleaned and disinfected after each use?	1. Clean 2. Clean and disinfected 3. No	32 (21.5%) 116 (77.8%) 1 (0.7%)	3 (1.97%)
X95. Are calves placed on individual pens?	1. No 2. Yes	20 (13.3%) 130 (86.7%)	2 (1.32%)
X96. Is the individual pen's material removed after each use?	1. No 2. Yes	2 (1.5%) 130 (98.5%)	20 (13.16%)
X97. Is the individual pen cleaned before other animal's introduction?	1. Always 2. Sometimes	122 (94.6%) 7 (5.4%)	23 (15.13%)
X97. Is the individual pen disinfected before other animal's introduction?	1. Always 2. Never	68 (62.4%) 5 (4.6%)	43 (28.29%)

	3. Sometimes	36 (33.0%)	
X97. Is the individual pen dried before other animal's introduction?	1. Always 2. Never 3. Sometimes	61 (64.9%) 16 (17.0%) 17 (18.1%)	58 (38.16%)
X98. Is contact between calves in individual pens possible?	1. No 2. Yes	3 (2.3%) 126 (97.7%)	23 (15.13%)
X99. Does each calf have its own feeding bucket?	1. No 2. Yes	19 (13.1%) 126 (86.9%)	7 (4.61%)
X100. Are calves fed with discarded milk?	1. Always 2. Never 3. Sometimes	31 (20.9%) 81 (54.7%) 36 (24.3%)	4 (2.63%)
X101. Are feeding buckets cleaned after each use?	1. No 2. Yes	7 (4.9%) 136 (95.1%)	9 (5.92%)
X102. How many calves are grouped together in collective pens?	Mean (sd) : 7.3 (4.6) min < median < max: 2 < 6 < 30 IQE (CV) : 5 (0.6)	15 values	8 (5.26%)
X103. Is the collective pen's material removed after each use?	1. No 2. Yes	13 (8.7%) 136 (91.3%)	3 (1.97%)
X104. Are collective pens cleaned before other animal's introduction?	1. Always 2. Never 3. Sometimes	126 (89.4%) 3 (2.1%) 12 (8.5%)	11 (7.24%)
X104. Are collective pens disinfected before other animal's introduction?	1. Always 2. Never 3. Sometimes	55 (48.7%) 13 (11.5%) 45 (39.8%)	39 (25.66%)
X104. Are collective pens dried before other animal's introduction?	1. Always 2. Never 3. Sometimes	62 (59.1%) 21 (20.0%) 22 (20.9%)	47 (30.92%)

G10

Variable	Statistic / Values	Freqs (% Valid)	Missing
X105. Milking system used	1. Manual 2. Robot	141 (92.8%) 11 (7.2%)	0 (0%)
X106. How many times per year do you take a milking equipment's static measurement ?	1. > 2 2. 1 3. 2	29 (19.9%) 91 (62.3%) 26 (17.8%)	6 (3.95%)
X107. How many cows are being milked?	Mean (sd) : 77.9 (84.2) min < median < max: 14 < 58 < 820 IQE (CV) : 45.5 (1.1)	76 values	8 (5.26%)
X108. How often are you milking per day (manually)/What is the average number of milking per day (robot)	1. 2 2. 2.3 3. 2.4 4. 2.5 5. 2.6 6. 2.7 7. 2.8 8. 3	139 (92.0%) 1 (0.7%) 2 (1.3%) 1 (0.7%) 1 (0.7%) 1 (0.7%) 1 (0.7%) 4 (2.6%)	1 (0.66%)

	9. 3.2	1 (0.7%)	
X109. How many milking points do you have in the milking parlor?	Mean (sd) : 10 (6.8) min < median < max: 1 < 8 < 36 IQE (CV) : 10 (0.7)	20 values	7 (4.61%)
X110. Do you use rubber or silicone teats?	1. Rubber 2. Silicone	134 (88.2%) 18 (11.8%)	0 (0%)
X111. After how many months do you replace milking teats?	1. 1 2. 10 3. 10000 4. 12 5. 12000 milking 6. 1500 in 1500 hours 7. 6 in 6 months 8. 2 9. 3 10. 4 11. 6 12. 8 13. 9 14. 24 15. 8 in 8 months 16. 4 in 4 months 17. It depends of number of hours 18. 3000 in 3000 hours 19. 50 in 50 days	3 (2.2%) 4 (3.0%) 3 (2.2%) 38 (28.3%) 1 (0.7%) 1 (0.7%) 21 (15.6%) 4 (3.0%) 3 (2.2%) 3 (2.2%) 29 (21.6%) 8 (5.9%) 1 (0.7%) 3 (2.2%) 2 (1.4%) 1 (0.7%) 2 (1.4%) 1 (0.7%) 1 (0.7%)	17 (11.18%)
X112. Do you disinfect milking teats between cows?	1. No 2. Yes, among cows with high somatic cell counts 3. Yes, among mastitic cows 4. Yes, between cows	47 (30.9%) 1 (0.7%) 29 (19.1%) 75 (49.3%)	0 (0%)
X113. How do you disinfect the equipment?	1. Hot water 2. Other 3. Steam	126 (84.6%) 19 (12.8%) 4 (2.7%)	3 (1.97%)
X114. Are teats cleaned before milking?	1. Dried 2. No 3. Washed and dried 4. Washed, without being dried 5. With foam	5 (3.3%) 1 (0.7%) 92 (60.5%) 19 (12.5%) 35 (23.0%)	0 (0%)
X115. Are the first milk jets inspected?	1. No 2. Yes	35 (23.0%) 117 (77.0%)	0 (0%)
X116. Do you usually disinfect teats after removing the milking teats?	1. No 2. Yes, with a spray 3. Yes, with an immersion	7 (4.6%) 11 (7.2%) 134 (88.2%)	0 (0%)
X117. Do cows stay up after milking?	1. No 2. Yes, between half an hour and an hour 3. Yes, for longer	59 (38.8%) 87 (57.2%) 6 (4.0%)	0 (0%)
X118. Is there a specific order for milking?	1. No 2. Yes, animals with	34 (22.4%) 118 (77.6%)	0 (0%)

	mastitis and with high somatic cell counts last		
X119. Do you trim udder's hair?	1. No 2. Yes, once a year 3. Yes, twice a year or more	131 (86.2%) 5 (3.3%) 16 (10.5%)	0 (0%)
X120. Do you trim tails?	1. No 2. Yes, once a year 3. Yes, twice a year or more	34 (22.4%) 39 (25.7%) 79 (52.0%)	0 (0%)
X121. Is a milk bacteriological test made at least once a year to every lactating cow?	1. No 2. Yes	103 (68.7%) 47 (31.3%)	2 (1.32%)

G11

Variable	Statistic / Values	Freqs (% Valid)	Missing
X122. How often are adult animal facilities cleaned and disinfected/year?	**Cleaning:** Min-0 Average- 231 Max -1095 **Disinfection:** Min-0 Average- 86 Max -1095		
X123. Groups of animals present on the farm	1. Dry cows, Newborn calves 2. Dry cows, Newborn calves, Single group 3. Dry cows 4. Dry cows, High and low lactation 5. Dry cows, Newborn calves, First lactation 6. Dry cows, Newborn calves, First lactation, High and low lactation 7. Dry cows, Newborn calves, High and low lactation 8. Dry cows, Single group 9. High and low lactation 10. Single group	4 (2.6%) 3 (2.0%) 18 (11.8%) 9 (5.9%) 1 (0.7%) 4 (2.6%) 7 (4.6%) 55 (36.2%) 1 (0.7%) 50 (32.9%)	0 (0%)
X124. The footbath is applied with a specific frequency?	1. No 2. Yes	41 (27.2%) 110 (72.9%)	1 (0.66%)
X124.1. Does the footbath have its own outflow?	1. No 2. Yes	38 (29.9%) 89 (70.1%)	25 (16.45%)

G12

Variable	Statistic / Values	Freqs (% Valid)	Missing
X125. Are animals grouped by age?	1. No 2. Yes	8 (5.3%) 144 (94.7%)	
X126. Is there a complete separation between age groups?	1. No 2. Yes	29 (20.1%) 115 (79.9%)	8 (5.26%)
X127. Do workers change boots between age groups?	1. Always 2. Never 3. Sometimes	3 (2.3%) 124 (96.1%) 2 (1.6%)	23 (15.13%)
X127. Do workers change clothes between age groups?	1. Always 2. Never 3. Sometimes	2 (1.6%) 126 (97.7%) 1 (0.8%)	23 (15.13%)
X127. Do workers change gloves or wash hands between age groups?	1. Always 2. Never	18 (14.0%) 111 (86.1%)	23 (15.13%)
X128. Is there a work routine?	1. From the oldest to the youngest 2. From the youngest to the oldest 3. There is no specific order	45 (31.2%) 23 (16.0%) 76 (52.8%)	8 (5.26%)
X129. Is there equipment separation between animal groups?	1. No 2. Yes 3. Yes, but it is not easily recognizable	15 (10.3%) 122 (84.1%) 8 (5.5%)	7 (4.61%)
X130. Do you share material or equipment with other farms?	1. No 2. Yes	147 (96.7%) 5 (3.3%)	0 (0%)
X131. What steps do you take when the material enters the farm?	1. Cleaning 2. Cleaning and disinfection 3. Nothing	5 (50.0%) 1 (10.0%) 4 (40.0%)	142 (93.42%)

G13

Variable	Statistic / Values	Freqs (% Valid)	Missing
X132. Are antibiotics used on the farm?	1. No 2. Yes	1 (0.7%) 151 (99.3%)	0 (0%)
X133. Are there vaccination protocols on the farm?	1. No 2. Yes	32 (21.2%) 119 (78.8%)	1 (0.66%)
X134. Which diseases are mentioned on the vaccination protocol?	1. Bovine respiratory disease 2. BVD 3. BVD, Clostridiosis 4. BVD, Clostridiosis, Bovine respiratory disease 5. BVD, IBR 6. BVD, IBR, Bovine respiratory disease 7. BVD, IBR, Clostridiosis 8. BVD, IBR, Clostridiosis, Bovine respiratory disease 9. BVD, Neonatal Diarrhea, IBR	1 (0.8%) 1 (0.8%) 2 (1.7%) 1 (0.8%) 42 (35.0%) 6 (5.0%) 5 (4.2%) 8 (6.7%)	32 (21.05%)

	10. BVD, Neonatal Diarrhea, IBR, Bovine Respiratory Disease 11. IBR 12. BVD, Neonatal Diarrhea, IBR, Bovine Respiratory Disease, Clostridiosis 13. Neonatal Diarrhea 14. BVD, Neonatal Diarrhea, IBR, Clostridiosis 15. Clostridiosis 16. Clostridioses, Bovine Respiratory Disease 17. Bovine Respiratory Disease 18.IBR, Bovine Respiratory Disease	7 (5.8%) 13 (10.8%) 3 (2.5%) 14 (11.8) 5 (4.2%) 4 (3.4%) 5 (4.2%) 2 (1.7%) 1 (0.8%) 1 (0.8%)	
X135. Do you implement protocols for antibiotic's responsible use? (If do not go to 141)	1. No 2. Yes	80 (52.6%) 72 (47.4%)	0 (0%)
X136. Does the protocol have diseases into account?	1. No 2. Yes	4 (5.6%) 68 (94.4%)	80 (52.63%)
X137. Which diseases are mentioned in antibiotherapy the protocol?	1. Another 2. Clinical Mastitis 3. Clinical Mastitis, Placental retention, Uterine disease 4. Clinical Mastitis, Pneumonia 5. Clinical Mastitis, Pneumonia, Other 6. Clinical Mastitis, Pneumonia, Placental retention 7. Clinical Mastitis, Pneumonia, Placental Retention, Uterine disease 8. Clinical Mastitis, Pneumonia, Placental retention, Uterine disease, Foot disease 9. Clinical Mastitis, Pneumonia, Placental Retention, Uterine disease, Other 10. Clinical Mastitis, Pneumonia, Placental Retention, Other 11. Clinical Mastitis, Pneumonia, Foot disease, Uterine disease 12. Clinical Mastitis, Uterine disease 13. Pneumonia	1 (1.4%) 14 (20.3%) 1 (1.4%) 9 (13.0%) 1 (1.4%) 6 (8.7%) 7 (10.1%) 20 (29.0%) 2 (2.9%) 1 (1.4%) 3 (4.4%) 1 (1.4%) 1 (1.4%)	83 (54.61%)
X138. How often is this protocol reviewed?	1. > 12 months 2. 12 in 12 months 3. 3 in 3 months 4. 6 in 6 months	11 (18.0%) 22 (36.1%) 14 (22.9%) 14 (22.9%)	91 (59.87%)
X139. How often do you use antibiotic treatments different from the ones recommended by the veterinarian? (Other antibiotics or different doses)	1. > Once a month 2. 1 time in 3 months 3. 1 time in 6 months 4. 1 time per month 5. Never	1 (1.4%) 7 (9.6%) 36 (49.3%) 2 (2.7%) 27 (37.0%)	79 (51.97%)

X140. Which of these aspects are defined in the protocol? **Situations in which antibiotics should be used**	1. No 2. Yes	1 (1.5%) 66 (98.5%)	85 (55.92%)
X140. Which of these aspects are defined in the protocol? **The type of antibiotic to use**	1. Yes	71 (100.0%)	81 (53.29%)
X140. Which of these aspects are defined in the protocol? **Animal's age**	1. No 2. Yes	3 (4.3%) 67 (95.7%)	82 (53.95%)
X140. Which of these aspects are defined in the protocol? **The production phase**	1. No 2. Yes	2 (3.1%) 63 (96.9%)	87 (57.24%)
X140. Which of these aspects are defined in the protocol? **The dose to be used**	1. Yes	69 (100.0%)	83 (54.61%)
X140..Which of these aspects are defined in the protocol? **Disease severity**	1. No 2. Yes	2 (3.5%) 55 (96.5%)	95 (62.5%)
X140. Which of these aspects are defined in the protocol? **Number of treatments already performed**	1. No 2. Yes	3 (6.1%) 46 (93.9%)	103 (67.76%)
X141. How often do you use antibiotic sensitivity tests?	1. Few times 2. Never 3. Oftentimes 4. Sometimes	30 (20.1%) 27 (18.1%) 28 (18.8%) 64 (43.0%)	3 (1.97%)
X142. Do you have selective treatment? Treating only some cows with antibiotics	1. No 2. Yes	68 (44.7%) 84 (55.3%)	0 (0%)
X143. What criteria do you use for selective treatment?	1. Occurrence of mastitis during lactation 2. Occurrence of mastitis during lactation, Presence of clinical signs 3. Occurrence of mastitis during lactation, Presence of clinical signs, Decrease in milk yield 4. Occurrence of mastitis during lactation, Presence of clinical signs, Decrease in milk yield, Somatic cell count 5. Occurrence of mastitis during lactation, Presence of clinical signs, Decrease in milk yield, Antibiotic sensitivity test 6. Occurrence of mastitis during lactation, Presence of clinical signs, Decrease in milk yield, Somatic cell count, Antibiotic sensitivity test 7. Occurrence of mastitis during lactation, Presence of clinical signs, Somatic cell count 8. Occurrence of mastitis during	4 (4.7%) 22 (25.9%) 9 (10.6%) 9 (10.6%) 1 (1.2%) 4 (4.7%) 6 (7.1%) 3 (3.5%)	67 (44.08%)

	lactation, Presence of clinical signs, Antibiotic sensitivity test 9. Occurrence of mastitis during lactation, Presence of clinical signs, Antibiotic sensitivity test, Somatic cell count 10. Occurrence of mastitis during lactation, Somatic cell count 11. Occurrence of mastitis during lactation, Antibiotic sensitivity test 12. Somatic Cell count 13. Presence of clinical signs 14. Presence of clinical signs, Somatic cell count 15. Presence of clinical signs, Antibiotic sensitivity test 16. Presence of clinical signs, Decrease in milk yield	2 (2.4%) 4 (4.7%) 2 (2.4%) 2 (2.4%) 10 (11.9%) 1 (1.2%) 2 (2.4%) 1 (1.2%)	
X144. What tests do you use to detect mastitis?	1. Dairy contrast 2. Dairy contrast, Electrical conductivity 3. Clinical examination 4. Electrical conductivity 5. Electrical conductivity, Somatic cell reader 6. TCM 7. TCM, Dairy contrast 8. TCM, Dairy contrast, Electrical conductivity 9. TCM, Dairy Contrast, ZOETIS Test 10. TCM, Electrical conductivity	22 (15.1%) 1 (0.7%) 1 (0.7%) 3 (2.0%) 1 (0.7%) 53 (36.3%) 60 (41.1%) 3 (2.0%) 1 (0.7%) 1 (0.7%)	6 (3.95%)
X145. What diagnostic test do you use to detect the causative agent of mastitis?	1. BOVICONTROL 2. Culture 3. Nothing 4. SEGALAB LABORATORY 5. Zoetis OFC 6. Zoetis OFC, Culture	1 (0.8%) 121 (92.4%) 2 (1.5%) 1 (0.8%) 5 (3.8%) 1 (0.8%)	21 (13.82%)