UNIVERSIDADE DE LISBOA

FACULDADE DE MEDICINA VETERINÁRIA

IMPLEMENTATION OF PARATUBERCULOSIS CONTROL MEASURES IN AN ENDEMICALLY INFECTED DAIRY HERD IN IRELAND

MARIA RITA SANTOS HORTA ANTUNES LEITÃO

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RESUMO

Implementação de Medidas de Controlo da Paratuberculose num Efetivo Leiteiro Endemicamente Infetado na Irlanda

A doença de Johne, ou paratuberculose, é uma enterite crónica causada pelo *Mycobacterium avium paratuberculosis* (Map), endémica em vários países. Uma vez que esta doença tem um impacto económico significativo, foram estabelecidos programas de controlo voluntário para melhorar o estado da paratuberculose em vários países afectados, como a Irlanda. Este estudo tem como objetivo definir os aspectos responsáveis pela limitação do desempenho do Irish Johne's Control Programme (IJCP) através da análise da implementação deste programa, durante 3 anos, num efetivo leiteiro. A análise que decorreu neste projeto poderá contribuir para melhorar a compreensão e o desempenho dos programas de controlo voluntários.

A pesquisa foi realizada através da análise de cada requisito do programa cumprido pelo efetivo em questão. Esta análise foi efectuada para os 3 anos de participação do efetivo no IJCP, recorrendo à base de dados oficial do programa, a Irish Cattle Breeding Federation (ICBF), para recolher a informação necessária. Os dados recolhidos foram depois comparados de um ano para o outro para investigar a relação entre cada ano e elaborar uma explicação fundamentada para o resultado observado.

Os resultados indicam que a prevalência aparente da doença está a aumentar anualmente, de 3,37% em 2020 para 5,21% em 2021 e 11,46% em 2022. A análise dos resultados do Plano de Avaliação e Gestão dos Riscos Veterinários (VRAMP) indica várias questões, principalmente relacionadas com a falta de cumprimento por parte do proprietário do rebanho e dos trabalhadores agrícolas, que afectam as medidas de higiene e de gestão das explorações agrícolas cruciais para alcançar um desempenho ótimo do programa. O aumento da prevalência aparente também pode ser influenciado pelo facto de o efetivo só ter estado no IJCP durante 3 anos e este período de tempo pode não ser suficiente para observar mudanças significativas no desenvolvimento e prevalência da doença.

Através da análise e reflexão realizadas ao longo deste estudo, é possível identificar os factores que comprometem o desempenho do PCIJ, contribuindo para um melhor conhecimento e para uma melhor implementação de programas de controlo voluntários. Tem ainda o potencial de ter um impacto positivo na gestão da doença de Johne nos países onde esta está presente.

Palavras-chave: paratuberculose, programa de controlo, conformidade, prevalência, Irlanda

ABSTRACT

Implementation of Paratuberculosis Control Measures in an Endemically Infected Dairy Herd in Ireland

Johne´s disease, or paratuberculosis, is a chronic enteritis caused by *Mycobacterium avium paratuberculosis* (Map), endemic in several countries. Since this disease has a significant economic impact, voluntary control programmes were established to improve the paratuberculosis status in several affected countries, such as Ireland. This study aims to define the aspects responsible for limiting the Irish Johne's Control Programme (IJCP) performance through the analysis of this programme implementation, for 3 years, in a dairy herd. The analysis that took course in this project could offer valuable insights for better understanding and enhancing the performance of voluntary control programs.

The research was performed through the analysis of each requirement of the programme accomplished by the herd in question. This analysis was conducted for the 3 years of the herd's participation in IJCP, resorting to the official programme's database, the Irish Cattle Breeding Federation (ICBF), to gather the information required. The data collected was then compared from one year to another to investigate the relation between each year and elaborate a reasoned explanation for the observed outcome.

The results indicate that the disease's apparent prevalence is increasing yearly, from 3.37% in 2020, to 5.21% in 2021, and 11.46% in 2022. The analysis of the Veterinary Risk Assessment and Management Plan (VRAMP) results indicates several issues, mainly related to the lack of compliance from the herd owner and farm workers, that affect hygiene and farm management measures crucial to achieving optimum programme performance. The increase in the apparent prevalence could also be influenced by the fact that the herd has only been on the IJCP for 3 years and this time frame might not be enough to observe significant changes in the disease's development and prevalence.

Through the analysis and reflection conducted over the course of this study, it is possible to identify the factors that compromise the performance of the IJCP, contributing to a better understanding and enhancing the implementation of voluntary control programmes. It also has the potential to make a positive impact on the management of Johne's disease in countries where it is present.

Key-words: paratuberculosis, control programme, compliance, prevalence, Ireland

RESUMO ALARGADO

Implementação de Medidas de Controlo da Paratuberculose num Efetivo Leiteiro Endemicamente Infetado na Irlanda

A doença de Johne, ou paratuberculose, é uma enterite crónica provocada por *Mycobacterium avium paratuberculosis* (Map). A principal via de infeção é a ingestão de alimentos contaminados com fezes de animais infetados, no entanto, este agente também se pode transmitir verticalmente *in utero*, ser excretado no colostro e leite de vacas infetadas ou por ingestão de bioaressois contaminados. Os animais infetados apresentam geralmente diarreia crónica, perda de peso e diminuição da produção leiteira. Visto que a doença tem um impacto económico significativo, foram estabelecidos programas de controlo voluntários em vários países nos quais a doença é endémica, tais como a Irlanda, com o intuito de melhorar a sua situação epidemiológica. Este estudo teve como objetivo definir os principais aspectos responsáveis por limitar o desempenho do Programa de Controlo de Johne Irlandês (PCJI), através da análise da implementação deste programa, durante três anos, num efetivo de vacas leiteiras no qual a doença é endémica.

O trabalho foi realizado através da análise de cada requisito do programa de controlo cumprido pelo efetivo em estudo. O PCJI consiste num momento de Testagem de Todo o Rebanho (TTR) e na elaboração de um Plano Veterinário de Análise e Gestão de Risco (PVAGR), efetuados anualmente pelo Médico Veterinário Responsável (MVR). Na TTR, todos os animais com mais de 2 anos de idade são submetidos a um teste *Enzyme-Linked Immunosorbent Assay* (ELISA). Caso este seja positivo ou inconclusivo, é realizado um teste Polymerase Chain Reaction (PCR) fecal, para confirmação. Relativamente ao PVAGR, este consiste num questionário para posterior cálculo da pontuação de risco. No final do questionário, é acordado entre o dono da exploração e o MVR um máximo de três medidas que possam reduzir a probabilidade de transmissão da doença na exploração. A análise foi relativa aos 3 anos de participação do rebanho no PCJI e os dados foram recolhidos da base de dados ofical do programa, que integra o *website* da Federação Irlandesa de Criação de Bovinos (FICB). Foram recolhidos dados relativos aos questionários preenchidos para completar o PVAGR e aos resultados obtidos na TTR. Estes foram comparados entre os vários anos, de modo a investigar uma relação entre os mesmos, se os resultados melhorararam ou pioraram, de forma a elaborar uma justificação plausível para essa relação.

Os resultados da TTR, neste rebanho em particular, indicam, a cada ano, um aumento na prevalência aparente serológica da doença. Em 2020 este valor era 3,37%, em 2021 5,21%, e 11,46% em 2022. Também se observou um aumento no número de animais com paratuberculose, através de testes de PCR fecal. Relativamente à análise dos resultados do

PVAGR, foram identificados vários problemas, maioritariamente relacionados com a falta de colaboração por parte do dono do efetivo e dos trabalhadores da exploração, que afetam o cumprimento de medidas de higiene e maneio cruciais para atingir o melhor desempenho do programa.

O aumento da prevalência aparente pode estar relacionado com o facto de o período de estudo ser de apenas 3 anos, o que poderá não ser suficiente para observar alterações significativas na evolução da prevalência da doença no efetivo. Outros factores como a falta de informação relativamente a esta doença na exploração, previamente à implementação do programa de controlo, e a falta de dados do PVAGR, relativo ao ano de 2021, também podem ter um impacto negativo na qualidade desta análise e nos resultados obtidos. Não obstante, devido às falhas de colaboração identificadas e suprarreferidas, seria possível justificar que não haja uma progressão positiva no controlo da doença nesta exploração. Não retirar os vitelos às mães em menos de 30 minutos, não alimentar apenas com leite de substituição, não manter os vitelos em estábulos individuais e partilhar as áreas designadas para partos com vacas doentes, expôr vitelos e novilhas após o desmame a fezes de vacas e as condições de limpeza e higiene terem piorado ao longo dos anos, são algumas medidas identificadas neste estudo, consideradas fraturantes para uma evolução positiva do programa. Estas medidas resultam num aumento da pontuação do PVAGR, demonstrando também um aumento do risco de transmissão desta doença na exploração. Outras recomendações do programa que não estão a ser cumpridas, tais como a medida de “testagem e abate”, manter o rebanho fechado ou introduzir apenas animais provenientes de explorações em que se conheça o estatuto da doença, podem ser colmatadas pelo uso de sémen sexado em primíparas, em combinação com o uso de sémen de raças de carne em vacas mais velhas, diminuindo a descendência com maior risco de estar infetada e transmitir a doença.

Em síntese, face às limitações do estudo e à complexidade dos vários fatores envolvidos na transmissão e contenção da doença, é difícil definir com certeza qual o fator responsável pelo aumento dos casos de paratuberculose neste efetivo. No entanto, sendo que a colaboração do dono da exploração e dos seus trabalhadores tem um impacto significativo em todos os fatores relacionados com o maneio da exploração que poderão estar a afetar o desempenho deste efetivo no PCJI, se este aspeto fosse melhorado seria possível melhorar a contenção da doença nesta exploração. Para este propósito, seria crucial que as recomendações e sugestões comunicadas pelo MVR fossem cumpridas. Com o intuito de colmatar este problema, surge a possibilidade de atribuir incentivos pela implementação de medidas que integrem o programa de controlo. Ultrapassando esta limitação, os resultados da implementação do PCJI irão melhorar e a exploração encaminhar-se-á para um futuro promissor com a redução da prevalência e controlo da doença de Johne.

Este estudo representa um importante e útil contributo para uma melhor compreensão e melhoramento da implementação do PCJI. Além disso, tem potencial para registar um impacto positivo na gestão e controlo da doença de Johne em outros países nos quais a doença esteja presente.

Palavras-chave: paratuberculose, programa de controlo, conformidade, prevalência, Irlanda

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LIST OF ABREVIATIONS AND SYMBOLS

AGID – Agar Gel Immunodiffusion

AHI – Animal Health Ireland

AVP – Approved Veterinary Practitioner

BTM – Bulk Tank Milk

BVD – Bovine Viral Diarrhoea

C – Cattle strain of *Mycobacterium avium* subsepecies *paratuberculosis*

DAFM – Department of Agriculture, Food and the Marine

ELISA – *Enzyme-Linked Immunosorbent Assay*

HTST – High Temperature Short Time

ICBF – Irish Cattle Breeding Federation

IG – Implementation Group

IgG – Immunoglobulin G

IJCP – Irish Johne's Control Programme

JD – Johne's Disease

LRs- Likelihood Ratios

MAP – *Mycobacterium avium* subspecies *paratuberculosis*

PCR – Polymerase Chain Reaction

SNPs – Single Nucleotide Polymorphisms

S – Sheep strain of *Mycobacterium avium* subsepecies *paratuberculosis*

TASAH – Targeted Advisory Service on Animal Health

TWG – Technical Working Group

WHT – Whole Herd Test

VRAMP – Veterinary Risk Assessment and Management Plan

1. Externships During the 6th Academic Year (2022/2023)

1.1. Vettotal ambulatory clinic (São Teotónio, Portugal)

The curricular externship took place at Vettotal, in São Teotónio, Odemira, Portugal, from September 19th until December 17th of 2022. During this period, I have shadowed Doctor Nuno Raposo in his day-to-day clinic activities with several farm animal species, such as bovine, caprine, ovine and swine.

From this experience, it is relevant to highlight some procedures in reproduction and obstetrics, such as an andrologic exam in bovine, with the supervision and support of Dr Rui Silva and several parturitions. The distocic parturitions observed and assisted were caused by foetal-maternal disproportion and bad foetal presentation and were solved by manual traction (observed in bovine and ovine) and mechanical (observed in bovine). Manual traction was also used in ovine and caprine with foetal death and mechanical traction in bovine cases of foetal death. I observed several uterine and vaginal prolapses, in both ovine and bovine. In bovines, I had the opportunity to assist in providing neonatal care, most frequently in cases of neonatal diarrhoea, through the administration of intravenous and oral fluids. I also observed some cases of clinic mastitis and pneumonia in both bovine and ovine.

During this trainship, I also observed several pathologies affecting the species mentioned above. In swine, the most observed was swine erysipelas and in bovine the ruminal atony due to indigestion, most frequently caused by intoxication, and pneumonia. In ovine, the pathology most observed was secondary and systemic infection due to retained foetal membranes.

In parallel with the clinical observation, I was able to assist Dr Nuno Raposo and Dr Rui Silva in activities related to herd health management, within the scope of a sanitary campaign proposed by the local association responsible for these procedures (Agrupamento de Defesa Sanitária do Litoral Alentejano).

1.2. Ruminant referral hospital – Vetsuisse-Fakultät, Universität Bern (Bern, Switzerlad)

From August 2nd to 30th of 2022, I enrolled on a 4-week externship programme in the Veterinary Medicine Faculty of Bern's University, in Bern, Switzerland. It consisted of assisting the day-to-day clinic activity, following closely the cases that were assigned to me. With each case, I had the opportunity to present it daily in the morning rounds, perform its admission exam and the daily physical exams, and also administer the necessary medication during its hospitalisation period. I also assisted in the execution of complementary exams (haematology, imagiology – X-ray and computerized tomography scan) and I was able to participate in the surgeries as an assistant surgeon.

In bovine, I followed a wound in the metacarpus, to which a drain was applied, and a wound in a claw, having participated in several bandage changes and wound cleaning procedures. I also followed several cases that required surgical intervention where I participated as an assistant surgeon: an abomasum left displacement with omentopexy, a cecum displacement and retroflexion with cecostomy and a fracture of the mandibular ramus with the application of an external fixator. I was also able to assist the anesthesiology team in a marsupialisation surgery of a calve with omphalophlebitis, a case that I was following. Additionally, I observed several other cases that were submitted to surgery, for instance, left and right abomasum displacements, teat amputations, a sigmoid flexure displacement and a claw amputation.

Regarding New World camelids, I followed a llama with anaemia and assisted its blood transfusion, having accompanied its cria during the hospitalisation period, feeding and controlling its weight gain. I performed an alpaca admission exam, that was later diagnosed with an ulcer in the gastric compartment C3 that ruptured causing peritonitis, I assisted in the attempt to stabilise it but it had to be euthanised. I also observed other cases, such as an alpaca with rectal prolapse, an infection of a cesarian section suture in an alpaca and dental extraction of a molar in a llama.

Regarding ovine, I only followed a case of urolithiasis with surgical intervention through a urostomy and the application of a Foley catheter. I also observed a tail amputation on a lamb.

In this externship, I also participated in several procedures that were part of the hospitalised patient's routine, such as passive milking, bandage changes and wound-cleaning procedures.

1.3. Bluestack Veterinary Clinic – mixed practice clinic (Donegal, Ireland)

The ERASMUS scholarship externship took place at Bluestack Veterinary Clinic, a mixed practise clinic in Donegal, Ireland, from February 15 to 20th of April of 2023. During this experience, I was able to follow and assist consults, surgeries and hospitalised animals at the small animal clinic, to shadow the large animal veterinary John Mcaloon and the equine veterinary Kathryn Mcaloon.

In small animals, I followed several cases, such as osteosarcoma, a dog with leukaemia, a tail amputation and exploratory enterotomy with foreign body removal in dogs. I observed first consults, assisting in the vaccination programme and identification with microchip placement and consults of dogs with acute diarrhoea, vomiting and seizures. I also monitored and followed hospitalised animals, having observed cases of acute exacerbation of chronic disease in a dog, a cat with acute kidney disease, an intoxicated dog, possibly with dicumarinics and a pyometra in a female dog.

In bovine, I followed reproductive and obstetric procedures, such as the application of progesterone intravaginal devices, ultrasound pregnancy diagnosis, uterine prolapses and distocic parturitions, caused by foetal-maternal disproportion and bad foetal presentation. These cases of distocic parturitions were solved by manual traction and mechanical. I also observed several cesarian section surgeries and cases of toxic puerperal metritis. In bovine, I also assisted cases of mild and severe clinical mastitis and milk fever. In young bulls, I observed cases of polioencephalomalacia induced by thiamine deficiency and a case of listeriosis caused by the ingestion of poor-quality sillage. In calves, I assisted cases of neonatal diarrhoea with intravenous fluids and medication administration, pneumonia cases and a fractured front limb with consequent bandaging and splinting.

In ovine, similar to bovine, I assisted distocic parturitions caused by foetal-maternal disproportion and situations of foetal death solved by manual traction. I also observed and assisted several cesarian section, pneumonia cases, a case of a fractured front leg in a lamb and a wound with a pen rose drain application, also in a lamb.

In horses, I observed several horse castrations, standing up and laying down, assisted in mesotherapy treatment for back pain and several X-ray exams of limbs, head and spine, and ultrasound exams of tendons and ligaments. I also observed ultrasound exams for pregnancy diagnosis in mares and several pre-purchase examinations.

2. Introduction

Johne's disease, or paratuberculosis, is a chronic enteritis caused by *Mycobacterium avium paratuberculosis* (Map) (Stevenson 2015). The main route of infection is through oral uptake via ingestion of materials contaminated with faeces of infected animals (Harris and Barletta 2001), however, it can also be transmitted vertically in utero (Whittington and Windsor 2009), excreted in colostrum and milk from infected dams (Constable et al. 2016) and through contaminated bioaerosols (Eisenberg et al. 2012). The infected animals are commonly presented with chronic diarrhoea, weight loss and decreased milk production (Whitlock and Buergelt 1996). Since the disease has a significant economic impact (Ott et al. 1999), voluntary control programmes have been established in several countries in which the disease is endemic (Geraghty et al. 2014). Based on reviews of other countries which have already established control programmes, more countries affected by Johne's disease are implementing voluntary Johne's control programmes, such as Ireland (Gavey et al. 2021).

Since the implementation of the Irish Johne's Control Programme (IJCP), research has been carried out regarding the efficiency of its surveillance methods (Sergeant et al. 2019) and testing strategies (Meyer et al. 2019). Furthermore, recommendations for enhancing the programme were published (Jordan et al. 2020; Gavey et al. 2021). However, an efficient method of measuring the progress in achieving the programme's objectives has not yet been established (Gavey et al. 2021).

This study focuses on the analysis of the implementation of a voluntary Johne's control programme in Ireland, in an endemically infected dairy herd, and it aims to contribute to a better understanding of the impact of the control programme and the challenges along its implementation. This analysis will contribute to identifying the factors potentially responsible for limiting the programme's results, and present solutions, in order to facilitate the improvement of the programme's performance. The analysis will be performed through the evaluation of each requirement of the programme accomplished by the herd in question. This evaluation was performed along the 3 years of the herd's participation in the IJCP and then compared from one year to another, to investigate the relation between each year, if the situation has improved or worsened, and elaborate a justification for that outcome.

Despite the value and utility of this study, some limitations were identified. For instance, the fact that the herd has only been on the IJCP for 3 years might have an impact on the results obtained, since this time frame might not be enough to observe significant changes in the disease's development and prevalence (Meylan et al. 2021). Additionally, the fact that there is no information regarding the disease's status in the herd prior to enrolling in the programme, and there is also a lack of data regarding one of the programme’s requirements in 2021. Nevertheless, this study allows a useful reflection on the subject.

This study is organised in three main sections. The first section contains a review of the existent literature regarding Johne’s disease, its epidemiological status in Ireland and the control programme implemented in this country. The following section mentions the materials and methods employed to conduct the study, and the obtained results. The final section addresses the discussion of the results and culminates in a conclusion of this study.

3. Literature review

3.1. Johne's Disease

3.1.1. Aetiology

Johne's disease, or paratuberculosis, is a chronic progressive granulomatous enteritis caused by *Mycobacterium avium* subspecies *paratuberculosis* (Map) (Stevenson 2015). *Mycobacterium avium* subspecies *paratuberculosis* is an aerobic microorganism and it is one of the subspecies composing the *M. avium* Complex (Biet et al. 2012). Even though Map is taxonomically described as an obligate pathogen for animals (Thorel et al. 1990), it can survive up to 55 weeks in a dry fully shaded environment (Whittington et al. 2004).

Mycobacterium avium subspecies *paratuberculosis* was divided into two main groups according to the species in which each strain was first isolated: the cattle group (C) and the sheep group (S) (Collins et al. 1990). The S-type is more difficult to isolate and has an extremely slow growth rate in laboratory culture, and the C-type is considered a faster-growing type, and even though each strain is associated with specific species, they are not species-specific, and they can affect all types of ruminants (Biet et al. 2012). Two more groups were discovered, one of them is termed "intermediate" or "Type III" and is believed to be a subtype of the S-type Map and the other group is termed "Bison" or "B-type". The S-type, also designated as Type I, was proved to have a host preference for sheep and to be more virulent in this species, while, the C-type also identified as Type-II is more commonly found in cattle and other species (Stevenson 2015).

3.1.2. Pathogenesis

Following the oral ingestion of Map, the tonsil was considered by Payne and Rankin (1961) a common portal of entry for the agent, that would spread to the suprapharyngeal lymph node, presenting lesions on both sites at a later stage of the disease, similar to the "primary complex" in tuberculosis. Since the dose of Map used in this study was abnormally large, this could have affected the natural response of the animal's immune system and condition the results. Later, Gilmour et al. (1965) used a smaller dose of the agent and demonstrated that the primary portal of entry would be the small intestine. This study also showed that the agent would then spread to the mesenteric lymph nodes and disseminate to other extra-intestinal sites later on, through blood or lymph. A study (Sweeney et al. 2006) confirmed this hypothesis. It has also been shown that the instillation of Map directly into the tonsillar crypts causes infection (Waters et al. 2003). The translocation of the agent through the intestinal epithelium was experimentally proved (Momotani et al. 1988) to be facilitated by M cells, specialised epithelial cells found in the ileum's Peyer's patches. The M cells take up Map and release it on the submucosa layer, where the macrophages phagocyte the agent. Since Peyer's patches can also be found throughout the jejunum, the culture of multiple sites confirmed the presence of the agent in jejunal and ileal sites.

A study performed by Sweeney et al. (2006) determined that the most sensitive mean for early detection of paratuberculosis infection, 3 weeks after inoculating the agent, was the sampling of multiple sites of jejunum and ileum.

After being exposed to the agent, animals could be divided into three main groups, according to the host-bacteria interaction. The first group can be defined as infected-resistant animals. They rapidly develop resistance to the infection and do not become shedders. The second group includes both animals capable of partially controlling the infection, shedding the agent intermittently, and animals that become intermediate cases, incubating the disease to become heavy shedders later on. The last group consists in animals that are most likely to develop clinical signs since the agent persists in their intestinal mucosa (Constable et al. 2016).

3.1.3. Transmission

It is consensual that the main method of infection of Johne's disease is through oral uptake of Map by susceptible animals, either via ingestion of contaminated milk, water and other feed, or environmental products by the manure of infected animals (Harris and Barletta 2001). Since the incubation period of the disease is normally long, infected animals may excrete Map in their faeces for 15 to 18 months before clinical signs appear, making it harder to prevent new infections (Constable et al. 2016). It was also observed that a cow residing in a heavily Map-contaminated environment could excrete the agent in its faeces without contracting the disease, becoming a "pass-through shedder" (Sweeney et al. 2012).

Contaminated colostrum and milk are the main sources of infection in newborn calves, the most vulnerable age group. The introduction of the agent in milk or colostrum can occur, not only through contaminated teats, but also directly through the excretion of Map in the milk or colostrum. This last option can occur during the late dissemination stage of the infection, either in cows or other species. According to Constable et al. 2016, "Up to 45% of clinically affected cows may excrete the organism in milk, which was isolated from 36% of colostrum samples from heavy shedders and 9% of samples from light shedders."

Another alternative route of infection detected on dairy farms is via contaminated bioaerosols, through its ingestion after inhalation or ingestion by licking and suckling. When considering the inhalation of these particles through the respiratory tract, only a small amount of dust can be taken, nonetheless, when there is continuous exposure, it is possible to reach a concentration of agent capable of causing infection (Eisenberg et al. 2012).

In utero infection is also established as a plausible method of transmission of Map in cattle. Data in a dairy herd has shown that about 9% of foetuses from subclinically infected cows and 39% from clinically affected cows were infected with Map (Whittington and Windsor 2009). Even though transmission of this organism via trophoblast in an animal considered a moderate shedder is unlikely before the development of cotyledons (placentation) and the epitheliochorial placenta is hypothesized to be impermeable to Map from 42 to 49 days post insemination, that could change after 60 days. Considering this, it is unlikely that transferring embryos from infected to uninfected dams could be responsible for the transmission of this agent. Another unlikely route of transmission would be through semen since the isolation of Map in bulls' and rams' semen is unusual and represented by anedoctal reports (Constable et al. 2016).

Concerning the spread of Map from farm to farm, it is usually due to the trading of livestock, mostly unknown infected animals, either shedding or during the incubation period. It could also happen through the lateral spread of faeces across boundary fences, or the exchange of manure between farms. There are some vectors of Map studied, such as the nymphs of the Oriental cockroach (*Blatta orientalis*) and earthworms and adult forms of Diptera on cattle farms, where the disease is already established. Other parasites such as ovine trichostrongylid larvae (*Haemonchus contortus*, *Ostertagia circumcincta*, and *Trichostrongylus colubriformis*) may also be important in the transmission of this organism. There is also evidence that Map can survive in amitraz-based dip fluid for up to 2 weeks, suggesting that dips could be important in the transmission of the disease, mainly if a cow recently dipped, with residual dip fluid covering the udder, is suckling calves (Constable et al. 2016).

3.1.4. Risk Factors

3.1.4.1. Animal Risk Factors

It is well established in the past by some authors (Deans Rankin 1961; Whitlock et al. 1996; Mortier et al. 2013) that resistance to infection with Map increases with age. Most studies have observed that between 4 months and 1 year of age, it becomes more difficult for cattle to get infected, and that by 1 year of age, the susceptibility of an animal is similar to an adult. The exact mechanism behind the increased susceptibility of neonatal calves comparatively to adults is yet to be defined. Nonetheless, it is hypothesized that the "open" intestinal barrier during the first 24 hours of life may allow, not only the absorption of beneficial macromolecules such as immunoglobins, but also the absorption of Map. Other hypotheses could be the immaturity of the innate or adaptive immune response in young calves, or even the previous exposure of adults who did not get infected but developed acquired immunity (Sweeney 2011). Some studies (Deans Rankin 1961; Mortier et al. 2013) have also observed that adult cattle resistance to Map infection is most likely related to containment or elimination of the organism once it penetrates the intestinal mucosa rather than a failure of Map to enter the tissue. Another factor to consider besides age at exposure is the quantity of agent ingested. Animals exposed to high doses of Map will become more susceptible to infection and are expected to have a shorter incubation period, than the ones ingesting lower doses. Apart from age-related resistance, cattle infected at an older age are more likely to leave the herd due to culling, either for production-related problems or other diseases, before even exhibiting clinical signs of the disease, due to its long incubation period (Sweeney 2011).

Different breeds and different genetics may also affect an animal's susceptibility to Map infection and the incidence of the disease (Constable et al. 2016). The heritability of cattle's susceptibility to Johne's disease was estimated to range from 1 to 18% with the majority of estimates between 9 and 12%. This genetic effect was quantified at the sire level using the daughter's phenotype. There have also been similar heritability estimates reported in sheep (Sweeney et al. 2012). Another recent study (Mallikarjunappa et al. 2020) intended to validate the multiple Single Nucleotide Polymorphisms (SNPs) that were previously demonstrated to be associated with Map infection. This study was successful, which means, even though it is still necessary to explore this field and validate more SNPs, genetic selection and breeding towards Johne's disease resistance in cattle, could be an alternative option to control the disease (Mallikarjunappa et al. 2020).

Other factors that seem to affect cattle susceptibility to Map infection, but are not well documented yet, include the amount of dietary iron intake, stress and infection with agents that might compromise the immune system, such as Bovine Viral Diarrhoea (BVD) virus. Stress due to parturition, transportation and nutritional deficiencies or excess might influence the development of clinical disease, as detected in field observations. Additionally, the agent survives during long periods in protected sites, leaving the housed animals at a higher risk of infection due to the heavy contamination by faeces (Constable et al. 2016).

Herd characteristics also affect the course of the disease. This was observed using a computer simulation model of paratuberculosis in dairy cattle that were operating with seven variables at an initial stage: herd size, annual herd birth rate and annual replacement date, number of infected cows at time zero, number of herd replacements purchased each year, risk of purchasing an infected heifer and number of effective cow-calf contacts per year (Collins and Morgan 1991). Age-specific culling rates are also important in the development of the model. Even though all the factors affect the spread of the disease in herds, the model is most sensitive to the effective cow-calf contacts per year, which is consistent with the results of other infectious disease models and with the main recommendation on Map control (Collins and Morgan 1991). The prevalence of infection in purchased cattle directly affects the factor "risk of purchasing an infected heifer" and the rate of herd infection in the model. Acquiring a large number of replacement heifers from herds with high infection rates will quickly result in the infection of a herd. The rate at which infected cattle leave the herd was an important factor in the development of the disease because if an infectious cow remained in the herd several years, it contributed to a new generation of infected calves and also to a rise in the number of infected herd replacements. The model also showed a continued increase in the prevalence of the disease in infected herds, until it reached a plateau generally at 40 to 60% of the herd, suggesting that the infection is spreading quickly in dairy cattle (Collins and Morgan 1991).

3.1.4.2. Environmental Risk Factors

Some management factors were identified as important in the prevalence of infection, based on observations in dairy herds, such as the management of newborn calves and growing calves, heifer breeding, environmental conditions, and manure management.

As mentioned before, the most important route of Map transmission is the oral uptake of infected faecal material, and the most susceptible age group are neonatal calves. Therefore, according to Sweeney et al. (2012), some risk factors for this susceptible group related to the environment would be the sanitary conditions of the maternity pen or calving area, the contamination of colostrum with manure and the contact of the calf with infected cattle, which means, calves should be isolated or removed from the dam before making attempts to stand up and calves should not contact with other adult animals in the calving area as they might be infected. According to Sweeney (2011), the susceptibility to infection with Map remains higher for calves up to 1 year of age than for adults. In this age range the most concerning environmental risk factors would be feeding whole milk, feed or water contaminated with the agent and contact with infected adult's manure (Sweeney et al. 2012).

The spread of manure to grazing pasture, wildlife access to feed supplies, and the number of crows and lagomorphs, since they might be infected and contaminate food and water, are some of the other environmental risk factors to be considered, found in a survey of farms in Scotland (Constable et al. 2016).

There is evidence that the prevalence of Johne's disease is related to soil acidification, excesses of iron and molybdenum and deficiencies in copper and selenium. The soil pH may also influence the severity of the clinical signs, with herds raised in alkaline soil having less severe clinical disease, despite the high incidence of infection. Furthermore, Map's survival could also be enriched by silt or sand content in loamy soils. This means the soil type is a risk factor that should be considered (Constable et al. 2016).

Since the faecal-oral route is the most common route of infection, incorrect handling of infected manure on a farm is an important environmental risk factor. A study in Minnesota dairy farms (Raizman et al. 2004) analysed the faecal pool prevalence of the agent in the environment, in both herds testing positive and negative for paratuberculosis. This study concluded that it is possible to use targeted samples of cow alleyways and manure storage areas as an alternative method for herd screening, assessing Johne's disease infection status and herd faecal prevalence. In the referred study, 78% of the environmental samples from positive herds were culture- positive, 7% of the negative herds had one positive faecal pool, and one herd had one positive environmental sample. Environmental samples were culture-positive in cow alleyways (77% of the herds), manure storage (68%), calving area (21%), sick cow pen (18%), water runoff (6%) and post-weaned calves' area (3%). It was also observed that herds with both culture-negative environmental samples had an estimate of 0.3 to 4% faecal pool prevalence, on the contrary, herds with high environmental bacteria growth had an estimate of 53 to 73% faecal pool prevalence (Raizman et al. 2004).

3.1.4.3. Pathogen Risk Factors

Pathogenic characteristics of Map also represent risk factors for cattle infected with Johne's disease, for example, the dormancy-related genes discovered in a study (Whittington et al. 2004). As previously mentioned, the agent is known to be an obligate pathogen for animals, nonetheless, it is also known to survive for long periods in the environment, which means that removing all infected animals to eradicate the disease is not a viable option. Considering this, and the protective effect of a shaded environment on the survival of the agent, a study conducted in Australia has suggested that selective grazing with non-susceptible hosts or mechanical slashing might be a good alternative to accelerate the decontamination of pasture, by maintaining low levels of shade at the soil surface (Whittington et al. 2004).

Another intrinsic characteristic of Map that is known to be disadvantageous in the elimination of the agent is its thermal resistance. This *Mycobacterium* is proved to be more thermoresistant than *Mycobacterium bovis* (Ayele et al. 2005). A study comparing two standard heat treatments, the low temperature, 63ºC for 30 minutes, and the high-temperature short time (HTST), 72ºC for 15 seconds, has shown that even though most of the organisms were destroyed with both methods, with the low-temperature protocol 5 to 9% of the organisms were still viable after treatment, whereas with HTST pasteurisation only 3 to 5% survived. Another study, referring to colostrum, concluded that pasteurisation of 30 litre batches, in a commercial dairy farm, at 63ºC for 30 minutes, should be enough to eliminate MAP in most situations without compromising the Immunoglobulin G (IgG) integrity in passive immunity. Nevertheless, the authors recommend further research into this subject (Godden et al. 2006).

3.1.5. Clinical Findings and Diagnosis

In cattle, there are four documented stages of Johne's disease. This disease creates an "iceberg effect", since for every clinical case in an advanced stage it is expected that 25 other animals in the herd are infected (Whitlock and Buergelt 1996).

Stage one of the disease is known as "silent" infection and it is usually the stage in which young livestock are up to 2-year-old, such as calves and heifers. These individuals seem similar to non-infected animals, they do not present clinical signs nor variations in their outward appearance, body weight gain and growth. Both routine and clinical pathology tests fail to identify the infection, however, the animal might be shedding the agent into the environment. The only reliable method of detecting infection at this stage is by culture or histologic demonstration of Map in tissues (Whitlock and Buergelt 1996).

Stage two, also known as subclinical disease, mostly occurs in adult animals that present no clinical signs, even though, they may be prone to other disorders such as infertility or mastitis. Most of these cases will test negative on faecal culture, nonetheless, they might still shed the agent to the environment in small amounts, presenting a risk to the herd. Only 15 to 25% of these cases will test positive on faecal culture and most of the infected animals at this stage are undetectable by any method. It is suggested that infected individuals in this stage will evolve for stage three, even though, most cases are culled from the herd before that, for other reasons (Whitlock and Buergelt 1996).

Stage three or clinical disease corresponds to initial clinical signs after a long incubation period of 2 to 10 years. The clinical signs may include gradual weight loss with normal or increased appetite, diarrhoea that can be intermittent, with periods of normal consistency, or continuous, increased water consumption, and normal vital signs (heart and respiratory rate and temperature) (Whitlock and Buergelt 1996). There can also be a decrease in milk production, which often occurs before the diarrhoea commences (Constable et al. 2016). This stage usually lasts 3 to 4 months until the disease progresses to stage four. Some rare cases regress to the second stage of the disease, other rare cases could suddenly present persistent loose manure or a watery scour. Commonly, at this stage, there is no tenesmus and, despite the loose consistency, the faeces appear to be normal, with no signs of blood or excess mucus (Whitlock and Buergelt 1996). The diarrhoea tends to improve in late pregnancy and then reappear in a severe form, after parturition. It can also improve temporarily when the animals are removed from the pasture and start eating dryer food (Constable et al. 2016). The faecal cultures are usually positive for paratuberculosis and the majority of the individuals with clinical disease have increased antibody detection when using commercial *Enzyme-Linked*

Immunosorbent Assay (ELISA) and Agar Gel Immunodiffusion (AGID) tests (Whitlock and Buergelt 1996).

Stage four, alias advanced clinical disease, is characterised by the progression of the disease with aggravation of clinical signs. Animals are progressively more lethargic, emaciated and cachectic. The diarrhoea also aggravates and is described as "water hose" or "pipe stream" diarrhoea, leading to hypoproteinemia, which causes intermandibular oedema. Both clinical signs indicate a terminal stage of paratuberculosis and when they occur, the animal's condition can decay significantly, within a few days. Animals in this stage often die from dehydration and cachexia, if not, they can be sent for slaughter for salvage value and may not pass inspection for human consumption (Whitlock and Buergelt 1996).

There are several methods available for paratuberculosis diagnosis. When using faecal samples, one option is bacterial culture. This method is advantageous since a positive result indicates the presence of viable Map and it also allows to identify the strain of the organism for molecular epidemiologic purposes. It is also possible to know the relative amount of agent that is being excreted by the animal and therefore evaluate its transmission risk (Sweeney et al. 2012). This method has an estimated sensitivity of 60% and a specificity of 99.9%, having necropsy has a reference with 100% sensitivity and specificity (Collins et al. 2006). The faecal samples could be collected from individual animals, pooled samples, or environmental samples. This method requires a long incubation period, which could be considered a disadvantage when compared to other methods (Sweeney et al. 2012). Another option regarding faecal samples is Map DNA detection. Compared with culture, this method is quicker, and even though the sensitivity and specificity can vary, the values are usually similar to the ones estimated with the faecal culture method. This method uses real-time polymerase chain reaction (PCR) and it also allows to estimate the relative amount of agent that is being excreted, and according to Aly et al. (2010), the results have a significant association with the ones obtained with the culture method. This method does not allow strain identification nor does it confirm the presence of viable Map (Sweeney et al. 2012). "Microscopic examination of Ziehl-Neelsen stained faecal smears for the presence of clumps of acid-fast Map organisms" (Weber et al. 2009) is another method available when using faecal samples. It can be used as an alternative to faecal culture since the results are available within an hour (Ris et al. 1988). Nevertheless, the estimated sensitivity of this test is 49.3% (Zimmer et al. 1999) and the specificity is 82.7% (Ris et al. 1988), meaning that, from all the referred options, this test might not be the most reliable (Ris et al. 1988).

Another method, studied by Amemori et al. (2004) mentions the use of tissue from the jejunum, ileum, adjacent lymph nodes, ileocecal valve and cecal lymph nodes collected through laparoscopy and biopsy as a useful technique to detect the organism, via culture or PCR in dubious cases, in early stages of the disease. It is estimated to have a sensitivity of 90% and a specificity of 100% (Collins et al. 2006).

Detecting antibodies in serum and milk is a commonly used method since it is more economical than others and quicker than culture (Sweeney et al. 2012). The sensitivity of this method varies and is limited by factors such as the dose and age of exposure to Map, which could influence the antibody production, and by the possibility of faecal shedding being prior to seropositivity (Sweeney et al. 2006). According to Collins et al. (2006), the estimated sensitivity of serum or milk ELISA is 30%, having necropsy as a reference. Normally, ELISA is interpreted as a positive or negative result, but more recently, the application of likelihood ratios (LRs) allows to obtain results on a continuous scale, that can be correlated with the level of faecal shedding, and therefore contribute to a more complete diagnosis (Collins 2002). The AGID is one of the most adequate methods used for paratuberculosis diagnosis, when in the presence of clinical disease. The estimated sensitivity is 96% and the specificity is 94%, nevertheless, regarding subclinical disease, the sensitivity of faecal culture is 3 times higher than the AGID test. This method is not too expensive, and it is possible to obtain results within 48 hours, however, due to cross-reactions with animals positive for tuberculosis, it should only be applied in tuberculosis-free herds (Constable et al. 2016). The AGID method has been replaced with ELISA since it presents better results in subclinical cases (Sweeney et al. 2012).

Detecting the cell-mediated immune response could be a good alternative for identifying the disease during the “eclipse” phase before the antibodies or Map organisms start being detectable. These methods include the intradermal Johnin test and the *in vitro* assay of antigen-induced gamma-interferon release (Sweeney et al. 2012). The intradermal Johnin test is specific and not too expensive, whereas the gamma-interferon assay is more expensive. Nevertheless, when interpreted with the newly developed algorithm, the gamma-interferon assay has a high specificity and could be used as an alternative to the intradermal Johnin test or as a diagnosis confirmation for animals testing positive for the intradermal test (Kalis et al. 2003). Either methods are not highly recommended due to their cost and variable performance (Sweeney et al. 2012).

3.1.6. Prevention and Control

Controlling paratuberculosis in ruminants is not an easy task due to Map’s ubiquitous nature (Constable et al. 2016), its long incubation period and the high number of subclinical cases of infection that are difficult to diagnose with the available methods (Olsen et al. 2002).

If a herd is free of the disease, measures should be implemented in order to keep it negative for paratuberculosis. The measures recommended for controlling Map infection are not specific to this agent and are based on good hygiene and biosecurity habits (Sweeney et al. 2012). These measures include not introducing animals from herds with paratuberculosis nor herds with unknown paratuberculosis status; avoiding exposure of animals to other animals with unknown paratuberculosis status, for example, in shows or auctions (Sweeney et al. 2012), since they can get infected as adults as well (Kovich et al. 2006); being cautious when managing youngstock by rearing calves on the farm, following the hygiene procedures established, or rearing calves off-site, on a paratuberculosis-free facility that follows the appropriate biosecurity procedures; breeding instead of buying replacement heifers; not using manure from farms with positive or unknown Johne's disease status; demanding compliance from all visitors in terms of enforcement of biosecurity measures and monitoring the herd status. The documentation and monitoring could be accomplished through voluntary control programmes for Johne's disease. These control programmes have guidelines and consist of repeated testing, risk assessment and the formulation of a management plan, granting the farmer an official herd status (Sweeney et al. 2012).

If a herd is infected with paratuberculosis, the necessary measures will be defined according to the intervention purpose, whether it aims to stabilize the disease and decrease its prevalence in the herd or to eradicate Map. These goals can be achieved through combinations of three measures: prevention of new infections, through enforcement of biosecurity, hygiene and management procedures; correct management of infected individuals, through programmes such as testing and culling animals or testing and managing them and improvement of animal's resistance to the Map agent, through vaccination and genetic selection (Sweeney et al. 2012).

These measures are defined and applied through control programmes, as referred to earlier, and in order to be successful, they demand more on-farm time than usual for the responsible veterinarian and commitment from the producer (Sweeney et al. 2012). A study performed by Collins et al. (2010),suggested that it is possible to obtain good long-term results and decrease paratuberculosis incidence in a herd when using these voluntary management programmes.

Vaccination against paratuberculosis has been a controversial method of prevention of this disease. Both live and inactivated vaccines have proven to be successful (Olsen et al. 2002). A study performed by Lu et al. (2013) has shown that the use of a high-efficacy vaccine in calves, might prevent the infection with Map agent in negative herds. Nevertheless, this disease could become endemic in the herd due to vertical transmission. In the same study, Lu et al. (2013), also observed a decrease in the transmission rate of heavy shedders, in the number of infected heifers and a decrease in vertical transmission, which could translate into a decline in the number of infected animals and also in the probability of the agent's persistence in the herd. Even though these results seem promising, the use of paratuberculosis vaccines causes a cell-mediated immune response detectable for a long period, which will interfere with the methods used to diagnose bovine tuberculosis, such as the intradermal tuberculin test and the gamma-interferon assay (Köhler et al. 2001). This will interfere with the various national tuberculosis eradication programmes, which is probably the main reason restraining the approval of paratuberculosis vaccines by authorities all around the world (Constable et al. 2016).

3.1.7. Treatment

Johne's disease is a condition with no cure. Treatment is usually only considered in particular situations, such as a case of exceptional production, sports animals with genetic interest or pet animals when combined with biosecurity measures to avoid environmental contamination with the agent. Since it is a chronic disease, the treatment purpose is to improve the individual's clinical condition and it will not eradicate the Map agent from the animal's organism nor prevent the animal from shedding the agent. Contrariwise, by treating, the farmer is maintaining an infected and shedding animal in the herd for longer than expected, which could increase the risk of transmission and environmental contamination (Sweeney et al. 2012).

The drugs indicated for the treatment of paratuberculosis, such as isoniazid (isonicotinic acid), clofazimine, rifampin and monensin, are not approved for use in food animals, except for monensin, and the cost of these drugs for cattle is high. Isoniazid was a pioneer in the treatment of Johne's disease in bovine. This substance can only eradicate Map during its growing phase, acting like a bacteriostatic, causing remission of the disease when administered. It can be intoxicating at a dosage of 30 mg/kg per day and the recommended therapeutic dosage varies from 10 to 20 mg/kg daily. Clofazimine is an anti-leprosy drug, frequently used in humans, and has been proven to be successful in the treatment of paratuberculosis in cattle. The recommended dosage is from 600 to 1000 mg, administered orally, daily, for the animal's lifetime. Rifampin, normally used in humans for the treatment of tuberculosis and in foals for *Rhodococcus equi* infections, when combined with other substances, such as streptomycin and levamisole, can be useful for the treatment of paratuberculosis in animals. The recommended dosage is 10 to 20 mg/kg, orally (Sweeney et al. 2012). A study has shown that levamisole, when administered intramuscularly every week, at a dosage of 2,5 mg/kg, might be helpful in the treatment of paratuberculosis (Senturk et al. 2009), nevertheless, further investigation on this subject is necessary. Gallium nitrate has also been studied as a chemoprophylactic treatment of this disease, and it was reported that could reduce Map tissue burden, however, it does not reduce faecal shedding of this agent (Fecteau et al. 2011). Another therapeutical option, recently studied, is dietzia subspecies C79793-74. It is proved that, when used as a probiotic, dietzia successfully treats asymptomatic and symptomatic infections, prolonging the animals' survival and regressing clinical signs, and its effects can be enhanced by dexamethasone in short treatment intervals. The same study also concluded that once the animal is Map negative, the treatment can be ceased. However, when in stage four of the disease, the therapy should be maintained daily (Click and Van Kampen 2010; Click 2011). Regarding the prevention of Johne's disease in calves, Click (2011) has also proven that dietzia is safe and sufficient for this purpose. Even though there seems to be a conflict of interest, due to the authors' commercial interest in this product, the presented evidence is enough to justify further study (Sweeney et al. 2012). Monensin is another drug that seems to be promising for the treatment of paratuberculosis since it is not toxic if used as recommended, nor expensive (Sweeney et al. 2012). According to Brumbaugh et al. (2000), the administration of this substance at a dosage of 450 mg daily, for 120 days, seems to have a beneficial effect, regarding histopathological lesions. Another study in neonatal calves (Whitlock et al. 2005), has shown that monensin intake, at a dosage of 70 mg daily, reduced the faecal shedding of Map and also decreased tissue colonisation. However, a study performed on cows (Hendrick et al. 2006) contradicts this information, since it has not found any relevant effect on faecal shedding of viable Map. It has also been studied in Canada (Hendrick et al. 2006) that this substance, when administered in breeding-age heifers and mature cows, is capable of reducing the number of positive ELISA tests, performed on milk, whether the herd is free of paratuberculosis, or it has already a history of the disease. If monensin reduces the test positivity and improves histologic lesions but has no effect on the amount of agent that is being shed, this could increase the viable Map presence in infected dairy farms, by maintaining infected animals for longer while they keep shedding the agent to the environment. In summary, based on experimental studies and clinical reports, the evidence for monensin recommendation is moderate and evidence for isoniazid and rifampin is weak (Sweeney et al. 2012).

3.1.8. Epidemiology and Global Prevalence

The disease has spread worldwide by the export of infected purebred stock with no clinical signs and it occurs most commonly in cattle rather than in other species, such as small ruminants. It is a globally endemic disease in livestock, and it has spread globally from Western Europe, in the last century, due to the intensification of live animal trade. Australia was the first country to be confirmed to have paratuberculosis in 1980, and there have also been reported cases, clinical and subclinical, in Mexico, Brazil and Argentina (Constable et al. 2016).

Estimating the prevalence of infection in cattle raises some complications, such as the difficulty in diagnosing subclinical infection and the failure to report diagnosed cases if the herd is not under any control programme (Constable et al. 2016). Despite this, a study previously published indicates that the prevalence of infected animals at herd level would be above 50% in many countries, or maybe higher (Nielsen and Toft 2009). Nonetheless, the levels of clinical disease are indicated as much lower (5%) (Bates et al. 2019). An overview of the global dairy herd level prevalence of infected animals can be observed in the following map (Figure 1).

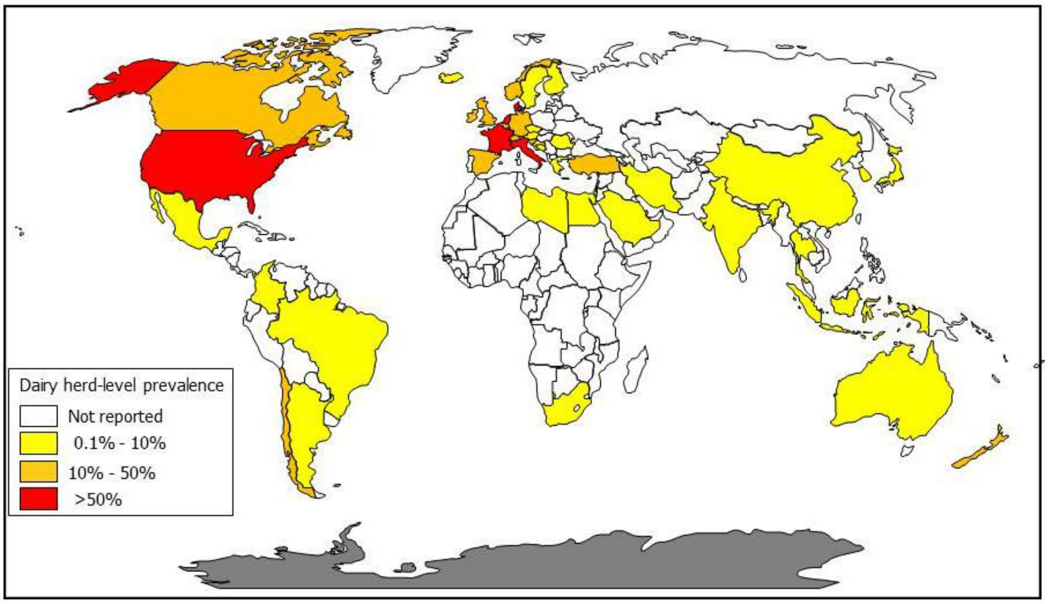


Figure 1 Map of the dairy herd-level prevalence of animals infected with Mycobacterium avium subspecies paratuberculosis (adapted from Collins and Manning, 2018)

3.1.9. Economic Impact

Johne’s disease is a condition capable of affecting economically the cattle industry. The economic impact on each herd depends on the number of individuals with clinical signs, subclinical disease and the number of animals shedding viable Map (Garcia and Shalloo 2015). According to Dufour et al. (2004), it also depends on other significant herd-related factors, such as its size, how it is managed and its production level. Due to the lack or imprecise data regarding paratuberculosis’ prevalence and the fact that most infections are subclinical, it is even harder to determine with precision the economic damage caused by this disease (National Research Council (U.S.), Committee on Diagnosis and Control of Johne’s Disease. 2003). According to a study conducted in the United States of America (Ott et al. 1999), a herd positive for paratuberculosis, compared to a negative herd, might suffer an economic loss of 100 US dollars per cow. Additionally, if the positive herd has at least 10% of its cows culled with clinical signs of the disease, it might suffer an even bigger loss (245 US dollars, per cow, annually). The same study estimated that Johne’s disease could cause the United States dairy industry an economic loss of up to 250 million US dollars annually (Ott et al. 1999). Another study (Dufour et al. 2004), conducted in France, estimated that each clinical case of paratuberculosis would cost around 1000 euros, considering the loss of the cow and its calf, costs of replacement, loss of milk production, veterinary visits and testing, and a subclinical case would cost 461 euros; attending the expected milk yield decrease.

When the positive herd has a low prevalence of the disease, with no clinical cases, or a small number of individuals with subclinical disease, it is more challenging to estimate the economic damage and it might not be too significant (Lombard 2011). However, in the presence of a large number of subclinical cases, the economic impact can be significant (Tiwari et al. 2008).

3.2. Johne's Disease in Ireland

3.2.1. Context

According to the Irish Department of Agriculture, Food and the Marine (DAFM 2022), the agri-food sector is a very important part of Ireland's economy, mainly the export of dairy products, which represent more than 50% of Ireland's exports (DAFM 2022). The production of dairy products is seasonal, coordinated with pasture growth, which is also seasonal. During winter, to avoid soil degradation, the cows are kept inside, and their milk production is less than 10% of the maximum production during the springtime (Gavey et al. 2021). Ireland is now exporting 90% of its production, the dairy exports' value in 2020 increased by 3% from 2019 and it is the second year that dairy exports have been worth more than 5 billion euros (Bord BIA - Irish Food Board 2021).

The reputation of Irish exported dairy products should be protected by engaging a programme that is scientifically proven to be effective in reducing the presence of this agent in dairy products. As mentioned before, despite being a threat to Ireland's trading success, Johne's disease also represents significant economic losses for affected herds (Gavey et al. 2021). In 2009, Animal Health Ireland (AHI) was established to cope with non-regulatory national animal health issues. In the same year, this organisation assembled experts and farmers, who defined Johne's disease as a biosecurity risk disease, that requires future management through the engagement of a long-term control programme, in order to reduce the risk of the disease and perpetuate the consumer's trust (More et al. 2010).

In late 2013, Ireland started a pilot Voluntary Johne's Control Program

me that would later evolve into a national voluntary programme. A review of other Johne's disease control programmes, in six endemically infected countries (Geraghty et al. 2014), was used as guidance to establish this pilot programme. The review indicated and compared the different testing methods, herd classification and recommended control measures used by each country, which helped to create a pilot programme more adequate to Ireland's circumstances. In order to guarantee the engagement of a solid Irish Johne's Control Programme (IJCP), AHI evaluated several surveillance methods (Sergeant et al. 2019) and testing strategies (Meyer et al. 2019). Jordan et al. (2020), have also reported some considerations to contribute to a more effective control programme.

3.2.2. Prevalence and Economic Impact

Johne's disease has been a notifiable disease in Ireland since 1955. In 1992, with the single European market, the probability of introducing the disease in Ireland, through the free movement of animals, increased significantly (Good et al. 2009). According to a survey conducted in 1997, in imported animals, 36% of the herds tested for Johne's disease, had at least one positive reaction to absorbed ELISA (Odoherty et al. 2002), and a few years later, it was reported by Cashman et al. (2008), a prevalence of 20% in dairy herds in Cork, south of Ireland. However, a recent study, conducted on animals enrolled on the national Johne's disease control programme, estimated a herd true prevalence of 28% in Irish dairy herds (McAloon et al. 2016).

Regarding the economic impact of this disease, a study conducted in a paratuberculosis-infected dairy herd, in Ireland, has reported a decrease of about 168 to 253 euros, in profit margin per cow (Barret et al. 2006). Even though these numbers may vary when referring to different herds, it applies to all situations the fact that the herds suffer economic damage with the introduction of Johne's disease.

3.2.3. Irish Johne's Control Programme (IJCP)

3.2.3.1. Introduction to IJCP

The Irish Johne's Control Programme is a voluntary programme, led by AHI, that emerged from a partnership between the Irish dairy industry and the Department of Agriculture, Food and the Marine (DAFM) since paratuberculosis is a notifiable disease and there is nothing described formally on its control or eradication (Gavey et al. 2021). The Rural Development Programme, DAFM, individual milk processors and farmers share the financial costs of this programme. The Technical Working Group (TWG) is formed by veterinary professionals and experts in Johne's disease who advise this programme, by guaranteeing that it is up to date regarding scientific knowledge and that it is well founded. The Implementation Group (IG) is responsible for the IJCP management, and it is formed by AHI, DAFM, representative organisations of milk processors, farmers and veterinarians, milk recording organisations, breed societies and the chair of the TWG and Animal Health and Welfare Northern Ireland (an organisation similar to AHI in Northern Ireland). The AHI is responsible for managing this programme's everyday activities and takes advice from TWG and IG (Gavey et al. 2021).

The IG has established four main objectives: “Enhance the ability of participating farmers to keep their herds clear of JD; assist participating farmers to reduce the level of infection in their herds; provide additional reassurance to the marketplace in relation to Ireland’s efforts to control JD” and “improve calf health and farm biosecurity in participating farms” (Gavey et al. 2021). In order to achieve the previously mentioned objectives, the herds enrolling in the IJCP are required to conduct four activities and the “annual herd level veterinary risk assessment and management plan (VRAMP)” (Gavey et al. 2021), is one of them. It is accomplished by a collaboration between an approved veterinary practitioner (AVP) and the farmer to establish the bioexclusion and biocontainment risks of Johne’s disease for the herd and agree on a maximum of three management measures to reduce Map’s probability of spreading and entering the herd. The “annual whole herd test (WHT) comprising ELISA screening tests with ancillary faecal culture or PCR testing of animals with positive or inconclusive ELISA results” (Gavey et al. 2021) represents another activity from the programme. The WHT is used as confirmation of herd-level for negative herds, as a way of prematurely detecting infection or as a way of monitoring the progression of the disease in positive herds. This measure requires that all 2-year-old animals or older, designated as eligible animals, have their milk or blood samples collected and tested with ELISA. The third criterion is that “ancillary testing is required for all animals with positive or inconclusive ELISA results unless the herd has a previous positive result for a faecal test” (Gavey et al. 2021). This measure’s purpose is to confirm the agent’s presence in the herd. The fourth and last requirement is that “an epidemiological investigation follows the first confirmation of infection in a herd under a Targeted Advisory Service on Animal Health (TASAH) programme” (Gavey et al. 2021), This investigation aims to identify the most likely source of infection and how it spread and then inform VRAMP to improve the management plan.

This programme offers protocols developed by veterinary practitioners and laboratory standards for testing and risk management, in order to keep accuracy and quality throughout the programme. To become an AVP and be able to conduct VRAMPs, sampling and test interpretation, veterinary practitioners must enrol a specific training offered by AHI. After completing the training, an AVP may also enrol on another training programme, that will qualify the practitioner to accomplish the TASAH epidemiological investigations (Gavey et al. 2021).

The DAFM offers National Reference Laboratory services, and all testing is performed in specific laboratories, that exclusively use test kits approved by the the Frederich-Loeffler-Institut. The test results are then transferred to the programme database (Gavey et al. 2021).

The activities fully funded by the programme include the ancillary PCR testing and the TASAH investigations. The ancillary faecal culture is not funded since it is rarely used. When a dairy herd completes the VRAMP and WHT requirements, DAFM and milk processors agree to share costs on the programme activities. DAFM funds the VRAMP and the milk processors fund the herd testing assistance. Compared to milk samples, testing blood samples is more expensive, and this cost difference is usually supported by the farmer (Gavey et al. 2021). For negative herds, the funding for testing decreases over 3 years. However, for positive herds the funding is maintained at the same rate. The programme advises the culling of positive animals, but there is no financial compensation for this activity since it is recommended and not mandatory (Gavey et al. 2021).

As referred previously, the WHT requires samples from all eligible animals, including animals usually missed in a herd test, such as bulls, sick animals, pre-calving heifers and dry cows, designated as "sweeper test". These animals are more likely to be tested through blood samples. Animals that are not breeding, allocated in a different facility, and epidemiologically separated from the breeding herd, can be excused from testing. Due to the risk of false positive results, it is recommended to avoid ELISA testing 90 days after the intradermal tuberculin test and 7 days after calving if using milk samples (Gavey et al. 2021).

The IJCP publishes, annually, a business plan with clear targets and also offers a variety of tools, such as guides, manuals and standard protocols, to farmers and their advisors enrolling on the programme, in order to promote its efficiency and success. To enhance the IJCP, the DAFM has surveillance measures for detecting several diseases, including Johne's disease, for example, testing bulk tank milk (BTM). When a herd is paratuberculosis-positive in BTM testing, the farmer is advised by a DAFM member to enrol the IJCP, allowing to confirm the infection and control the disease and its impacts (Gavey et al. 2021).

3.2.3.2. Farmers' Compliance and Approach to Testing

The number of new herds enrolling in the IJCP and the herds already registered in the programme, which accomplish the annual requirements, is decreasing. The members of the IG concluded that Brexit and COVID-19 had a negative effect on farmers' and veterinary practitioners' compliance. It also affected the stakeholders' promotion of this programme near their clients and suppliers in 2020. Even though the benefits of joining this programme surpass the costs, at the end of 2020, only 11% of the dairy herds, in Ireland, were registered in the IJCP. In 2019, 82% of the registered herds completed both VRAMP and WHT, and in 2020, only 75% did. From 2018 to 2020, the percentage of herds completing the required ancillary PCR tests improved, increasing from 30% to 67%. The ancillary testing is required when an animal has a positive or inconclusive ELISA result, and presuming this test is not performed, the IJCP considers the herd as infected, even if the disease is not confirmed, which could have a negative impact on the herd's future. According to the programme data, the number of herds conducting the ancillary tests is increasing since 2018. However, the incidence of positive PCR test results has decreased, most likely due to the exclusion of known-infected herds from the funded testing. A large number of herds registered in the IJCP did not conduct this ancillary PCR test, which makes it inaccurate to estimate the prevalence of infected herds using the programme data. Additionally, since the herds voluntarily joined this programme, the data available is not a random sample of Irish herds, therefore the extrapolation of a national prevalence would not be accurate (Gavey et al. 2021). Among the ELISA tests conducted in 2020, 47% were on milk samples and the rest on blood samples. It was observed that tests performed on milk samples had a higher rate of positive and inconclusive results and a lower specificity than blood samples (Gavey et al. 2021).

According to some researchers (Smith and Findeis 2013; Regan et al. 2021), in situations where there are no instant benefits, it is necessary to discuss with the farmers the risk of an outbreak of the disease, as well as the consequences of the introduction and spread of the disease, in order to motivate them to enrol in the programme or continue to accomplish its annual requirements. It is reported that, in voluntary programmes like IJCP, the farmers respond better to social and psychological factors than to extrinsic pressure applied through regulations (Gavey et al. 2021).

Testing should occur within the calendar year. Usually, ELISA testing occurs from April to October, for milk samples, and at the end of the calendar year, for blood samples. The collection of blood and faecal samples and the VRAMP activities should be performed ideally during the winter housing period, for logistical purposes. Many herds (30%) have not been able to accomplish the annual requirements before the end of the calendar year, extending the programme usually for one month. In the future, it would be interesting to include this extension in the programme, since farmers and AVPs frequently endeavour to accomplish the requirements in 12 months (Gavey et al. 2021).

3.2.3.3. Data Management and Communication

All the data regarding the IJCP is stored in the Irish Cattle Breeding Federation (ICBF) database. The designated laboratories upload the test results into this database and the AVPs upload VRAMP and TASAH reports. Information regarding the animals' genetics and production, such as birth dates, pedigrees, livestock movements and scheduled dates for intradermal tuberculin testing is also available on the database, in order to facilitate founded decision-making, interpretation of test results and assessment of the disease's progress in the herd (Gavey et al. 2021).

Testing for the IJCP can be rather complex, therefore, the AHI has a flowchart available on its website (figure 2) with the necessary information to achieve the programme's requirements. Primary, farmers should consult their AVPs for support and technical advice. Besides their specific training, the AVPs have useful resorts such as a more detailed flowchart and exclusive access to standard procedures, guidelines, training materials, protocols, and forms for laboratory submissions. Regardless of the tools offered for both AVPs and herd owners, this programme also provides useful instruments to improve compliance, such as automated text messages from ICBF to farmers suggesting the next steps in the programme and advising them to seek out their AVPs for more information; the release of brief communications with consistent and clear information for AVPs and farmers and webinars (Gavey et al. 2021).

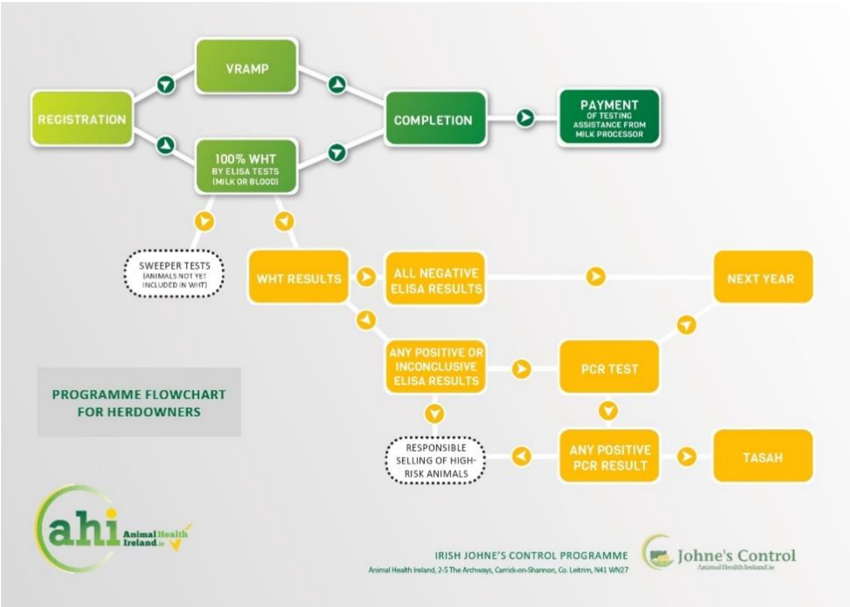


Figure 2 IJCP flowchart for herd owners available on AHI website (adapted from Animal Health Ireland, 2021)

3.2.3.4. Future Perspective

Regardless of the development of Johne's disease control programmes, some authors (Barkema et al. 2018) agree that there has not been sufficient progress, since the prevalence of this disease has not decreased in many countries and the disease has not yet been successfully eradicated in cattle. In order to continue increasing the number of participating herds in the future, it is necessary to innovate and improve communication methods to elucidate to conservative farmers the importance and relevance of these control programmes (Gavey et al. 2021).

In 2021, the IJCP released a practical protocol with the purpose of scoring herd risk. This protocol introduced objective measures of risk obtained from testing and keeping a livestock movement history in each herd. It also acknowledged the implementation of VRAMP measures that address each farm's priorities for the mitigation of the disease. This innovation may result in an additional motivation for herd owners to enrol on the programme since it provides empirical proof of a herd's level of assurance. Furthermore, these measures have the potential to provide voluntary marketing prospects for negative herds, thereby incentivizing the trade of low-risk breeding stock, to instigate farmers with positive herds to biocontain the infection and to enhance consciousness regarding the management of paratuberculosis (Gavey et al. 2021).

A study will be conducted in order to enhance understanding of the herd owners participating experience. This will allow to identify the farmers' motivations and obstacles in accomplishing the annual requirements, and therefore improve the recruitment strategies and motivate farmers to enrol on the programme, enhancing the way communication occurs within the programme, clarifying its benefits, and optimizing the completion of the annual programme's requirements. Another study is planned with the aim of improving the support provided to AVPs, based on their experience (Gavey et al. 2021).

A pertinent aspect that has a significant implication for the future course of the IJCP is establishing an objective method of measuring the progress and accomplishment in achieving the programme's objectives, with the assistance and contribution of herd owners, AVPs and other stakeholders (Gavey et al. 2021).

4. Materials and Methods

The aim of this study is to analyse the evolution of paratuberculosis in an endemically infected dairy herd and the implementation of the IJCP in the same herd. The study was based on retrospective information, with data regarding the IJCP from the years 2020 to 2022, collected from the AHI database, the ICBF, concerning a dairy farm, enrolled on this control programme. The data collected was qualitative, such as the questionnaires performed for the VRAMP, the recommendations formulated by the AVPs and the laboratory results, and quantitative, such as the VRAMP score. The study is considered to be inductive research since the hypothesis is formed using the collected data.

4.1. Dairy Farm

The study was based on data collected from a dairy farm in the west of Ireland (geographical coordinates: 54.5870990, -8.1519638) with a total area of about 162 ha. It is a pasture-based herd, that grazes for approximately 8 months per year, usually from mid-March to November, depending on the climate conditions. During the winter, the herd is fed with grass silage and remains inside, in order to avoid soil degradation.

The herd population varies between 150 and 200 animals, comprising a milking herd of 86 animals, which is divided into 71 spring calving and 15 autumn calving cows, all of which are Holstein Frisian breed. Each milking cow has an average productivity of 25 L per day and is fed approximately 1,5 tonnes of concentrate per year.

The farm slurry is collected in a tank and spread on the land during summer, which is used for grazing.

The animals are mostly bred on the farm and rarely purchased from other farms. Nevertheless, when it occurs, the animals are usually bought from herds whose paratuberculosis status is unknown. Regarding this disease, the herd owner does not cull all the animals testing positive, despite being advised to do so by the IJCP. The cull rate of the herd is 15%.

There are no individual maternity pens, the calving cows share a large pen that could have a maximum of 20 animals simultaneously. Calves are fed unpasteurised colostrum, nevertheless, no waste milk is fed to heifer calves. The youngstock mostly graze a different pasture from the adult milking herd, however, it is exposed to adult faeces in the calving shed, in yards and through the slurry that is spread on the whole farm.

Aside from the herd, the farm land is also grazed by a small sheep flock that belongs to the same owner.

This herd initially enrolled on the IJCP on the 22nd of May 2020.

4.2. Irish Johne´s Control Programme

The IJCP is a voluntary programme, created by AHI to control and decrease the Johne's disease impact in Ireland. In this research, this programme was used as a tool to assist the analysis of Johne's disease evolution on a dairy farm in Ireland.

The programme implies four main activities: VRAMP performed annually by the AVPs in collaboration with the herd-owner, where both agree on a maximum of three management measures to decrease the probability of spreading the disease in the herd; WHT, also performed annually, which consists in collecting blood or milk samples from all the eligible animals in the herd (animals over 2-year-old) for ELISA testing; ancillary testing with faecal PCR for positive or inconclusive ELISA results in the WHT and, when the infection in the herd is confirmed, an epidemiological investigation under a TASAH programme.

The information employed and analysed in this study is limited to the VRAMP, WHT and ancillary testing data available. The VRAMP is composed of a vast questionnaire, with various questions regarding biosecurity and management measures within the farm, in different herd groups (pre-weaned heifers, heifers, cows and calving area). Each option of answer represents a number, the higher the number selected, the higher the risk (figure 3). The numbers assigned to each question are selected according to criteria established by Animal Health Ireland, which are provided to the AVP during training for the Irish Johne's Control Programme.

Regarding pre-weaned heifers, each question has four answer options: number 1, 4, 7, and 10. The maximum score for this section is 80. The following questions were asked:

1) Are calves fed colostrum from own mother or from known low risk colostrum cows or artificial colostrum (artificial colostrum is only recommended in emergency situations)?
2) Are at least 3 litres of colostrum (first milking) consumed within the first 2 hours?
3) Are calves fed on low risk whole milk,
4) rised low risk milk or milk replacer?
5) How often is non-saleable whole milk (high risk) fed?
6) Are calves housed in individual or group pens in the first week?
7) Is there exposure to cow manure in the calf housing or grazing area?
8) Is there exposure to cow manure by watering or feeding utensils?
9) Are calves fed forages that have received slurry from adult animals within the last year?

This section also considers questions that are not included in the score calculation. These questions are if the herd-owner feeds colostrum from other herds; if he feeds milk from cows from other herds and if so, when was it last fed.

Regarding heifers, questions 1), 3), and 5) have three answer options: numbers 1,4, and 7; and questions 2), 4), and 6) have two answer options: numbers 1 and 4. The maximum score for this section is 33. The following questions were asked:

1) Are weaned heifers exposed to cows or their manure at any time?
2) Are maiden heifers exposed to cows or their manure at any time?
3) What is the overall hygiene and cleanliness score of weaned heifers?
4) What is the overall hygiene and cleanliness score of maiden or in-calf heifers?
5) Are weaned heifers (>=6 months) fed forages that have received slurry from adult animals within the last year?
6) Are maiden or in-calf heifers (>=6 months) fed forages that have received slurry from adult animals within the last year?

Regarding cows, questions 1) and 3) have four answer options: number 1, 4, 7, and 10; and questions 2) and 4) have three answer options: number 1, 4, and 7. The maximum score for this section is 34. The following questions were asked:

1) Dry cows area environment hygiene score (performed around calving, in the spring and autumn)
2) Milking cows' area environment hygiene score
3) Dry cows cleanliness
4) Milking cows cleanliness

Regarding the calving area, each question has four answer options: number 1, 4, 7, and 10. Similarly to the first section, the maximum score is 80. The following questions were asked:

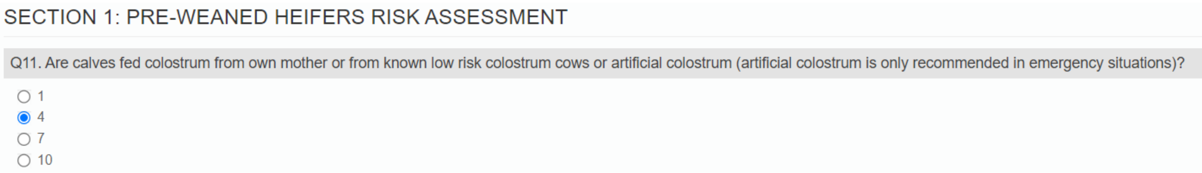
1) Single or multiple cows in calving areas?
2) Manure build up, risk for calf exposure?
3) Manure on soiled udders and legs of cows?
4) Calving area used for lame or sick cows?
5) Calving area used for JD clinical or JD test positive cows?
6) Birth of calves in areas other than designated calving area?
7) Likelihood of calf nursing cow(s)?
8) How fast are newborn dairy calves removed from their mothers?

After completing the questionnaire, it is possible to know the VRAMP score in each group and in total. There are also several questions regarding the herd history, farm bioexclusion measures and animal movements that are not included in the calculation of the VRAMP score, although, they are part of this activity.

Regarding the herd's history, subjects such as whether a Johne's herd test was completed, if so, which type of test was used, and the date of the last Johne's herd test, are adressed. It also questions if there was any suspect case of Johne's disease, for instance, any cow with clinical signs of the disease, or if there was ever a clinically confirmed Johne's disease or test-positive cow in the herd. If these two questions are answered positively, it is asked how many animals and in which year was the most recent case.

Regarding the bioexclusion questionnaire, it considers important topics regarding biosecurity and waste management routines within the farm. In this section, the herd-owner is questioned about the equipment used to spread slurry, if it is his own or not; if he spreads cattle slurry or manure from other herds on his pasture; if he grazes cattle purchased for fattening in his pasture; if the cattle are grazed with cattle from other herds; if he grazes on rented ground, if so, is slurry or manure from his farm spread on the rented ground; does he rears calves or heifers under a different herd number and does he cograze sheep on his farm.

The recommendation of a maximum of three management measures to decrease the probability of spreading the disease in the herd are also present in the VRAMP section. The WHT consists of ELISA testing blood or milk samples of all the eligible animals in the herd and it detects antibodies for *Mycobacterium avium* subspecies *paratuberculosis*. On the contrary, the ancillary test is a PCR and it will detect the agent in faeces of animals with positive or inconclusive ELISA results.


SECTION 1: PRE-WEANED HEIFERS RISK ASSESSMENT

Q11. Are calves fed colostrum from own mother or from known low risk colostrum cows or artificial colostrum (artificial colostrum is only recommended in emergency situations)?

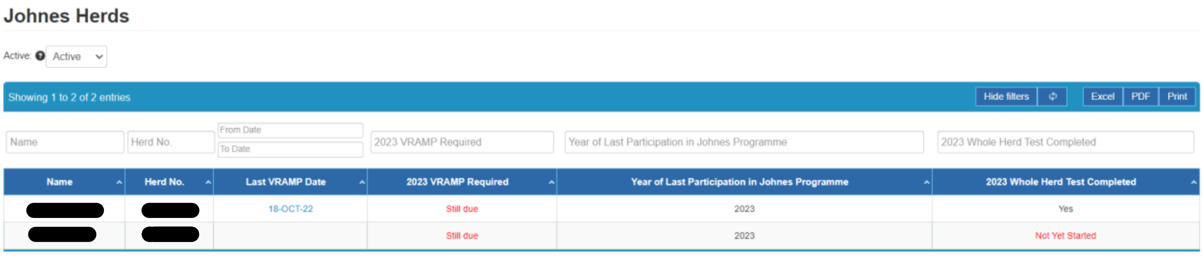
- ○ 1
- ● 4
- ○ 7
- ○ 10

Figure 3 Example of a question and its answer options from the VRAMP questionnaire (adapted from ICBF database, 2023)

4.3. Irish Cattle Breeding Federation

The ICBF owns a database used for storing and managing the data collected for the IJCP. This platform contains data regarding the animal's genetics, production information, date of birth, pedigree, livestock movement and schedule dates for intradermal tuberculin testing. It can also be used to keep BVD records and manage the data regarding this disease and save the milk recording results.

The platform has a dashboard with all the herds enrolled on the IJCP accompanied by the AVP (figure 4). The dashboard displays if each herd concluded the VRAMP and the WHT, and when the next VRAMP is due. When selecting the herd number, the platform will display each test result performed on the herd in a table (figure 5), along with a graphic representation of the results (figure 6), after selecting the year intended. In this section, there are also details available about the herd and herd owner (figure 7) and some additional information about the herd and Johne's status that are available for consultation by selecting the intended option (figure 8).


Johnes Herds

Active: Active

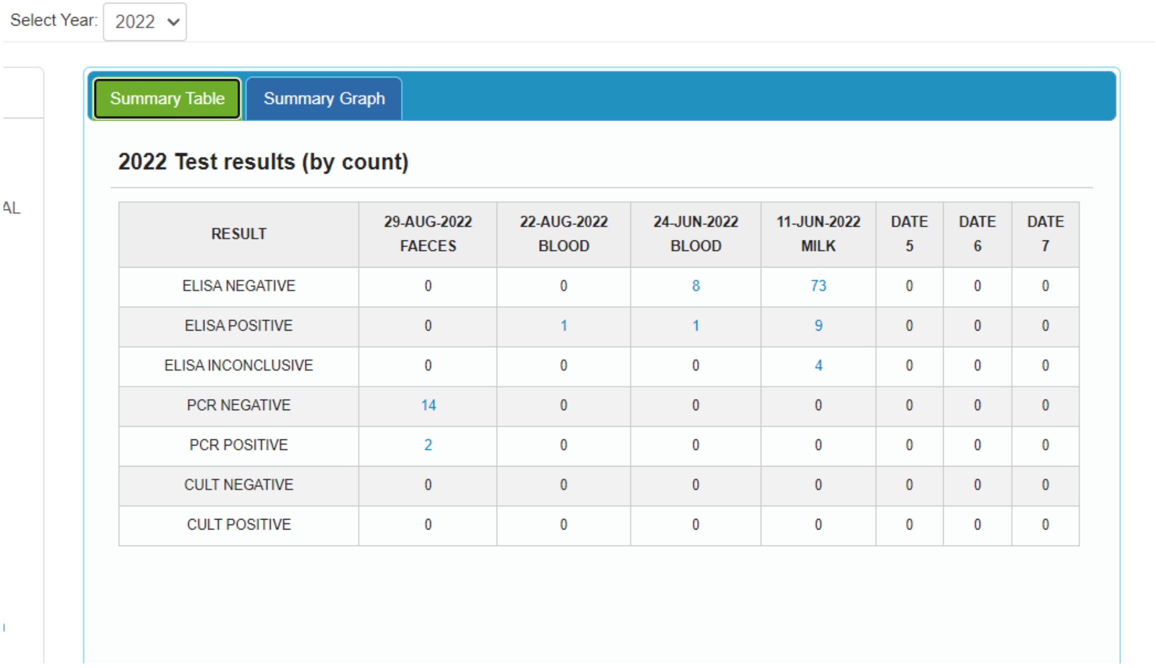
Showing 1 to 2 of 2 entries

Hide filters | Excel | PDF | Print

Name | Herd No. | From Date / To Date | 2023 VRAMP Required | Year of Last Participation in Johnes Programme | 2023 Whole Herd Test Completed

Name	Herd No.	Last VRAMP Date	2023 VRAMP Required	Year of Last Participation in Johnes Programme	2023 Whole Herd Test Completed
[redacted]	[redacted]	18-OCT-22	Still due	2023	Yes
[redacted]	[redacted]		Still due	2023	Not Yet Started

Figure 4 Johne's dashboard (adapted from ICBF database, 2023)



Select Year: 2022

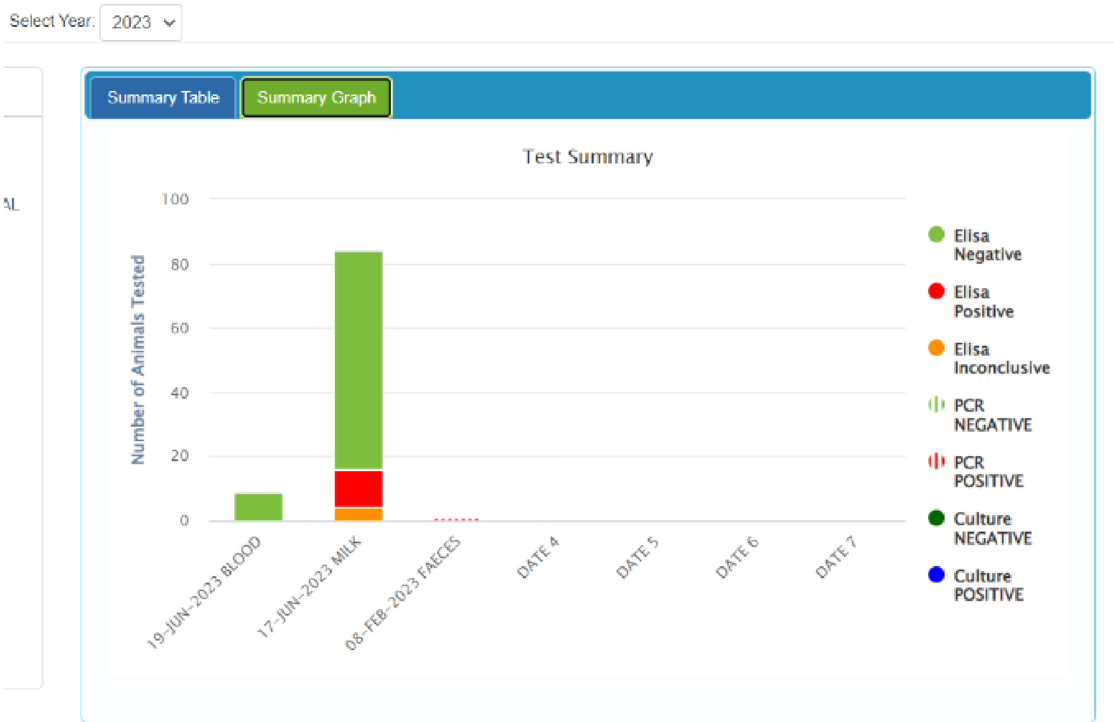
Summary Table | Summary Graph

2022 Test results (by count)

RESULT	29-AUG-2022 FAECES	22-AUG-2022 BLOOD	24-JUN-2022 BLOOD	11-JUN-2022 MILK	DATE 5	DATE 6	DATE 7
ELISA NEGATIVE	0	0	8	73	0	0	0
ELISA POSITIVE	0	1	1	9	0	0	0
ELISA INCONCLUSIVE	0	0	0	4	0	0	0
PCR NEGATIVE	14	0	0	0	0	0	0
PCR POSITIVE	2	0	0	0	0	0	0
CULT NEGATIVE	0	0	0	0	0	0	0
CULT POSITIVE	0	0	0	0	0	0	0

Figure 5 Test results in a table (adapted from ICBF database, 2023)

Figure 6 Test results in a graph (adapted from ICBF database, 2023)



Current Johnes Herd Status

Select

Herd Details

Herd Number:	[redacted]
Owner Name:	[redacted]
Address:	[redacted]
Mobile:	[redacted]
Date of most recent enrolment in the IJCP	01-JAN-23
Date of initial enrolment in the IJCP	22-MAY-20
Date of last positive faecal culture or PCR	10-FEB-23
Whole herd test completed?	Yes
Ancillary PCR testing required?	No
ELISA re-testing recommended?	No
Date of last VRAMP	18-OCT-22
VRAMP for 2023	VRAMP is required, still due
Last TB test date	22-JUN-2023
Next scheduled TB test date	16-DEC-2023

Figure 7 Herd details (adapted from ICBF database, 2023)

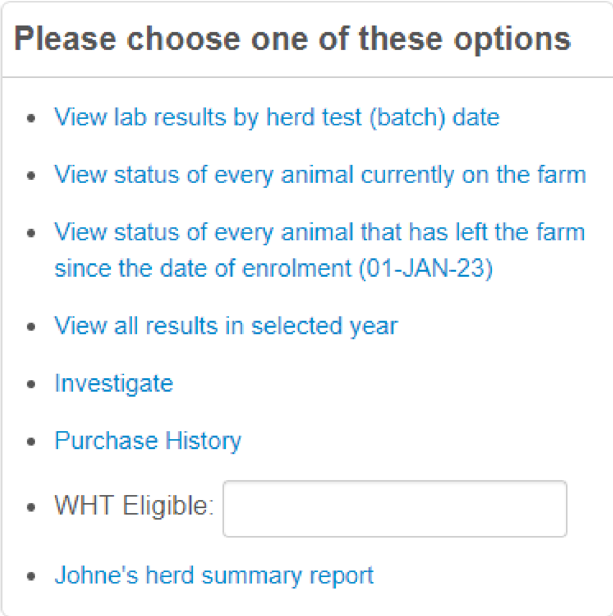


Figure 8 Additional information available for consultation (adapted from ICBF database, 2023)

5. Results

To analyse the evolution of paratuberculosis and the implementation of the IJCP in this herd, data regarding the VRAMP and the WHT annual requirements for three years (2020-2022) was collected from the ICBF database and will be described in this section.

5.1. VRAMP Results

As referred in the chapter "Materials and Methods", the VRAMP results include a questionnaire regarding biosecurity and management measures applied in the farm, in different groups, such as pre-weaned heifers, heifers, cows, and calving area. Since each answer represents a risk score, in this section the risk score for each herd group and the total herd risk score will be presented. The VRAMP results also include the answers to several questions regarding the herd history, farm bioexclusion measures, and animal movements that will not contribute to the score calculation. Likewise, the recommendation of a maximum of three management measures to decrease the probability of spreading the disease in the herd is also present in the VRAMP section and will be exposed in this chapter.

There is no VRAMP data available to the year 2021, therefore the only data present in this section will be from 2020 and 2022.

In order to provide a better understanding of the results, each part of the VRAMP activity, including the complete questionnaires, will be included in the appendix.

5.1.1. Herd History Questionnaire

In 2020, this herd already had a Johne's herd test completed, with milk samples collected individually, on the 12th of August 2014. There was 1 suspect case of Johne's disease in 2020 and there were two ELISA positive cases in the same year.

In 2022, the last Johne's disease herd test completed was registered on the 11th of June 2022, with milk samples collected individually. There were three suspect cases in 2022 and 10 positive cows in the same year.

5.1.2. Bioexclusion Questionnaire

In 2020, the herd-owner used his own equipment to spread slurry and used cattle slurry and manure from his farm only. His animals did not share pasture with other herds, and he did not graze animals purchased for fattening in his pasture. He grazes on rented ground and spreads slurry or manure from his farm on it. He does not rear calves nor heifers under a different herd number and has sheep cograzing on his farm.

In 2022, the answers to the questionnaire that reflect a few habits practised in this farm remained the same, except for the fact that the farm's slurry or manure is no longer spread on rented ground.

5.1.3. Risk Assessment and Scores

In the following sections, each question will be answered with a number that will be considered for calculating the risk score. The higher the score, the higher the risk.

5.1.3.1. Pre-Weaned Heifers (Section 1)

In 2020, the questions 2), 3), 6), 7), and 8) were answered with number 1, corresponding to the lower risk. Otherwise, question 5) was answered with number 4 and questions 1) and 4) were classified with number 7. Regarding the last questions, the answer was negative for both. The risk score of this section, in 2020, was 23.

In 2022, the questions 2), 3), 4), 5), 7), and 8) were answered with number 1, while questions 1) and 6) were classified with number 4. Similarly to 2020, the last questions were answered negatively. The risk score of this section was 14, which decreased about 9 points since 2020.

5.1.3.2. Heifers to First Calving (Section 2)

In 2020, questions 1), 3), and 4) have been answered with the number 1, questions 2) and 6) were answered with the number 4, and question 5) with the number 7. The risk score of this section, in 2020, was 18.

In 2022, question 4) was answered with the number 1, and questions 1), 2), 3), 5), and 6) were answered with the number 4. The risk score of this section was 21, it increased by 3 points since 2020.

5.1.3.3. Cows (Section 3)

In 2020, all the questions were classified with number 1. The risk score of this section in 2020 was 4.

In 2022, the questions were answered with the number 1, apart from question 2), which was classified with the number 4. The risk score increased by 3 points since 2020, reaching 7 points.

5.1.3.4. Calving Area (Section 4)

In 2020, questions 3) and 4) were classified with the number 1, questions 2), 7), and 8) were answered with the number 4, and questions 5) and 6) corresponded to the number 7. Only question 1) was considered as number 10. The risk score of this section was 38.

In 2022, questions 5) and 6) were answered with the number 1, questions 2), 3), 4), 7), and 8) were classified with the number 4, and only question 1) corresponded to the number 7. The risk score was 29 and it had decreased 9 points since 2020.

5.1.3.5. Total VRAMP Score

In 2020, the score in section 1 was 23/80, section 2 was 18/33, section 3 was 4/34 and section 4 was 38/80, summing up to a total of 83/227.

Year / ***Section***	**2020**	**2022**	**Difference between 2020-2022**
Section 1	23/80	14/80	Decreased risk 9

Section 2	18/33	21/33	Increased risk 3
Section 3	4/34	7/34	Increased risk 3
Section 4	38/80	29/80	Decreased risk 9
Total VRAMP	83/227	71/227	Decreased risk 12

In 2022, the score in section 1 was 14/80, section 2 was 21/33, section 3 was 7/34 and section 4 was 29/80, adding up to a total of 71/227. It has decreased by 12 points since 2020, which means that the risk is lower in 2022.

In the table below, it is possible to observe the score results with a schematic presentation, in order to facilitate the interpretation and comparison between the two years, 2020 and 2022.

Table 1. VRAMP scores of each section (1-4) and year (2020-2022), and comparison between the two years

5.1.4. Recommendations and Previous Recommendations

As previously mentioned, the VRAMP activity includes a section where the AVP elaborates on future recommendations, resulting in a maximum of three management measures that aim to decrease the probability of spreading the disease within the herd. The ICBF database has two tabs available: the "recommendations" tab, where the measures agreed upon for the current year are registered in detail, to which section of the VRAMP they refer and which question they refer to, and the "previous recommendations" tab, where the last recommendations are registered in detail, the action summary pointing the tasks that need to be started or continue to be performed, when are they due to be completed and if there has been compliance since the last VRAMP. This section's results will be addressed in this chapter.

5.1.4.1. Recommendations and Previous Recommendations for 2020

The recommendation details underline several aspects such as the managing of calves and their contact with cow's manure. For instance, Johne's test positive, suspect, or high risk cows cannot have contact with calving pens used by other cows - these high risk cows are calved in a separate location. It is not recommended to have multiple cows in one calving pen - it should be divided into smaller pens if possible. It should also be avoided to have calvings in any other place rather than the designated calving pen. The calves should be taken away from their mother in less than 30 minutes and heifer calves should be tagged and noted.

The other recommendation concerns calf feeding. It is recommended that heifer calves are fed colostrum from older cows since they present a higher chance of being identified as positive to paratuberculosis through each year WHT than younger cows, due to the disease's long incubation period and should be fed milk replacer instead of whole milk. In contrast, bull calves can get colostrum from younger cows and can be fed whole milk. No calf should be fed from the dump tank, containing waste milk (for example, milk from cows with mastitis, high cell counts or antibiotics residuals).

The third measure refers to workers' hygiene and calving area management. It is recommended for the workers to use foot baths at all pens' doors and regularly replace and refill them with disinfectant. Another important change is to keep calves in individual pens for the first 7 days to minimise the chance of a calf infected with Johne's *in utero* or with a contaminated coat carrying the disease from the maternity pens to their calf herd mates. When the calves are over 1 week old, they may move to group pens and if possible, the groups should have less than 10 calves.

Considering that the farm had only enrolled on the IJCP in 2020, there were no records of previous recommendations or action summary for that year.

5.1.4.2. Recommendations and Previous Recommendations for 2022

The recommendation details for the year 2022 comprise measures regarding animal movement, bioexclusion, and calving area sections.

When referring to animal movement, it was advised not to buy animals from herds with unknown Johne's status.

Regarding bioexclusion, the recommendations agreed upon were to continue annual testing and identify the animals with positive or inconclusive test results, by marking them with red tape around their tails. It was also recommended to calve these cows in an isolated area, snatching the calf from the cow immediately after birth and not to feed calves with their colostrum. It was also advised to cull these cows, as soon as possible.

The third recommendation concerned the calving area and it reinforced the need to remove the calf from the cow immediately after birth (snatch calving), not allowing the calf to suckle from the dam. It was also advised to feed the calves with colostrum from animals with several negative Johne's test results and to continue to feed calves with milk replacer (powder milk), especially when they were meant to be female replacements. It should also be ensured that dry cows are as clean as possible before entering the calving shed.

The previous recommendations from the 2020 VRAMP, were formerly mentioned. The action summary regarding the first measure from 2020 is to continue testing in order to identify

Johne’s positive cows in the herd and mark them with red tape on their tails. It also mentions that Jonhe’s cows can be bred for beef but only if they stay in the herd for a short period, and they should be calving in an isolated calving area or outside, if it is practical enough. Snatch calving should be practised in the whole herd and calves should only be fed colostrum from cows with negative Johne’s tests. The importance of continuing to practise good hygiene on the farm is underlined: cows should be as clean as possible, especially around calving time, it is important to keep the body and legs clean to avoid faecal-oral transmission and remain as a closed herd, without buying animals from other herds. The compliance referring to this subject, since the previous VRAMP, is positive.

The action summary of the second measure agreed upon in 2020, is to continue to work with the AVP on the bioexclusion plan for the farm, in order to decrease the number of animals with positive tests in this herd, in the future. It is also mentioned the need to discuss a breeding strategy for the herd, for instance, suspect cows can be bred for beef. It is also required to ensure that the suspect cows are clearly marked for the farm workers to notice. There has been compliance regarding these issues since the last VRAMP.

Regarding the last recommendation from the 2020 VRAMP, the action summary underlines the importance of avoiding grazing youngstock on land that received slurry, and there has been compliance since the previous VRAMP.

5.2. WHT Results

As mentioned previously in the chapter “Materials and Methods”, the WHT results will be presented in this section. For this test, the AVP collects milk or blood samples from all the eligible animals in the herd (more than 2-year-old) for ELISA testing, detecting antibodies for Map. Granting that the results of the ELISA testing are positive or inconclusive, an ancillary PCR test is required. Different from the ELISA test, the PCR test will detect the agent in faecal samples.

The WHT was performed in 2020, 2021, and 2022, however, in 2021 the required PCR tests were not performed, and 2023 results are incomplete. The results will be presented in a table for each year, in order to improve reading and interpretation (Tables 2, 3 and 4).

5.2.1. WHT Results for 2020

In 2020, a total of 89 animals were tested with ELISA, 7 animals were tested using blood samples, and 82 animals were tested with milk samples. Only 4 animals (3 ELISA positive and 1 inconclusive) required ancillary testing and the results were all PCR negative.

WHT Results for 2021

In 2021, a total of 96 animals were tested with ELISA, 5 animals through blood samples, and 91 animals through milk samples. Regardless of the requirement for conducting the ancillary PCR testing in the event of positive or inconclusive ELISA results, it was not carried out this year.

5.2.2. WHT Results for 2022

In 2022, a total of 96 animals were tested with ELISA, 10 using blood samples and 86 using milk samples. Fifteen animals required ancillary testing (11 ELISA positive and 4 inconclusive) and 2 of them were PCR positive.

6. Discussion

This chapter conveys a reflection on the evolution of Johne's disease in this herd along with the implementation of the IJCP. Limitations were identified in this study that will be discussed. A reflection on the results obtained from the implementation of the control programme will also be presented.

The apparent prevalence, which represents the percentage of animals with positive test results regardless of whether they are actually infected with the disease (Fegan 1999) (ELISA positive test results / total of animals tested x 100), is increasing yearly. The obtained result was of 3.37% (3/89x100), in 2020, 5.21% (5/96x100), in 2021, and 11.46% (11/96x100), in 2022. The results presented in the previous section also indicate a rise in the number of animals confirmed to be infected with paratuberculosis, through faecal PCR tests.

6.1. Action Plan Implementation

The dairy farm selected for this analysis did not follow the recommendation proposed by the IJCP of "*test and cull*". This measure, combined with measures that reduce the transmission of paratuberculosis, would be highly beneficial for containing the disease (Kudahl et al. 2011) and it would contribute to faster control (Collins et al. 2010), since the animals identified as positive for this disease would be removed from the herd, preventing them from infecting other herd mates and spreading the infectious agent to the environment. However, this measure would reduce the gross margin during the first years and the profits of its implementation would only be noticeable within 10 to 20 years, therefore, it is not feasible for this dairy farm to apply it to its herd (Kudahl et al. 2011). Another important recommendation is maintaining the herd closed and prioritising breeding within the existing herd. If it is necessary to purchase any animal, it should be bought from paratuberculosis-free herds. Even though the herd owner rarely acquires new animals from other herds, he buys animals from herds with unknown Johne's status. This procedure has also been identified before as a limitation to the implementation of Johne's control programmes (Meylan et al. 2021) and it might be responsible for introducing infected animals into the herd. Since the cull rate of this herd is 15%, using sexed semen in maiden heifers would be a good alternative to this issue. Additionally, this procedure could be combined with the use of semen from beef bulls in older cows, in order to decrease the offspring with a higher risk of carrying and spreading the disease.

Limitations related to IJCP were also identified in this study. The herd has only recently enrolled on the control programme, in 2020, and only 3 years of data were analysed in this study. As observed previously in another study, the three-year time frame might not be enough to witness any significant change in the herd disease prevalence (Meylan et al. 2021). Considering that Johne's disease has a long incubation period (that could be 2 to 10 years), it can resist up to 55 weeks in a dry and shaded environment, the WHT is an ELISA test, and it is possible for animals to be infected and shedding the agent but not having gone through seroconversion yet, a realistic evolution of the disease and evaluation of the efficiency of the IJCP would require more years following the programme requirements and activities.

Regarding the VRAMP data from 2020, there are no previous recommendations documented, since it was the first year of the programme, therefore there is no reference of the farm's management regarding this disease in the past. There is also no VRAMP data for 2021, which complicates the understanding of the disease establishment, and evolution from 2020 to 2022. Concerning the WHT data from 2021, the ancillary testing was not performed, which implies there was no confirmation of how many animals were infected, compromising, once again, the understanding of the disease evolution between the years 2020 and 2022.

The herd owner and farm workers' lack of compliance was also determined as a limitation. This was identified in management issues, such as not snatching the calves from their mothers in less than 30 minutes, not feeding milk replacer only, not keeping calves in individual pens and using the calving area for lame or sick cows, as well as in hygiene and biosecurity aspects, such as exposing the calves and weaned heifers to cow's manure and overall, the cleanliness of the facilities has worsened over the years. In spite of the fact that the herd owner demonstrated compliance with the recommendations presented in the year 2020, the repetitive advising of the same measures in 2022 as in 2020 and the increase of some risk scores in the VRAMP section from 2020 to 2022, demonstrate some lack of compliance and commitment to the IJCP.

6.2. Discussion of the Study Results

From the year the farm enrolled on the IJCP, in 2020, and the year 2022, the number of individuals positive in faecal PCR for Johne's disease increased from 0 to 2. However, due to the intrinsic factors of this infectious agent, such as its long incubation period and the possibility of an infected animal shedding the agent without testing positive for ELISA antibody, the results do not imply that no more animals are shedding the agent to the environment. Nevertheless, the number of animals infected with this disease increased in these three years, and the situation could be caused by many other factors that will be explained and detailed below.

It is important to reinforce that one possible scenario for raising the infections could be that some animals were already infected and excreting the agent to the environment, despite not being detected in the first WHT from the IJCP, in 2020. In this case, even if the control programme recommendations were being followed strictly, it would be expected to register more positive cases. Various factors related to the Map agent's intrinsic characteristics, for instance, the long incubation period and environmental resistance, or the farm management procedures identified in the VRAMP questionnaires, for example, not using low risk colostrum or milk replacer when necessary, and having young animals exposed to faeces from adult animals, whether directly or indirectly through spreading slurry on grazing areas, could be responsible for the disease increased prevalence in the herd.

Regarding the intrinsic factors of this agent, the fact that it can survive up to 55 weeks in a dry fully shaded environment could influence the obtained results. If there was an animal previously infected and shedding the agent on this farm, the agent might still be present and viable, being able to infect other animals. Furthermore, if the farm workers do not practice the correct hygiene and biosecurity procedures, such as using the footbaths available and different cleaning equipment for stables and feed rooms and alleys (Larsen and Johnson 1956), they might be responsible for carrying the agent within the farm between different groups. Another factor to consider is the various ways in which Map interacts with its host. The animal might become resistant to this infection, never shedding the agent, or it might control the infection and not present clinical signs while intermittently shedding the agent. This implies that an animal could be shedding the agent to the environment putting other herd mates at risk but not showing signs of infection. Moreover, when infected and not resistant, the host usually goes through a silent phase (stage one) and a subclinical stage of infection (stage two), in which most cases will not be detected by any diagnostic method, even though they could shed the agent in small amounts. These factors overlayed by the fact that the WHT consists of an ELISA test and that faecal shedding can occur prior to seropositivity, implying that an animal can test negative for ELISA but be infected and shedding the agent to the environment, also play an important role in the infection numbers rising. Regarding the transmission of this agent, it is most commonly through oral uptake of the agent, due to faecal contamination of the environment, food, milk and colostrum or water. Since newborn calves are the most susceptible group, they are more likely to be infected, particularly if the farm does not follow the strict conduct proposed by the IJCP on, for example, maintaining the calving and calves' pens clean, removing the calves from their mothers as soon as possible, feed the calves only pasteurised colostrum and avoid faecal contamination. The recommendation regarding not feeding colostrum from high risk animals, such as younger cows, as mentioned in the obtained results, might be controversial, due to ELISA's low sensibility. *In utero* infection is also a risk, implying that if any cow was infected, even if not detected on the WHT, the calf might become infected with Map, even when following the programme recommendations by snatching the calves from their mothers. Additionally, in case any calf was infected, due to the long incubation period and the fact that only 2-year-old animals or older are eligible for testing, the infected individuals will only be identified later. All these factors could be part of the reason for the rising of paratuberculosis cases in the herd.

Other reasons concerning the VRAMP responses to the questionnaires and risk scores might support the rise in the number of paratuberculosis cases. When comparing the recommendations for 2022 with the previous recommendations, which in this case translates as the recommendations for 2020 (two sections of the VRAMP), it is possible to identify some measures that are still mentioned in 2022, even though they were already mentioned in 2020. Additionally, when comparing the 2020 recommendations, which are mainly based on the risk score questionnaires, with the correspondent risk score for 2020 and 2022, it is possible to observe that some risk scores increased in 2022, despite the indicated recommendations, and others were not significantly minimised, as it would be expected and necessary in order to obtain favourable results. For instance, snatching the calves from their mothers, not feeding high risk colostrum (from infected or young cows), using pasteurised colostrum and milk replacer when necessary and having the calves in individual pens, are some of the measures repeatedly recommended in 2022. Concerning the risk scores' analysis, in 2020, it was recommended not to have multiple cows in one calving pen, however, the score for the question "*Single or multiple cows in calving area*?" in 2020 was maximum (10 points) and in 2022, only decreased to 7 points, which still represents a considerable risk for the herd. Another similar situation happened regarding the recommendation from 2020, relative to removing the calves from their mothers in less than 30 minutes. In both years, 2020 and 2022, the risk score was 4 points for both questions "*Likelihood of calf nursing cow(s)*?" and "*How fast are newborn dairy calves removed from their mothers*?". The result demonstrates that there has been no improvement in this matter, despite of the AVP's recommendation. Lastly, concerning the 2020 recommendation on feeding low risk colostrum and milk replacer, the question "*Are calves fed colostrum from own mother or from known low risk colostrum cows or artificial colostrum (artificial colostrum is only recommended in emergency situations*)?" was classified as a 7 and then decreased to a 4. This indicates that the situation was corrected, however, it was not evicted, as it ideally should be to reduce the risk of infecting calves; the question "*How often is non-saleable whole milk (high risk) fed*?" was previously classified with 7, in 2020, and decreased to 1, in 2022, and the question "*Are calves fed on low risk whole milk, pasteurised low risk milk or milk replacer*?" was classified with 1 point and remained a low risk situation for the year after as well. These situations, along with recommendations' repetition the year after, indicate that some of the required and advised measures were not followed nor accomplished completely. Additionally, despite the improvements observed in the risk score questionnaires, some of the remaining questions and respective risk scores increased from 2020 to 2022, demonstrating that some of the procedures were even performed in a way that presented higher risk of infection or spreading the disease within the herd. For instance, in the section "Pre-Weaned Heifers" the question "*Is there exposure to cow manure in the calf housing or grazing area*?" increased its risk score from 1 to 4, in 2022. This is a considerable change and implies that now the calf housing or grazing area is being significantly exposed to cow manure. Since the main route of infection is faecal-oral, the direct contact of the most susceptible group or other animals to faeces from other herd members, presents a high risk for the transmission of this agent. In the section "Heifers to First Calving" the questions "*Are weaned heifers exposed to cows or their manure at any time*?" and "*What is the overall hygiene and cleanliness score of weaned heifers*" increased the risk score, from 1 to 4. This is significant, since the weaned heifers used to be cleaner, more hygienic and not be exposed to cows or their manure constantly, avoiding the risk of infection through faecal contamination of the environment and their own coats, and recently this became a higher risk situation to the herd. In the "Cows" section the question "*Milking cows' area environment hygiene score*" has increased from 1 to 4. Similar to the previous situation, the hygiene score of the cows' area environment was minimal and has increased, which could be responsible for more infections due to the increased environment faecal contamination. Lastly, in the section "Calving Area" the questions "*Manure on soiled udders and legs of cows*?" and "*Calving area used for lame or sick cows*?" have increased the risk score from 1 to 4. Once more, this indicates the deterioration of the facilities' hygiene, having manure in contact with the cows' udders, which will increase the risk of calves getting infected by ingesting faecal particles when suckling or even cows getting infected by having their mouths in contact with parts of their bodies that might have faecal contamination. The mentioned situations are not desirable and might compromise the efficiency of the control programme.

The answers to the "Bioexclusion Questionnaire", in the VRAMP, are also considered important factors responsible for raising case numbers on the farm. As previously mentioned, farm management measures are an essential component in containing the disease, decreasing its incidence, and even preventing the introduction of the disease in the herd. As previously mentioned, studies have shown that Map can survive up to 55 weeks in faecal material when stored in a dry fully shaded environment (Whittington et al. 2004). In slurry, it can survive around 28 days, at 30°C air temperature, 98 days, at 15°C, and 252 days, at 5ºC (Jorgensen 1997). A study has also shown that Map was identified in soil samples until 100 days after applying infected slurry (Salgado et al. 2013). Therefore, when the herd owner states that he spreads manure and slurry on the pasture, even though it is from his own farm, it increases the risk of disseminating the Map agent, present in the faeces, through the land, potentially exposing the animals to the agent during grazing. In 2020, the herd owner was also spreading manure and slurry on rented land in which his animals graze currently, increasing the risk of disseminating the agent to an area that does not belong to the farm and might be used to graze other animals in the future. Even though the herd owner does not share the pasture with other herds, does not graze animals purchased for fattening, nor rears calves or heifers under a different herd number, he has sheep cograzing on his farm. As stated earlier, Map is not species-specific and can affect all ruminants, implying that when sharing land with sheep that are not paratuberculosis-free, the herd owner is increasing the risk of infecting the herd through contact with sheep faeces that might contain viable Map agent. If the silage is well fermented and well preserved, it can contribute to reducing the risk of Johne´s disease infection due to fermentation products' inactivation effect on the agent (Katayama et al. 2000). Therefore, an alternative to this management issue would be to ensilage the grass from the fields in which the slurry was applied.

In summary, due to the limitations of the study, Johne's disease complexity, and the various farm and herd management components that play an important role in Map's transmission and containment, it is difficult to conclude precisely which factor might have been responsible for increasing the cases of paratuberculosis in this herd. It seems highly probable that various of the mentioned components influenced the results observed in this study. Nonetheless, it is crucial to consider that a voluntary control programme, such as the IJCP, highly relies upon the herd owner, farm workers and AVPs compliance. Without the total commitment of all involved parts, it is difficult to improve a herd's situation or maintain its paratuberculosis-free status. In this situation, with the lack of information, such as the 2021 VRAMP questionnaires and the 2021 WHT, and the difficulty in completely accomplishing all the recommendations elaborated by the AVP, it is also more challenging to identify accurately the factors standing in the way of progress towards a paratuberculosis-free herd, and consequently, to correct them and obtain better results. However, since compliance has a great impact on the factors related to farm management that might be affecting the herd's IJCP results, the compliance's improvement, particularly from the farmer and farm workers, in accomplishing the recommendations and following the AVP suggestions, would allow to enhance the herd's paratuberculosis status. A solution for the lack of compliance could be, for example, the attribution of incentives for implementing the required control measures (Meylan et al. 2021). By overcoming this limitation, the results of the implementation of the IJCP would be promising and the farm would be heading to a brighter future with the reduction and control of Johne's disease.

7. Conclusion

This chapter will summarise the analysis performed during this study and reflect on the contribution of this work for the future of the IJCP implementation, as well as propose strategies to improve the identified issues.

This study aimed to define the aspects responsible for limiting the IJCP performance through the analysis of this programme's implementation, for 3 years, in a dairy herd. The obtained results in this particular herd indicate that the disease's apparent prevalence is increasing each year, from 3.37%, in 2020, to 5.21%, in 2021, and finally to 11.46%, in 2022. Through the analysis of the VRAMP results, the study also identified several issues, mainly related to the lack of compliance from the herd owner and farm workers, that affect hygiene and farm management measures crucial to achieving the optimum programme performance. This inference is consistent with the existing literature on the limitations of voluntary control programme implementation (Meylan et al. 2021).

Therefore, this research contributes to identifying the main factors that compromise the performance of the IJCP and offers suggestions considering other study cases, in order to improve Johne's disease control nationally, and possibly in other countries which also rely on voluntary control programmes to improve the disease's status. Besides the considered suggestions, related to specific issues identified in this farm, a broader solution to improve the lack of compliance, such as the attribution of incentives for implementing each control measure (Meylan et al. 2021), could be applied in this herd, and herds enrolling on these control programmes.

In future studies, it would be recommended to extend the time frame of the analysis, in order to increase the chances of registering significant changes in the disease prevalence (Meylan et al. 2021) and therefore to improve the quality of the analysed results and conclusions. In forthcoming research, it would also be interesting to develop a systematic methodology to evaluate the efficacy of the control programme and to identify the challenges associated with the implementation, improving the correction of the main identified issues.

To conclude, this study might be a helpful contribution to the understanding and improvement of the implementation of voluntary control programmes, adding a beneficial input to the control of Johne's disease in affected countries.

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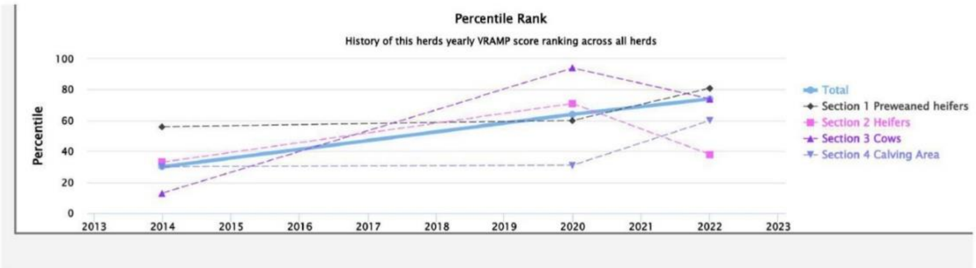
9. Appendix

Appendix 1 - Complete 2020 VRAMP questionnaire, page 2 (adapted from ICBF, 2023)



1. Have you ever completed a Johne's Disease herd test? *

YES NO

Type of test *

Milk (Individual) Blood (Individual) Milk (Bulk) Blood (Bulk)

Date of last Johnes Disease herd test *

2014-08-12

2. Has there been any suspect case(s) of JD - have any cows had chronic diarrhoea / or shown signs of wasting or other signs? *

YES NO DON'T KNOW

How many were there? *

1

Appendix 2 - Complete 2020 VRAMP questionnaire, page 3 (adapted from ICBF, 2023)

4. Do you use your own equipment to spread slurry on your farm? *

YES NO

5. Do you spread cattle slurry/manure from other herds on your pasture? *

YES **NO**

6. Do you graze cattle purchased by you for fattening on your pasture? *

YES **NO**

7. Do you graze cattle/cows on commonage or with cattle from other herds? *

YES **NO**

8. Do you graze on rented ground? *

YES NO

Is slurry or farm yard manure other than from your farm spread on this rented ground? *

YES NO

9. Do you use contract rearers or rear calves/heifers under a different herd number? *

YES **NO**

10. Do sheep cograze on this farm? *

YES NO

Appendix 3 - Complete 2020 VRAMP questionnaire, page 4 (adapted from ICBF, 2023)

What year was the most recent case? *

2020

3. Have you ever had any confirmed clinical JD or test positive cows in your herd? *

YES NO DON'T KNOW

If yes - how many were there? *

2

What year was the most recent case? *

2020

Appendix 4 - Complete 2020 VRAMP questionnaire, page 5 (adapted from ICBF, 2023)

Appendix 5 - Complete 2020 VRAMP questionnaire, page 6 (adapted from ICBF, 2023)

ANIMAL MOVEMENT

	Herd Size on 1st Jan		Animal Introductions During the Previous 12 months					Risk Assessment	
	Animals Over 2 Years of Age	Total Animals	No. of Animals	Male	Females	Overseas Direct Imports	No. of Herds Purchased from	Highest RA Score	Comments
2023	93	268	2	0	2	0	0		
2022	96	234	265	2	263	0	2		
2021	95	216	242	1	241	0	1		
2020	89	215	238	0	238	0	1		
2019	324	477	0	0	0	0	0		
2018	303	463	3	0	3	0	0		
2017	181	407	117	2	115	0	5		
2016	172	310	0	0	0	0	0		
2015	164	289	5	5	0	0	0		
2014	155	270	0	0	0	0	0		An almost entirely closed herd for almost 6 years (only 1 animal came back into the herd
2014	155	270	0	0	0	0	0		An almost entirely closed herd for almost 6 years
2013	151	256	0	0	0	0	0		

Section 1: Pre-weaned heifers risk assessment

Appendix 6 - Complete 2020 VRAMP questionnaire, page 7 (adapted from ICBF, 2023)

11. Are calves fed colostrum from own mother or from known low risk colostrum cows or artificial colostrum (artificial colostrum is only recommended in emerg
situations)? *

1 4 **7** 10

12. Are at least 3 litres of colostrum (first milking) consumed within the first 2 hours? *

1 4 7 10

13. Are calves fed on low risk whole milk, pasteurised low risk milk or milk replacer? *

1 4 7 10

14. How often is non-saleable whole milk (high risk) fed? *

1 4 **7** 10

15. Are calves housed in individual or group pens in the first week? *

1 **4** 7 10

16. Is there exposure to cow manure in the calf housing or grazing area? *

1 4 7 10

17. Is there exposure to cow manure by watering or feeding utensils? *

1 4 7 10

18. Are calves fed forages that have received slurry from adult animals within the last year? *

1 4 7 10

Appendix 7 - Complete 2020 VRAMP questionnaire, page 8 (adapted from ICBF, 2023)

Total Score 23. (Maximum score is **80**, the higher the score - the higher the risk)

19. Do you feed or have you fed colostrum from other herds? *

YES **NO**

20. When was this last fed?

21. Do you feed milk from cows from other herds *

YES **NO**

22. When was this last fed?

Appendix 8 - Complete 2020 VRAMP questionnaire, page 9 (adapted from ICBF, 2023)

Section 2: Heifers (>=6 months) to first calving risk assessment

23. Are weaned heifers exposed to cows or their manure at any time? *

1 4 7

24. Are maiden heifers exposed to cows or their manure at any time? *

1 **4**

25. What is the overall hygiene and cleanliness score of weaned heifers *

1 4 7

26. What is the overall hygiene and cleanliness score of maiden or incalf heifers? *

1 4

27. Are weaned heifers (>=6 months) fed forages that have received slurry from adult animals within the last year? *

1 4 **7**

28. Are maiden or incalf heifers (>=6 months) fed forages that have received slurry from adult animals within the last year? *

1 **4**

Total Score 18. (Maximum score is **33**, the higher the score - the higher the risk)

Appendix 9 - Complete 2020 VRAMP questionnaire, page 10 (adapted from ICBF, 2023)

Section 3: Cows risk assessment

29. Dry cows area environment hygiene score *

1 4 7 10

30. Milking cows area environment hygiene score *

1 4 7

31. Dry cows cleanliness *

1 4 7 10

32. Milking cows cleanliness *

1 4 7

Total Score 4. (Maximum score is **34**, the higher the score - the higher the risk)

Section 4: Calving area risk assessment

33. Single or multiple cows in calving areas? *

1 4 7 **10**

34. Manure build up, risk for calf exposure? *

1 **4** 7 10

Appendix 10 - Complete 2020 VRAMP questionnaire, page 11 (adapted from ICBF, 2023)

35. Manure on soiled udders and legs of cows? *

1	4	7	10

36. Calving area used for lame or sick cows? *

1	4	7	10

37. Calving area used for JD clinical or JD test positive cows? *

1	4	**7**	10

38. Birth of calves in areas other than designated calving area? *

1	4	**7**	10

39. Likelihood of calf nursing cow(s)? *

1	**4**	7	10

40. How fast are newborn dairy calves removed from their mothers? *

1	**4**	7	10

Total Score 38. (Maximum score is **80**, the higher the score - the higher the risk)

Appendix 11 - Complete 2020 VRAMP questionnaire, page 12 (adapted from ICBF, 2023)

Scoring Totals

Scoring Section	Totals
Section 1: Pre-weaned heifers risk assessment	23/80
Section 2: Heifers (>=6 months) to first calving risk assessment	18/33
Section 3: Cows risk assessment	4/34
Section 4: Calving area risk assessment	38/80
Total JD VRAMP Score	**83/227**

Recommendations for changes on this farm

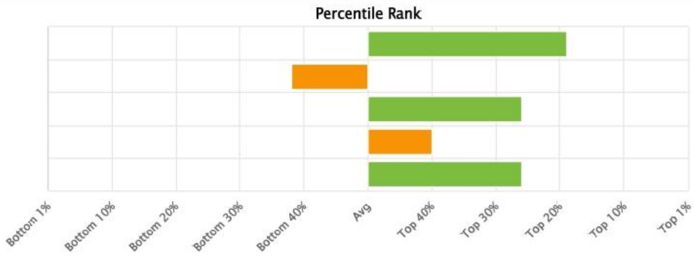
Recommendation Details	Section	Relative to which questions

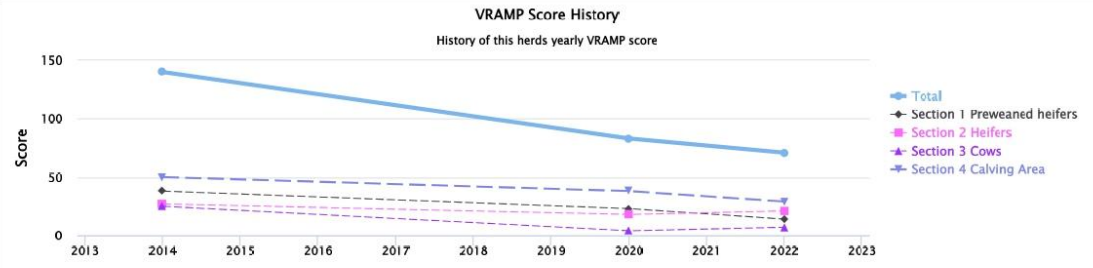
Appendix 12 - Complete 2022 VRAMP questionnaire, page 2 (adapted from ICBF, 2023)

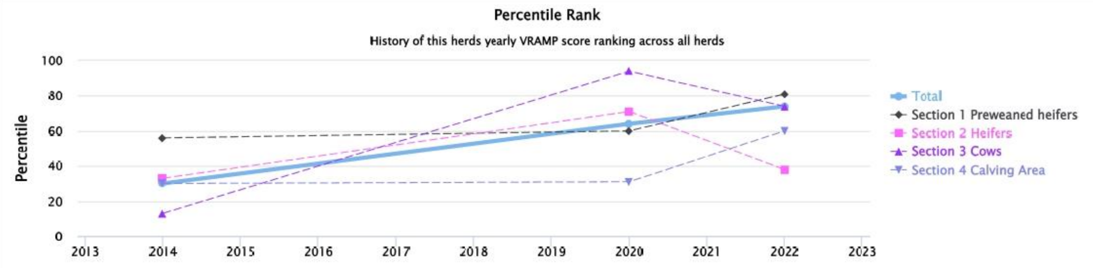
VRAMP Score and History Section

2022	Score
Section 1 Preweaned heifers	14/80
Section 2 Heifers	21/33
Section 3 Cows	7/34
Section 4 Calving Area	29/80
Total	71/227

Percentile <= 35 = Red
Percentile > 35 and <= 65 = Orange
Percentile > 65 = Green







Appendix 13 - Complete 2022 VRAMP questionnaire, page 3 (adapted from ICBF, 2023)

HERD HISTORY (SEPARATE TO VRAMP SCORE)

Q1. Have you ever completed a Johne's Disease herd test?

- [x] Yes
- [ ] No

Type of test

- [x] Milk (Individual)
- [ ] Blood (Individual)
- [ ] Milk (Bulk)
- [ ] Blood (Bulk)

Date of last Johnes Disease herd test

11-Jun-22

Q2. Has there been any suspect case(s) of JD - have any cows had chronic diarrhoea / or shown signs of wasting or other signs?

- [x] Yes
- [ ] No
- [ ] Don't know

How many were there?

3

What year was the most recent case?

2022

Q3. Have you ever had any confirmed clinical JD or test positive cows in your herd?

- [x] Yes
- [ ] No
- [ ] Don't know

If yes - how many were there?

10

What year was the most recent case?

2022

Appendix 14 - Complete 2022 VRAMP questionnaire, page 4 (adapted from ICBF, 2023)

BIOEXCLUSION SECTION (SEPARATE TO VRAMP SCORE)

Q4. Do you use your own equipment to spread slurry on your farm?

- ☑ Yes
- ☐ No

Q5. Do you spread cattle slurry/manure from other herds on your pasture?

- ☐ Yes
- ☑ No

Q6. Do you graze cattle purchased by you for fattening on your pasture?

- ☐ Yes
- ☑ No

Q7. Do you graze cattle/cows on commonage or with cattle from other herds?

- ☐ Yes
- ☑ No

Q8. Do you graze on rented ground?

- ☑ Yes
- ☐ No

Is slurry or farm yard manure other than from your farm spread on this rented ground?

- ☐ Yes
- ☑ No

Q9. Do you use contract rearers or rear calves/heifers under a different herd number?

- ☐ Yes
- ☑ No

Q10. Do sheep cograze on this farm?

- ☑ Yes
- ☐ No

Appendix 15 - Complete 2022 VRAMP questionnaire, page 5 (adapted from ICBF, 2023)

ANIMAL MOVEMENT

Year	Herd Size on 1st Jan		Animal Introductions During the Previous 12 months					Risk Assessment	
	Animals Over 2 Years of Age	Total Animals	No. of Animals	Male	Females	Overseas Direct Imports	No. of Herds Purchased from	Highest RA Score	Comments
2023	93	268	2	0	2	0	0		
2022	96	234	265	2	263	0	2		
2021	95	216	242	1	241	0	1		
2020	89	215	238	0	238	0	1		
2019	324	477	0	0	0	0	0		
2018	303	463	3	0	3	0	0		
2017	181	407	117	2	115	0	5		
2016	172	310	0	0	0	0	0		
2015	164	289	5	5	0	0	0		
2014	155	270	0	0	0	0	0		An almost entirely closed herd for almost 6 years (only 1 animal came back into the herd
2014	155	270	0	0	0	0	0		An almost entirely closed herd for almost 6 years
2013	151	256	0	0	0	0	0		

Appendix 16 - Complete 2022 VRAMP questionnaire, page 6 (adapted from ICBF, 2023)

SECTION 1: PRE-WEANED HEIFERS RISK ASSESSMENT

Q11. Are calves fed colostrum from own mother or from known low risk colostrum cows or artificial colostrum (artificial colostrum is only recommended in emergency situations)?

☐ 1
☑ 4
☐ 7
☐ 10

Q12. Are at least 3 litres of colostrum (first milking) consumed within the first 2 hours?

☑ 1
☐ 4
☐ 7
☐ 10

Q13. Are calves fed on low risk whole milk, pasteurised low risk milk or milk replacer?

☑ 1
☐ 4
☐ 7
☐ 10

Q14. How often is non-saleable whole milk (high risk) fed?

☑ 1
☐ 4
☐ 7
☐ 10

Q15. Are calves housed in individual or group pens in the first week?

☑ 1
☐ 4
☐ 7
☐ 10

Q16. Is there exposure to cow manure in the calf housing or grazing area?

☐ 1
☑ 4
☐ 7
☐ 10

Q17. Is there exposure to cow manure by watering or feeding utensils?

☑ 1
☐ 4
☐ 7
☐ 10

Q18. Are calves fed forages that have received slurry from adult animals within the last year?

☑ 1
☐ 4
☐ 7
☐ 10

Total Score 14. (Maximum score is **80**, the higher the score - the higher the risk)

Q19. Do you feed or have you fed colostrum from other herds?

☐ Yes
☑ No

Q20. When was this last fed?

Q21. Do you feed milk from cows from other herds

☐ Yes
☑ No

Q22. When was this last fed?

Appendix 17 - Complete 2022 VRAMP questionnaire, page 7 (adapted from ICBF, 2023)

SECTION 2: HEIFERS (>=6 MONTHS) TO FIRST CALVING RISK ASSESSMENT

Q23. Are weaned heifers exposed to cows or their manure at any time?

- ☐ 1
- ☑ 4
- ☐ 7

Q24. Are maiden heifers exposed to cows or their manure at any time?

- ☐ 1
- ☑ 4

Q25. What is the overall hygiene and cleanliness score of weaned heifers

- ☐ 1
- ☑ 4
- ☐ 7

Q26. What is the overall hygiene and cleanliness score of maiden or incalf heifers?

- ☑ 1
- ☐ 4

Q27. Are weaned heifers (>=6 months) fed forages that have received slurry from adult animals within the last year?

- ☐ 1
- ☑ 4
- ☐ 7

Q28. Are maiden or incalf heifers (>=6 months) fed forages that have received slurry from adult animals within the last year?

- ☐ 1
- ☑ 4

Total Score 21. (Maximum score is **33**, the higher the score - the higher the risk)

Appendix 18 - Complete 2022 VRAMP questionnaire, page 8 (adapted from ICBF, 2023)

SECTION 3: COWS RISK ASSESSMENT

Q29. Dry cows area environment hygiene score

- ☑ 1
- ☐ 4
- ☐ 7
- ☐ 10

Q30. Milking cows area environment hygiene score

- ☐ 1
- ☑ 4
- ☐ 7

Q31. Dry cows cleanliness

- ☑ 1
- ☐ 4
- ☐ 7
- ☐ 10

Q32. Milking cows cleanliness

- ☑ 1
- ☐ 4
- ☐ 7

Total Score 7. (Maximum score is **34**, the higher the score - the higher the risk)

Appendix 19 - Complete 2022 VRAMP questionnaire, page 9 (adapted from ICBF, 2023)

SECTION 4: CALVING AREA RISK ASSESSMENT

Q33. Single or multiple cows in calving areas?

- ☐ 1
- ☐ 4
- ☑ 7
- ☐ 10

Q34. Manure build up, risk for calf exposure?

- ☐ 1
- ☑ 4
- ☐ 7
- ☐ 10

Q35. Manure on soiled udders and legs of cows?

- ☐ 1
- ☑ 4
- ☐ 7
- ☐ 10

Q36. Calving area used for lame or sick cows?

- ☐ 1
- ☑ 4
- ☐ 7
- ☐ 10

Q37. Calving area used for JD clinical or JD test positive cows?

- ☑ 1
- ☐ 4
- ☐ 7
- ☐ 10

Q38. Birth of calves in areas other than designated calving area?

- ☑ 1
- ☐ 4
- ☐ 7
- ☐ 10

Q39. Likelihood of calf nursing cow(s)?

- ☐ 1
- ☑ 4
- ☐ 7
- ☐ 10

Q40. How fast are newborn dairy calves removed from their mothers?

- ☐ 1
- ☑ 4
- ☐ 7
- ☐ 10

Total Score 29. (Maximum score is **80**, the higher the score - the higher the risk)

Appendix 20 - Complete 2022 VRAMP questionnaire, page 10 (adapted from ICBF, 2023)

Scoring Totals

Scoring Section	Totals
SECTION 1: PRE-WEANED HEIFERS RISK ASSESSMENT	14 / 80
SECTION 2: HEIFERS (>=6 MONTHS) TO FIRST CALVING RISK ASSESSMENT	21 / 33
SECTION 3: COWS RISK ASSESSMENT	7 / 34
SECTION 4: CALVING AREA RISK ASSESSMENT	29 / 80
Total JD VRAMP Score	71 / 227