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Proceedings of the 6th Paratuberculosis Forum

Riviera Maya, Mexico, 4 June 2018

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Proceedings of the 6th Paratuberculosis Forum

Riviera Maya, Mexico, 4 June 2018

Hosted by
Dr. David Kelton, University of Guelph
Guelph, Ontario, Canada
In association with the International Dairy Federation

PROCEEDINGS OF THE 5TH PARATUBERCULOSIS FORUM

ABSTRACT

The 6th ParaTB Forum, sponsored by the International Dairy Federation (IDF), was held on June 4th, 2018 at the International Convention Center in Riviera Maya, Mexico. The Forum was comprised of more than 25 delegates, representing 13 countries (Germany, Italy, Spain, the Netherlands, Ireland, Czech Republic, Brazil, Colombia, Canada, Australia, Argentina, Slovenia, and the United Kingdom). The Forum presents an opportunity for delegates to discuss and report on the current state of paratuberculosis research and control programs in their home nations. This IDF publication reviews some of the common themes and takeaways that emerged from the presentations.

Keywords: *Johne's disease, paratuberculosis, MAP, disease control, national programmes*

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PROCEEDINGS OF THE 6TH PARATUBERCULOSIS FORUM

TABLE OF CONTENTS

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FOREWORD

The ParaTB Forum is an initiative of the International Dairy Federation (IDF), with the first Forum held in Shanghai, China in 2006. The second ParaTB Forum took place in Minneapolis, United States in 2009, the 3rd ParaTB Forum in Sydney, Australia in 2012, the 4th ParaTB Forum in Parma, Italy in 2014 and the 5th ParaTB Forum in Nantes, France in 2016. The 6th ParaTB Forum is being convened in Riviera Maya, Mexico in June 2018.

The Forum provides an opportunity for people involved in the coordination and management of national and regional Johne's disease programs to engage in a frank and open discussion about methods used, progress towards program objectives and lessons learned.

This publication contains 13 papers, all of which are being presented by program representatives on the day (June 4th, 2018).

Acknowledgements go to Dr. David Kelton from the University of Guelph in Ontario, Canada who is hosting the Forum and to Dr. Steven Roche for assistance in organizing the Forum Program and compiling the Proceedings.

The Proceedings of earlier ParaTB Forums have been published in the Bulletins of the International Dairy Federation:

5th ParaTB Forum (2016) – Bulletin of the International Dairy Federation No 484-2016
4th ParaTB Forum (2014) – Bulletin of the International Dairy Federation No 475-2014
3rd ParaTB Forum (2012) – Bulletin of the International Dairy Federation No 460-2012
2nd ParaTB Forum (2009) – Bulletin of the International Dairy Federation No 441-2009
1st ParaTB Forum (2006) – Bulletin of the International Dairy Federation No 410-2007

Caroline Emond
Director General
International Dairy Federation
Brussels, September 2018

PRESENTATIONS

1

PARATUBERCULOSIS IN GERMANY - NEXT STEP FORWARD TO CONTROL IN CATTLE HERDS

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1. INTRODUCTION

In Germany, paratuberculosis is a notifiable disease, without any mandatory control regulations at national level. In view of the regulation (EU) 2016/429 of the European Parliament and of the council on transmissible animal diseases and the amendment and repeal of certain acts in the area of animal health ('Animal Health Law'), which will harmonize animal disease control in the European Union, paratuberculosis was evaluated by the European Food Safety Agency (EFSA) (More et al., 2017).

This scientific evaluation concluded that the disease should be listed and would comply with the criteria for the application of the disease prevention and control referred to optional control in member states (category C), trade restriction (category D) and monitoring (category E). Although the result of the discussion between the member states is not published yet, the controversy regarding paratuberculosis could result in the compromise that the disease will be listed with category E due to expected difficulties in diagnosis and implementation of control measures. Therefore, it is unlikely that in the near future an obligatory federal MAP control programme will be initiated in Germany.

In 2014, the German federal ministry of agriculture released recommendations for paratuberculosis control (FMFA, 2014). At the moment, eight different MAP control programmes for cattle are operating (one mandatory, seven voluntary) in different federal states but none for other ruminants such as sheep, goats or deer. The federal recommendations are meant to sensitize farmers and veterinarians to the disease and give guidance on a similar control approach. Three stages in MAP control are promoted starting with a control stage, leading to a certification stage and finally a farm can be certified as MAP non-suspected. However, since participation is not obligatory MAP control in the federal states varies from non-existent up to an obligatory approach.

When a paratuberculosis control is implemented in the region, the Animal Disease Fund of this federal state offers financial aid for farmers who participate. However, the extent of financial benefits and the conditions which have to be met differ widely between regions. In the following section, the MAP control programmes as initiated in Thuringia and in Lower Saxony will be discussed in more detail. MAP control in Thuringia has been implemented for 15 years, participation is voluntary and, since 2008, has been based on faecal culture, whereas MAP control in Lower Saxony is based on serology, has been initiated only recently and is the first programme of which dairy farms have a legal obligation.

2. AIMS AND OBJECTIVES

The programme period 2008–2014 was reviewed in 2015 and the results were published (Donat, 2017) and presented at the 4th ParaTB forum in 2016 (Donat et al., 2016). The aim of the revised Thuringian control programme is to reduce within-herd prevalence of MAP shedders in enrolled farms and to eventually eradicate MAP in those herds where the owner is committed to this aim (TMLSHWF, 2015). The programme consists of a control phase with four levels. Each level has its own testing scheme. Levels 1–3 are in line with the federal recommendations. Starting with MAP identification (level 1) where semi-annually environmental samples are tested by PCR or culture. Level 2 consists of identification of “high risk animals” by serological testing of all cows, the focus of level 3 is on identifying subclinical shedders by MAP detection in faecal samples of all cows by PCR or faecal culture and herds in level 4 must additionally remove identified MAP shedders within one month (exceptions for pregnant cows and cows suckling a calf). Level 4 is open for herds with an incidence of MAP shedders lower than 3% during the last year and controlled trading. To support herds in level 4, a 200€ incentive for each removed cow is offered. The key element for each level is improvement of biosecurity on the farm. Cattle showing clinical symptoms of paratuberculosis have to be tested immediately at each level. Herds enter the certification stage when no MAP shedding animals can be detected during the last 12 months. When faecal tests have been negative for a period of three years, herds are certified as MAP-unsuspected.

So far, 77 dairy farms are participating in the programme, which represents about 40% of all dairy cows in Thuringia. At the end of 2017, eight (six dairy and two beef herds) of the initially MAP positive herds are certified as MAP-unsuspected. Furthermore, 50 originally MAP-negative herds are certified to date. Most of the initially MAP positive herds have reached level 4 (eradication stage).

		Dairy herds		Beef cow-calf herds		Total	
Control programme phase		Initially MAP-positive	Initially MAP-negative	Initially MAP-positive	Initially MAP-negative	Initially MAP-positive	Initially MAP-negative
Control stage	Level 1	5	3	1	0	6	3
	Level 2	1	0	1	0	2	0
	Level 3	10	3	7	0	17	3
	Level 4	28	0	11	0	39	0
Certification stage (3 years)		0	4	0	4	0	8
Certified MAP-non suspect		6	17	2	33	8	50
Total		50	27	22	37	72	64

Table 1. Results of the regional paratuberculosis control programme in Thuringian Cattle herds (status as of 31st December 2017).

In a subset of 27 dairy herds that have been enrolled since 2008 or before, the cumulative incidence was determined for the period 2008–2017. The mean cumulative incidence started at 14 % whereas nine years later, only a cumulative incidence of 2% was detected indicating a successful reduction of MAP transmission in participating herds. These results show that MAP reduction up to the point of eradication at herd level is possible when measures are continued for several years. A tailored approach of management measures by trained veterinary advisors is important and financial incentives are helpful to enhance the success of the programme.

To initiate the Johne's control, the Farmers' Association and the Dairy Association of Lower Saxony approached the Animal Disease Fund when the BHV1 eradication in Lower Saxony became evident. The Animal Disease Fund supported the initiative and was willing to take the lead during development and implementation of the programme. Stakeholders from many different dairy organizations were involved in the development. The aim of the newly implemented paratuberculosis control programme in Lower Saxony is a reduction in prevalence of paratuberculosis in high risk herds.

The programme started on a voluntary basis in 2016 and in 2017, regulations were enacted by the federal state authority. Since then, MAP diagnostics and a biosecurity analysis on farms with a MAP positive test outcome are mandatory for dairy farms. The regulations intend to alert farmers to the infection and its consequences, reducing MAP transmission in infected herds by improving biosecurity on dairy farms, identifying MAP antibody positive cows and to train and motivate farmers and veterinarians to realize the advantages of the control efforts. First herd screening is performed using pool milk samples (n ≤ 50), which has been recommended to identify highly prevalent herds. However, low prevalence herds will not be detected. Pool milk samples are collected routinely for IBR-monitoring three times a year at all dairy farms in Lower Saxony. When a not-negative result is obtained, all animals >24 months of age have to be individually tested by ELISA either on blood or individual milk samples. A biosecurity analysis and a MAP reduction plan must be produced when at least one animal has been identified to be MAP antibody positive. The biosecurity analysis has to be evaluated each year until MAP prevalence declines below 2%. In addition, in Lower Saxony only animals >24 months can be purchased when they have a negative MAP ELISA outcome. Costs for sampling and diagnostics are covered by the Animal Disease Fund and the biosecurity analysis is financially supported as well. In addition, farmers can join a programme offered by the Animal Disease Fund for a period of at least five years. After subscription, ELISA positive cows have to be removed within 18 months following detection. Animal losses due to MAP infection are reimbursed by the Animal Disease Fund of Lower Saxony. Farmers are entitled to the market value of these cows taking the slaughter value into account.

Within the first eight months of the voluntary programme, already more than 3,000 dairy herds had their animals tested for MAP antibodies. At the end of 2017 the number of participating dairy farms increased to 4,800, which is 50% of the dairy farms of Lower Saxony. At that time, testing was mandatory for only two months. First analysis of highly prevalent herds in Lower Saxony shows that about 14% of herds are identified as MAP positive by the pool milk ELISA, which is consistent with the results of the individual ELISA when grouped by MAP prevalence. 14% of the herds are categorized as having a MAP seroprevalence of 5% or higher.

3. LESSONS LEARNED

The national approach shows that federalism in Germany causes diversity in the control approach between different regions. This can be seen as a positive if the control strategies are implemented regionally and the results shared nationally so that the different experiences can help to identify the best approach. However, in all strategies a herd-status approach should be introduced, since the herd information is the most certain data available and can give important information to potential buyers. The biological characteristics of paratuberculosis, limited diagnostic sensitivity and the economic and social impacts of disease control on producers can present significant challenges to measuring success in controlling Johne’s disease.

The experiences of the Thuringian control programme show that most farmers are interested in eradicating the disease from their herd. As expected, for a long period of time, additional measures are necessary to be able to actually gauge successful MAP reduction; a decade is still too short. Sustaining motivation for such a long time is the challenge. A voluntary setting can only be partially successful. To reach the next step, a mandatory programme is necessary. An easier entrance to the programme might be helpful as well. Over time, the defensive attitude of breeding organisations against MAP control is declining.

Although MAP control in Lower Saxony has only recently started, a few conclusions can already be drawn. Firstly, when different stakeholders are involved in programme development and before implementation, the acceptance of a control programme increases. Secondly, farmer and dairy associations are more open to the challenge of MAP control compared to veterinary officers who are more difficult to get on board. Thirdly, sensitization for the disease by cattle breeding and trading organizations is important for general acceptance. These organizations, however, were on board during the development stage in Lower Saxony and helped to promote the programme. Last, but not least, communication and motivation with all stakeholders is the key to success before and during programme implementation and remains a continuing challenge.

4. IMPLICATIONS FOR THE FUTURE

In the near future, MAP categorization at EU level will not change and official measures will not go beyond monitoring. Therefore, a mandatory control due to a national control regulation cannot be expected. A possible alternative might be a combination of mandatory monitoring enacted by the government with an optional voluntary control initiated by the farmer. A second alternative could be the involvement of the industry requesting milk only from herds with known MAP status.

Samples already collected for the monitoring of other diseases (BHV1, Leucosis, Brucellosis or others) might give easy access to MAP monitoring and once regular monitoring is performed, MAP control might be an easy next step. The collection of environmental samples such as sock samples, known for monitoring salmonella in poultry herds, might also be a useful tool for ongoing analysis.

In addition, the effect of repeated checks on test reliability should be evaluated with available field data. The “Herd risk status” based on repeated testing might improve safety in animal trade and, therefore, reduce MAP transmission between farms. This analysis might be sector driven or based on the official cattle database, both options are possible.

The tailored communication and motivation of all stakeholders has to be continued and further improved.

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2

BOVINE PARATUBERCULOSIS IN ITALY: RESULTS AFTER FOUR YEARS OF APPLICATION OF THE NATIONAL GUIDELINES

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1. INTRODUCTION

Paratuberculosis is widespread in Italy, where over 50% of bovine herds are infected (3, 4, 5). In order to improve the health status of dairy herds and to protect the dairy export market, the Italian Ministry of Health issued the “National guidelines for the control of bovine paratuberculosis and for assigning the health ranking of herds” (1). The National Guidelines represent the first nationally coordinated control and certification programme against bovine paratuberculosis in Italy. The main components of the guidelines have been previously described (2). All of the regions in Italy have formally adopted the guidelines but, their application, being voluntary, is not uniform throughout the Italian territory.

2. AIMS AND OBJECTIVES

Presented here are the results of the first four years of application of the National guidelines in Lombardy, a Northern Italian region where nearly 40% of the Italian milk is produced and the guidelines are widely applied.

In 2017, in the 11 provinces of the Lombardy region, there were 5,673 dairy herds and 1,000,000 total heads, including 500,000 dairy cows. Of these, 4,157 herds officially adopted the guidelines (Table 1). The herds can only join the plan for application of biosecurity measures, and are visited by Public Service veterinarians to verify the absence of clinical cases, thus obtaining the certification necessary for the export market. The number of herds joining the plan rose from 56.0% of total dairy herds in 2014 to 73.3% in 2017.

Accreditation for the health ranking (PT1–PT5) is based on the results of standardized serological testing schemes, to be repeated annually. From 2014 to 2017, a rise in the number of tested herds (from 7.2% to 20.9% of total dairy herds) was observed and a reduction of both infected herds (from 74.2% to 56.0% of tested herds) and positive cows (from 4.2% to 1.9% of tested cows) (Tables 1 and 2).

The herd-level prevalence is based on serology, where a herd is classified as "infected" if at least one sample gives positive result (ELISA, IDEXX confirmation test).

The prevalence of herds with a percentage of seropositive animals >5% was 28.8% in 2014 and gradually decreased to 8.4% in 2017.

Year	Total herds	Herds joining the plan	Tested herds	Infected herds	% herds with AP>5%
2014	6,445	3,608 (56.0%)	466 (7.2%)	346 (74.2%)	28.8
2015	6,336	4,001 (63.1%)	881 (13.9%)	629 (71.4%)	18.8
2016	6,022	4,230 (70.2%)	1,052 (17.5%)	815 (77.5%)	23.0
2017	5,673	4,157 (73.3%)	1,183 (20.9%)	663 (56.0%)	8.4

(AP: Apparent Prevalence)

Table 1. Herd-level prevalence in Lombardy region from 2014 to 2017.

Year	Tested cows (ELISA screening)	Positive cows (ELISA confirmation)	% positive cows
2014	52,963	2,230	4.2
2015	105,466	3,363	3.2
2016	124,750	4,170	3.3
2017	141,330	2,651	1.9

Table 2. Animal-level prevalence in Lombardy region from 2014 to 2017.

This improvement is also evident considering the variations occurring in frequency distribution of within-herd seroprevalence from 2014 to 2017 (see Figure 1).

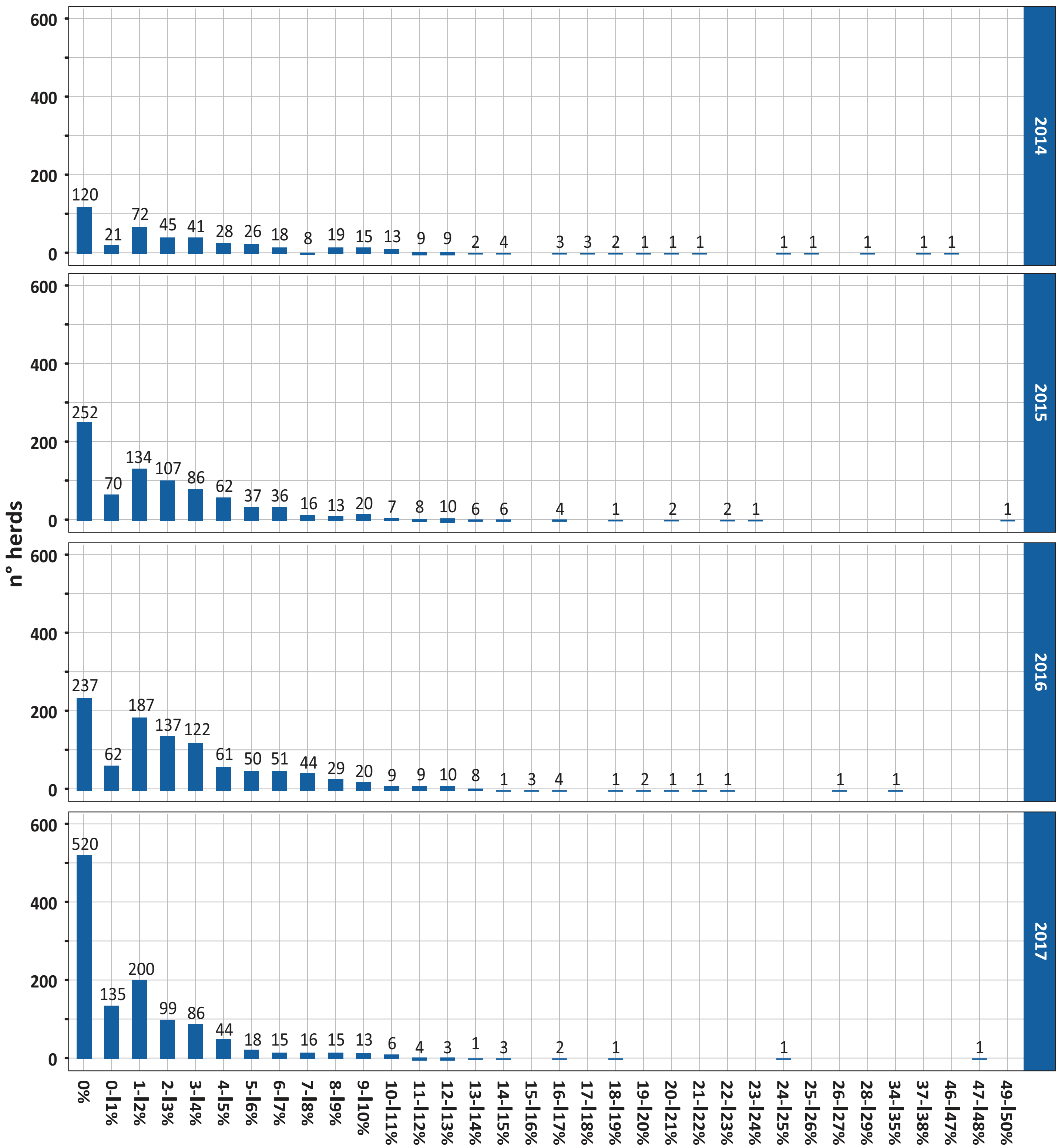

Figure 1. Variation in within-herd seroprevalence of paratuberculosis in Lombardy herds from 2014 to 2017.

In parallel with a decrease of paratuberculosis prevalence, a rise in the number of herds obtaining a health ranking, in particular PT1 (low risk herds) and PT2 (herds negative to one or two controls) was observed. (Table 3, Figures 2, 3 and 4).

		Health ranking				
Year	Total herds	PT1 Low risk	PT2 Negative	PT3 Certified	PT4 Certified	PT5 Certified
2014	6,445	187	137	10	2	4
2015	6,336	354	268	6	4	4
2016	6,022	567	303	22	5	7
2017	5,673	577	393	57	24	11

Table 3. Paratuberculosis in Lombardy region: total number of herds and relative health ranking.

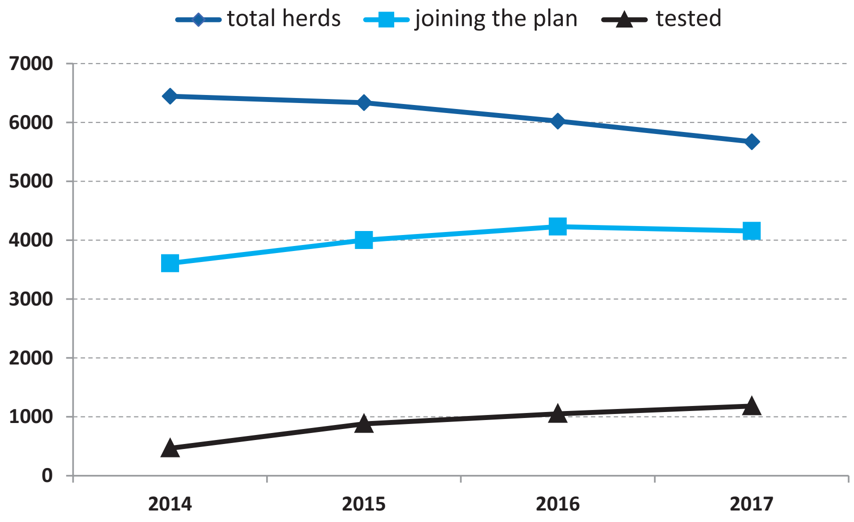

Figure 2. Number of total herds, herds joining the plan and tested herds in Lombardy region.

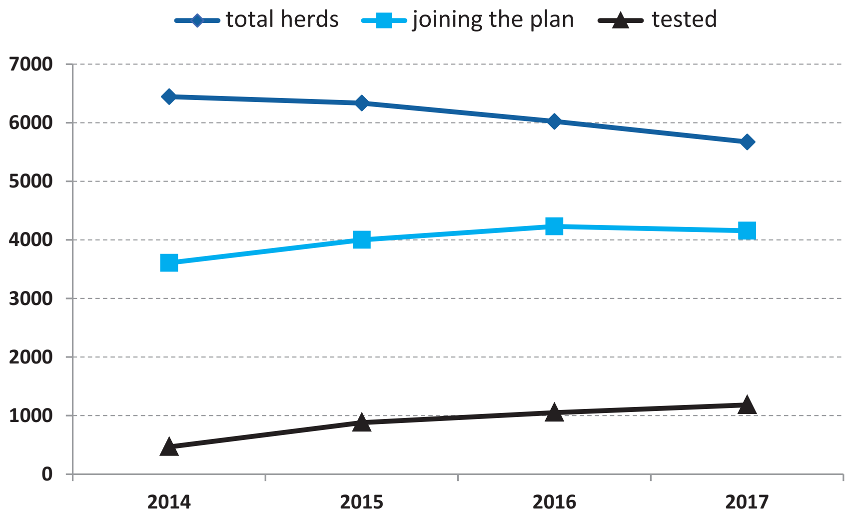

Figure 3. Percentages of herds joining the plan and tested herds in Lombardy region.

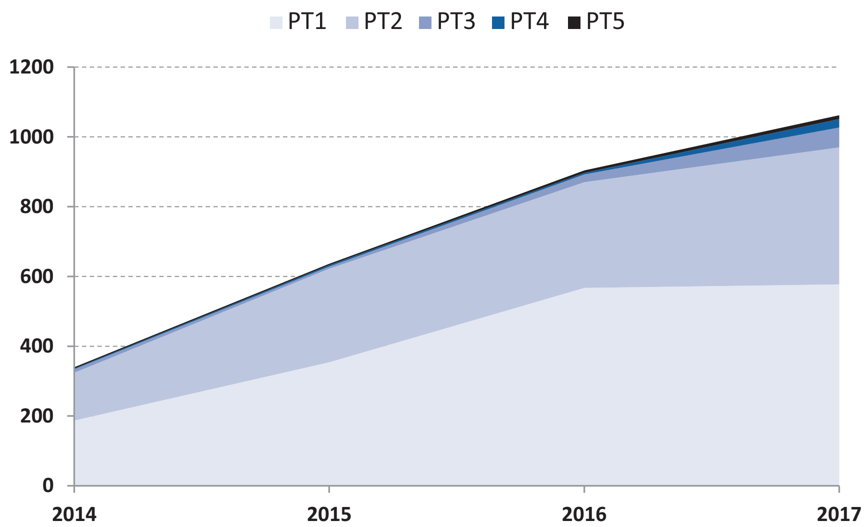

Figure 4. Number of herds obtaining a health ranking in the period 2014–2017.

3. LESSONS LEARNED AND IMPROVEMENTS MADE

In the Lombardy region, during the period 2014–2017, a general improvement in the situation of both prevalence of infected herds and infected animals was recorded, despite the absence of economical compensations for culled animals and financial support for the ELISA tests, which are paid for exclusively by the farmers.

Among the critical points, there is the under-reporting of clinical cases (102 in the considered period), partially due to the fact that farmers tend to cull seropositive cows, in particular with high S/P values, anticipating the onset of clinical disease. In fact, culling of seropositive cows was strongly recommended, independently from the within-herd seroprevalence, and was fulfilled in 70% of cases within a few months.

Moreover, the presence of suspect false positive results of ELISA tests must be highlighted, in herds which repeatedly tested negative to previous controls. In 35% of cases, a positive ELISA result was not followed by a further positivity in the following year's test. This is poorly understood by the farmers and can be a cause for dropping out of the programme. In the guidelines, the possibility of a confirmatory test (PCR on faeces of seropositive animals) is only provided for certified herds (from PT3 to PT5). To define a possible threshold of within-herd seroprevalence for extending to lower levels (PT1 and PT2) the possibility of a confirmatory test, in 2017, 652 faecal samples of seropositive animals coming from 126 herds were tested by PCR.

Overall, setting a cutoff of 3% of seroprevalence, the results of faecal PCR were:

Positive in 22/221 cows (10.0%) from herds with a seroprevalence <3%

Positive in 130/431 cows (30.2%) from herds with a seroprevalence>3%

The results are shown in more detail in Table 4.

Ranking	Number of herds	Herds confirmed by faecal PCR	Tested faeces	PCR positive faeces
PT0 >5% S+	18	12 (66.7%)	251	65 (25.9%)
PT1 3–5% S+	24	18 (75.0%)	180	65 (36.1%)
PT1 < 3% S+	43	12 (27.9%)	160	22 (13.7%)
PT2	38	0 (0.0%)	58	0 (0%)
PT3	3	0 (0.0%)	3	0 (0%)
Total	**126**	**42 (33.3%)**	**652**	**152 (23.3%)**

Table 4. Results of faecal PCR in seropositive cows of herds with different within-herd seroprevalence. On the basis of the results obtained, we intend to extend the possibility of a PCR confirmatory test on seropositive cows in herds showing a within-herd seroprevalence < 3%.

The main strengths of the improvements recorded in Lombardy region are:

Synergic collaboration among health veterinary services, laboratories, practitioners and farmers through the specific creation of support groups, in order to sustain joining and participation in the programme.

Communication, education and awareness of the stakeholders; the information resources created by the National reference center for paratuberculosis were most useful (biannual national conferences for all the stakeholders; online course for veterinarians, manuals for the control of paratuberculosis in dairy and beef herds, a risk assessment application for tablet devices, explanatory brochure and movies for farmers), all freely available online at the link of the National reference Centre (6).

Strong engagement of the farmers' associations in enhancing the health level of bovine population and of the dairy industry in guaranteeing food safety to consumers.

Standardization of diagnostic tests throughout the Italian territory, thanks to the laboratories network of veterinary public institutes (Istituti Zooprofilattici Sperimentali), submitting annually to the proficiency interlaboratory tests organized by the National Reference Center for paratuberculosis.

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3

LONG-TERM RESULTS OF AN EXPERIMENTAL VACCINATION TRIAL IN DAIRY CATTLE IN THE BASQUE COUNTRY: DID WE REACH ERADICATION?

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1. INTRODUCTION

In Spain, paratuberculosis (ParaTB) appears to be widely distributed in cattle but, there are few studies aimed at establishing its prevalence. After screening 1,031 slaughtered cattle, Vazquez et al. [1] found an individual animal prevalence of 46.7% according to histopathological lesions and 39.1% according to tissue PCR. In a similar study by Balseiro et al. [2], the estimated prevalence was 28.4%.

Although it is common knowledge that ParaTB causes huge economic losses to the livestock sector, in Spain there is no national control programme for ParaTB. However, there are different voluntary control plans based on test-and-cull strategies (TCS), as well as good management practices linked to livestock health defence groups.

In the Basque Country, according to cost-benefit analyses, vaccination against ParaTB is considered the best way of controlling the disease; however, vaccination for cattle is not allowed in Spain because of possible interference with the diagnostic tests used in the bovine tuberculosis (bTB) eradication programme. Notwithstanding, bovine tuberculosis is close to being deemed eradicated in the Basque Country, with only 0.09% of herds affected in 2017. This fact, together with the high prevalence of clinical cases of ParaTB on some farms, has led the local animal health authorities to support a vaccination trial in farms with a history of heavy clinical incidence.

2. AIMS AND OBJECTIVES

The aim of this work was to evaluate ParaTB vaccination strategy (VS) and TCS, considering *Mycobacterium avium* subsp. *paratuberculosis* (MAP) faecal shedding as an epidemiologic indicator of the efficacy of each control strategy. At the same time, the effect of the vaccine and MAP infection on the bTB diagnostic tests used in the eradication programme was analysed.

3. LESSONS LEARNED

Even though VS and TCS have reduced ParaTB prevalence, the economic costs associated with TCS herds were much higher because ELISA or PCR positive animals have to be slaughtered. Therefore, this field trial shows that vaccination is a highly efficient strategy for ParaTB control in terms of epidemiologic and economic interests. It demonstrates that ParaTB eradication can be achieved at a reasonable cost, without extreme management changes, in a short period of time and, moreover, consistently maintained afterwards.

4. IMPROVEMENTS MADE

An experimental field trial has been carried out for more than 13 years in the Basque Country, Spain. The trial began in 2005 with three Friesian herds recruited for the VS and two for the TCS, and currently 19 VS and nine TCS herds are participating. In VS herds, all animals over two months of age are vaccinated with an inactivated vaccine (Silirum^{TM}, CZ Veterinaria, Spain) at the time they join the programme and afterwards, only replacer animals between two and six months old. Blood and faecal samples from animals older than 24 months are collected annually for ELISA and PCR testing. To date, more than 15,000 samples have been analysed using these methods.

ELISA testing presented a high percentage of positives after vaccination (Figure 1). The percentage was lower when the animals were vaccinated at under six months of age. However, ELISA is not a useful tool for the detection of ParaTB natural infection in vaccinated animals in any case.

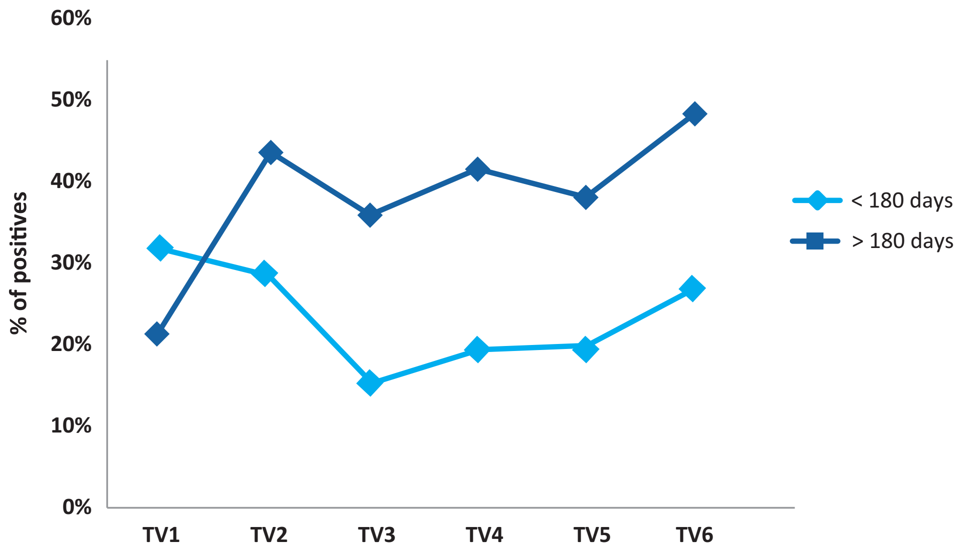

Figure 1. Percentage of positives to the ELISA considering the age at vaccination.
TV: time since vaccination (years).

The initial shedding average prevalence in VS and TCS herds was 12.20% and 8.27% respectively. A sharp decline in the proportion of shedders was observed in both groups between the first and the second annual sampling (AS). This trend continued in VS herds until the 6th AS when the proportion of shedders was 0.74%. In contrast, this figure settled at around 4.13% in TCS herds during the same period (Figure 2). The number of animals and herds included in the programme changed from sampling to sampling, mainly due to the incorporation of new herds and to others that left the programme because owners retired or changed their activity. After vaccination, only 1.98% of the total number of animals included in VS herds was shedding MAP at some moment during their lifetime.

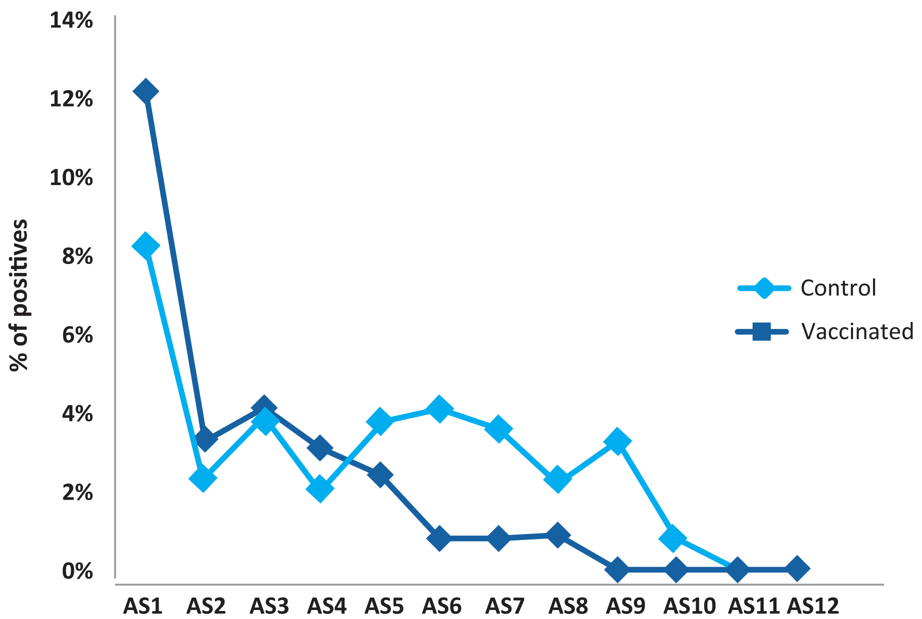

Figure 2. Percentage of MAP shedders per group detected by real-time PCR in each of the annual samplings.
AS: Annual sampling. After AS8 in TCS herds and AS11 in VS herds less than 200 animals were tested annually.

However, for animals vaccinated when younger than two months of age this figure further decreased to only 0.67% and to 1.04% when the age of vaccination was below six months old.

The proportion of MAP shedders according to the age at vaccination and the time since vaccination are shown in Figure 3. Since only animals older than 24 months are sampled, no animal vaccinated at less than six months has PCR results at TV1. It is important to note as well, that the group of animals vaccinated at more than six months included only a total of 25 animals from TV7 to TV9. Reducing the proportion of animals shedding MAP is achieved in both groups. However, vaccinating before the age of six months seems to yield better results because the prevalence of shedders is lower and more stable across time.

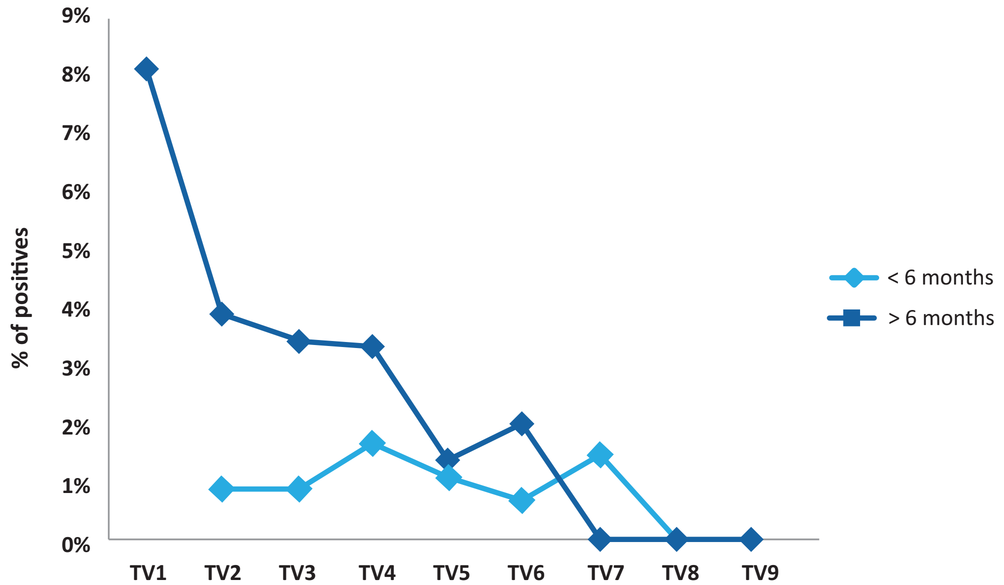

Figure 3. Percentage of MAP shedders considering the age at vaccination and the time since vaccination at the sampling date. TV: time since vaccination (years).

More importantly, at the moment, 12 herds have been subjected to more than six AS in the VS group and eight (66.7%) out of 12 of these herds have had no shedders since their 5^{th} AS. Four (50%) of them had no positive animals during the last five samplings. In the TCS group, in contrast, only one herd had negative results for three consecutive samplings but one animal became positive in the following year.

5. REFERENCES

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4

RESULTS OF MILK QUALITY ASSURANCE PROGRAMME FOR PARATUBERCULOSIS IN DUTCH DAIRY HERDS INDICATE REDUCED TRANSMISSION OF THE INFECTION

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1. INTRODUCTION

In 2006, a milk quality assurance programme (MQAP) for paratuberculosis in Dutch dairy herds was initiated. The aim of this MQAP is to reduce the concentration of *Mycobacterium avium* subsp. *paratuberculosis* (MAP) in bulk milk (van Roermund et al., 2005; Weber et al., 2008; Geraghty et al., 2014). The MQAP is run alongside the pre-existing 'Intensive Paratuberculosis Program' (IPP; aiming at elimination of MAP and low-risk trade of cattle; Benedictus et al., 2000). At present, approximately 99% of Dutch dairy herds participate in either of the two programmes (Anon, 2018).

Herds participating in the MQAP are assigned a herd status (A, B or C) based on the results of herd examinations (Weber et al., 2014). In short, the initial assessment consists of a single herd examination. Test-negative herds enter a surveillance procedure and are assigned status A. The surveillance of herds with status A consists of biennial herd examinations. Test-positive herds at the initial assessment or surveillance procedures enter a control procedure and are assigned status B (if all test-positive cattle have been removed from the herd) or status C (if any test-positive cattle are retained in the herd). If the annual herd examination in a herd with status B yields negative results only, then the herd progresses to status A.

Each herd examination consists of testing either individual milk samples of all lactating cattle or serum samples of all cattle ≥ 3 years of age by ELISA. Farmers are entitled to confirm positive ELISA results by individual faecal PCR assay or culture. Further details of the herd examinations have been described previously (Weber et al., 2014).

Results obtained in the first cohort of 718 herds that voluntarily entered the MQAP in 2006–2007 have been presented before (Weber et al., 2014; Weber et al., 2016). The aims of this paper are to present an update on the results achieved in this cohort of herds in a twelve-year period (2006–2017) and to evaluate the effect of control on the age at onset of ELISA positivity in cattle in participating infected herds.

2. MATERIALS AND METHODS

Results of the initial assessment, surveillance and control procedures of the MQAP obtained between January 2006 and March 2018 by all Dutch dairy herds that entered the MQAP in 2006 and 2007 without prior participation in the IPP were retrieved from the certification operating system and the laboratory information system of GD Animal Health.

To analyse the results of the surveillance and control procedures, non-parametric Kaplan-Meier survival analyses were performed. In the analysis of the results of the surveillance procedure, loss of status A due to positive test results in the surveillance procedure was considered an event. In the analysis of the results of the control procedure, progression of a herd from status B or C to status A was considered an event. In the analysis of the results of the surveillance procedure, only the first period that a herd was assigned status A was included. Similarly, in the analysis of the results of the control procedure in herds with status B or C, only the first continuous period that a herd was assigned status B and/or C was included. Thus, repeated measurements of individual herds were excluded. In both survival analyses, loss to follow-up (i.e., at the end of the study period, cessation of participation in the MQAP, herds changing production type to non-dairy or herds ceasing to exist) was taken into account as right censoring. Loss of status A due to non-adherence to the regulations of the MQAP (such as the requirement to test cattle introduced from a herd with a lower status) was also considered as right censoring.

To analyse the proportion of herds with status A, B or C over time after the first herd examination, the status of each herd was retrieved at one-month intervals.

To assess the effect of the programme on the transmission of MAP, the course of the apparent prevalence over time was calculated. This calculation was complicated by the fact that the cut-off of the milk ELISA had been changed during the study period[1]. Therefore, the S/P ratios of tested samples were retrieved. Apparent prevalences were calculated at a cut-off S/P ratio of 1.00 for both milk and serum samples. The apparent ELISA prevalence of heifers (24–36 months of age) was used as a proxy-parameter for the spread of MAP. By restricting the analysis on the prevalence in heifers to the first test

1 In the MQAP, individual milk samples are tested with the IDEXX Paratuberculosis Screening Ab Test using a cut-off S/P ratio = 1.00. Initially, a cut-off S/P ratio = 0.25 was used. In August 2007, this cut-off was raised to 1.00 to increase the specificity of the test. Individual serum samples are tested with the IDEXX Paratuberculosis Screening Ab Test using cut-off S/P ratios of 0.90 and 1.10, with samples with an S/P ratio between 0.90 and 1.10 being reported as inconclusive.

result of individual animals, a direct effect of culling test positive individuals on the course of the apparent prevalence was eliminated. Time of submission of samples was related to the initial assessment (year 0: 0.5 years prior to 0.5 years after the initial assessment; year 1: 0.5 to 1.5 years after the initial assessment, *etc.*).

The efficacy of long-standing participation in the programme on the transmission of MAP in infected herds was evaluated by a logistical regression analysis of the probability for a heifer to be ELISA positive at its first test. For this analysis, a dataset was used consisting of all ELISA results of samples submitted between Jan 2006 and March 2018 from all dairy herds that were assigned status B or C at the initial assessment in 2006–2007, whilst at least 2% of all samples submitted in year 0 (i.e., 0.5 years prior to 0.5 years after the initial assessment) resulted in an S/P ratio ≥ 1.00. From this dataset, the first ELISA result of each individual was selected, provided this first result was obtained from a milk sample tested at 24–36 months of age. In the logistic regression, ELISA result (positive if S/P ≥1.00) was used as the outcome whereas the interval between birth of the heifer and the date of the initial assessment was entered as the explanatory variable (born before the initial assessment, within five years after the initial assessment and five to ten years after the initial assessment). The effect of this interval was estimated whilst correcting for effects of other available putative risk factors.

Finally, the effect of long-standing participation in the programme on the age at onset of ELISA positivity was evaluated with a Weibull proportional hazards model, using the same dataset of all ELISA results of samples submitted between Jan 2006 and March 2018 from all dairy herds that were assigned status B or C at the initial assessment in 2006–2007 with at least 2% of all samples submitted in year 0 resulting in an S/P ratio ≥ 1.00. ELISA results of both milk and serum samples were included in the analyses. For the purpose of this analysis, an S/P ratio ≥ 1.00 was considered as an event, irrespective of the sample type. The asynchronous interval censored nature of the data was taken into account in the analysis. Three groups of cattle were distinguished: cattle born within five years before the initial assessment of the herd, cattle born within five years after the initial assessment and cattle born five to ten years after the initial assessment.

3. RESULTS

3.1. Participating dairy herds

Of the 718 dairy herds in the cohort, 551 herds (77%) were still classified as a dairy herd at April 1st, 2018. The remaining herds ceased to exist or ceased to produce milk. Therefore, and because the herds entered the initial assessment of the MQAP at varying time points in 2006–2007, the number of herds for which data were available decreased over time after the initial assessment (Figure 1).

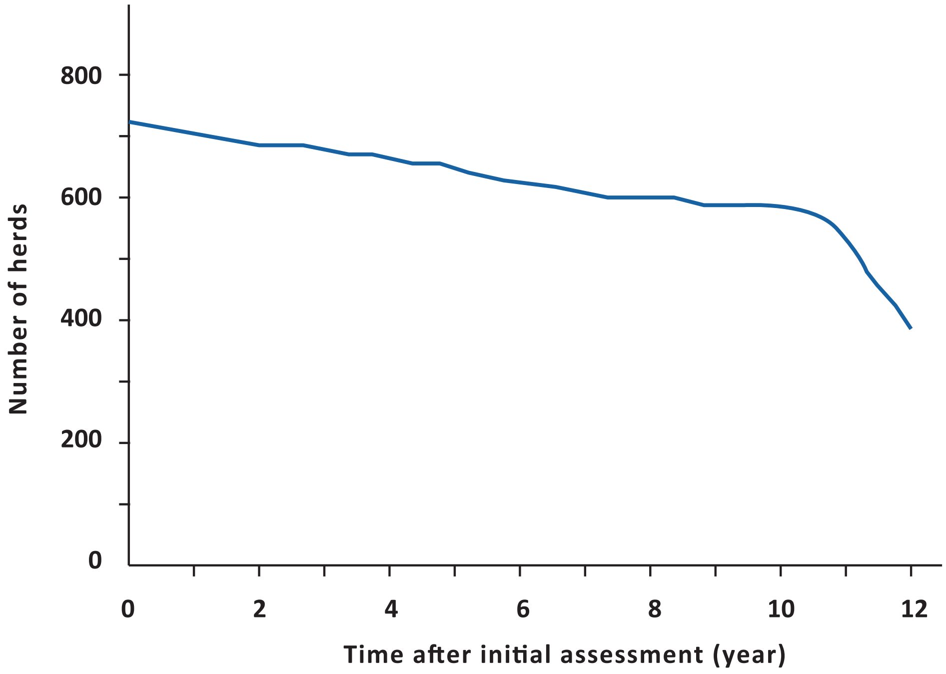

Figure 1. Number of dairy herds for which data were available over time after the initial assessment of the MQAP.

3.2. Surveillance of herds with status A

At completion of the initial assessment, 420 herds achieved status A (Weber et al., 2014). These herds had a probability of 57% to retain status A for at least ten years (Figure 2). Of the 298 herds that were assigned to status B or C at the completion of the initial assessment, 246 herds (83%) progressed to status A at a later stage. Subsequently, these herds had a probability of 19% to retain status A for at least eight years (Figure 2).

3.3. Control in herds with status B or C

At completion of the initial assessment, 298 herds were assigned status B or C (Weber et al., 2014). These herds had a cumulative probability of 84% to progress to status A within ten years after completion of the initial assessment (Figure 2). Of the 420 herds that were assigned status A at completion of the initial assessment, 123 herds shifted to status B or C at a later stage. These herds had a cumulative probability of 96% to regain status A within seven years after shifting to status B or C (Figure 3).

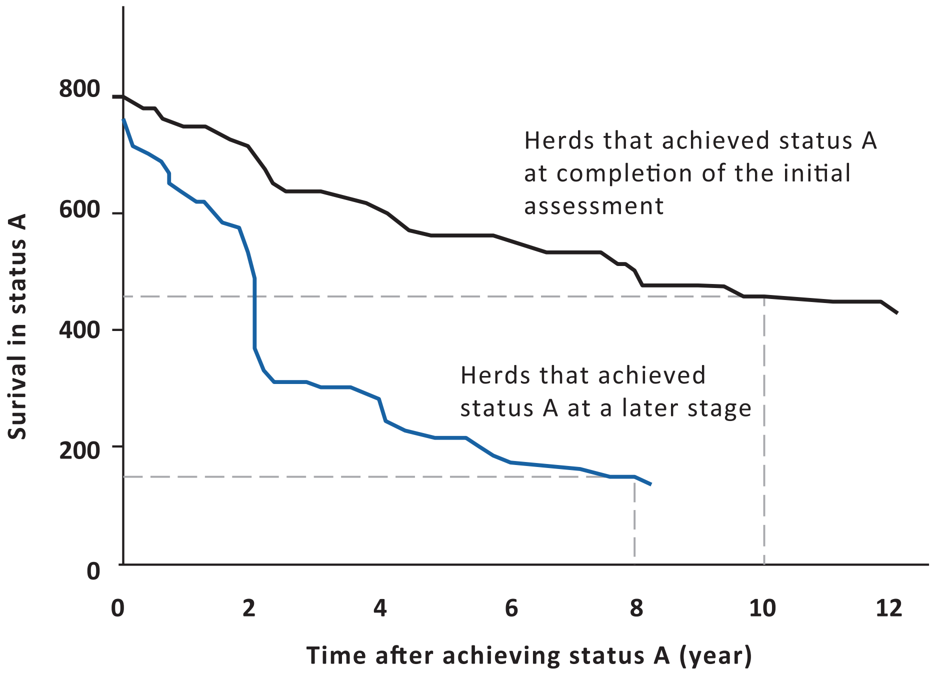

Figure 2. Survival curve for Dutch dairy herds in status A. Loss of status A due to positive test results in the surveillance procedure was considered an event; loss of status A due to other reasons and loss to follow-up were considered as censoring. The survival curves are based on data of 420 herds that achieved status A at the completion of the initial assessment and 246 herds that achieved status A at a later stage.

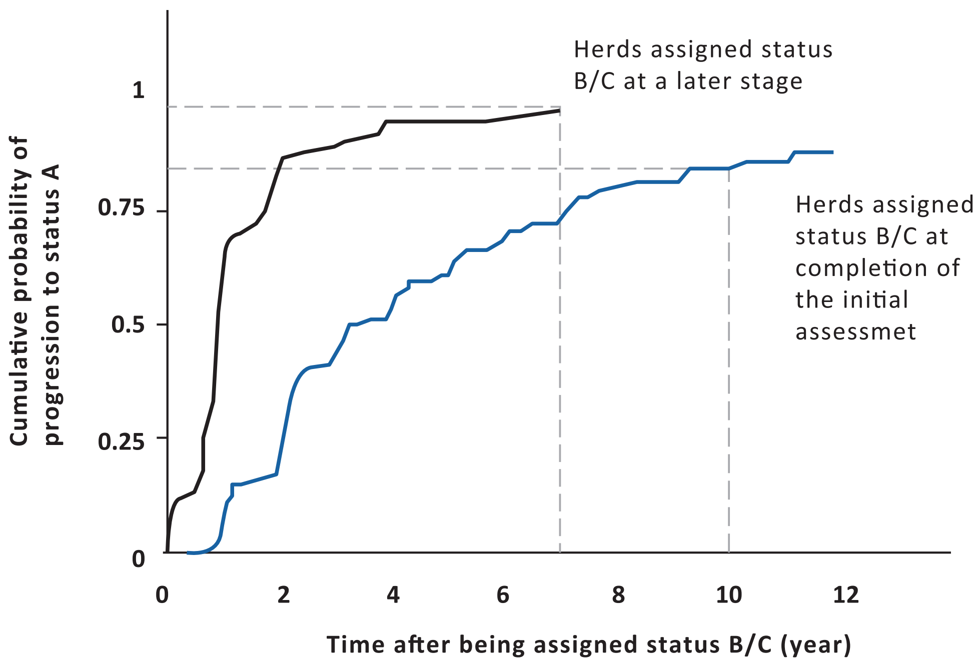

Figure 3. Cumulative probability of progression to status A over time after a herd is assigned either status B or C. The curves are based on data of 298 Dutch dairy herds that were assigned status B or C on completion of the initial assessment, and 123 Dutch dairy herds that were assigned status B or C at a later stage.

3.4. Proportion of herds with status A, B or C over time

The assignment of the 718 dairy herds to status A, B or C over time after the first herd examination of the initial assessment is shown in Figure 4.

The proportion of herds with status A increased from 45% immediately after the first herd examination (i.e., prior to any confirmatory testing of faecal samples of ELISA positive cattle) to 75% at ten years after the first herd examination (Figure 4B). In part, this increase could be explained by an increase in the cut-off of the individual milk ELISA from 0.25 to 1.00 in August 2007, i.e., up to 19 months after the first herd examination of participating herds. However, even after the increase of the cut-off, the proportion of herds with status A consistently increased from 65% at two years after the first herd examination, to 75% at ten years after the first herd examination (proportions test, $p<0.001$).

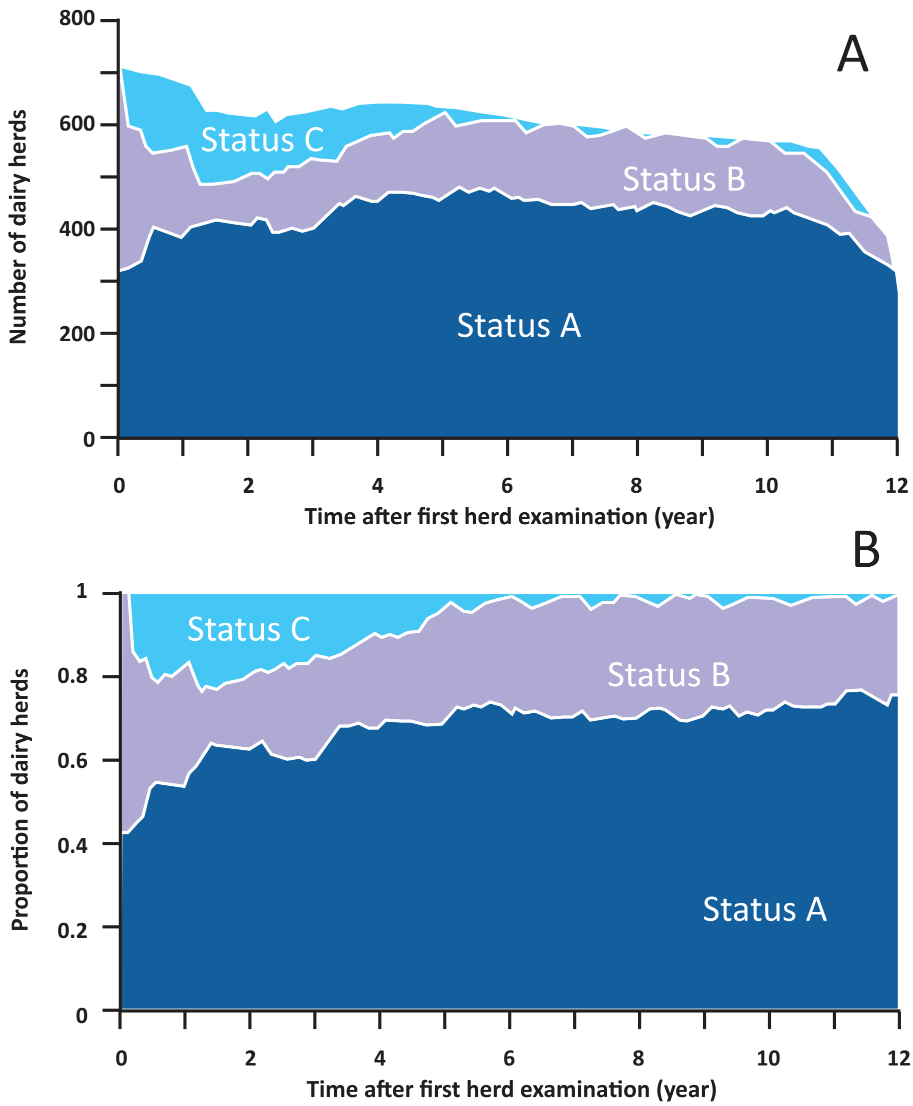

Figure 4. Assignment of 718 Dutch dairy herds that entered the Milk Quality Assurance Programme in 2006 or 2007 to status A, B or C over time after the first herd examination of the initial assessment. (A) Number of herds per status, (B) Proportion of herds.

3.5. Apparent prevalence

During the study period, over 430,000 samples from the study herds were tested by ELISA (95% milk samples, 5% serum samples). The S/P ratio could be retrieved for 99.5% of the samples tested. The course of the apparent prevalence over time is shown in Figure 5, assuming a cut-off S/P ratio of 1.00 for both serum and milk samples.

To eliminate the direct effects of culling test positive cattle on the apparent prevalence, a similar analysis was performed in the subset of the first test results of individual animals tested at two years of age (Figure 5B). At the initial assessment, 0.5% of these heifers were ELISA positive (i.e., S/P≥1.00) and in subsequent years, this proportion fluctuated between 0.1% and 1.1%. However, after year 7, less than 0.3% of heifers were ELISA positive (Figure 5B).

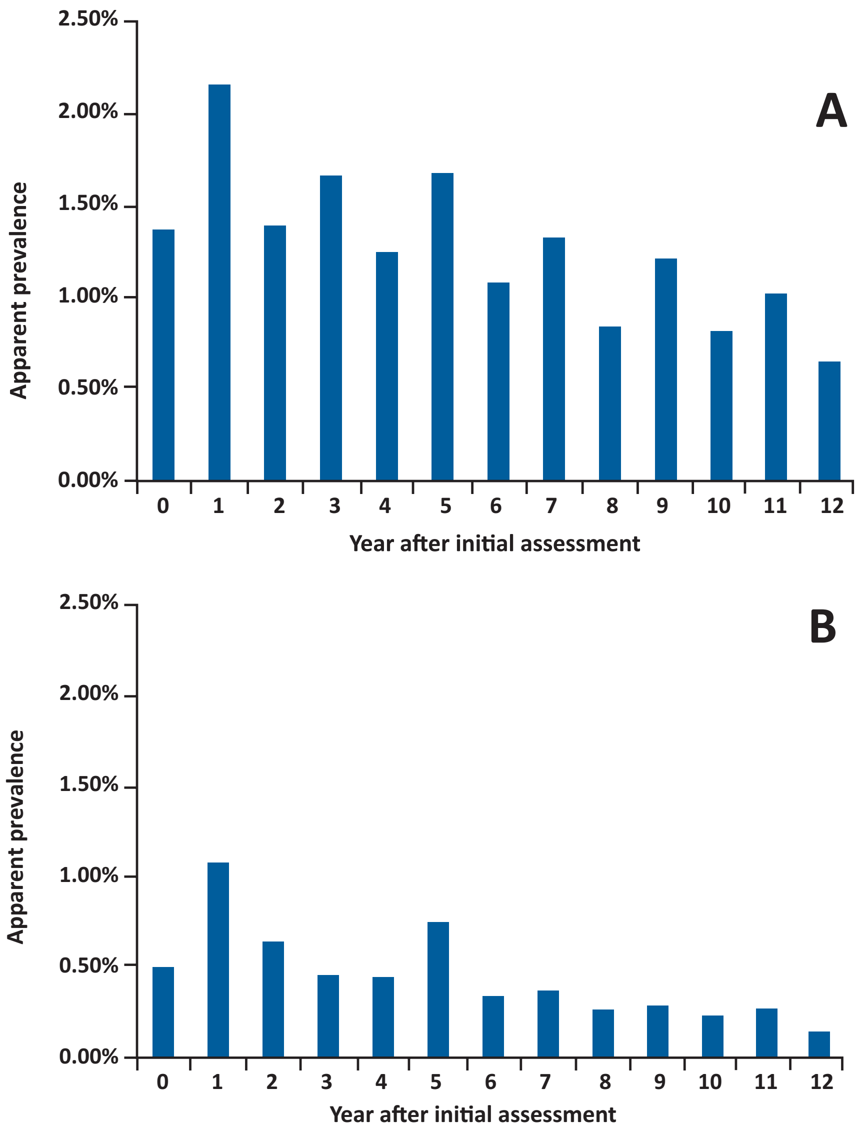

Figure 5. Apparent prevalence at a cut-off S/P ratio of 1.00 for both serum and milk samples in a cohort of 718 Dutch dairy herds that entered the Milk Quality Assurance Programme in 2006 or 2007. (A) all age groups; (B) heifers tested for the first time at two years of age.

3.6. Probability of a heifer being ELISA positive at its first test

Of the 298 dairy herds that were assigned status B or C at the completion of the initial assessment of the herd in 2006–2007, 136 herds had at least 2% of samples submitted in year 0 with an S/P ≥ 1.0. Complete records (including explanatory variables) were available for 24,342 heifers from 129 of the 136 herds. Of these 24,342 heifers, 231 heifers (0.9%) had a positive milk ELISA result with an S/P ≥1.0. With reference to heifers born prior to the initial assessment of the herd, heifers born within five years after the initial assessment of the herd had an OR = 0.45 (95% CI: 0.33, 0.60) of a positive ELISA result with an S/P ≥1.0, whereas heifers born five to ten years after the initial assessment had an OR = 0.17 (0.11, 0.26; Table 1).

Variable	Number of observations	β	S.E.	P (Wald)	OR (95% CI)	P (LR test)
Time at which heifer is born:						
Prior to initial assessment of the herd	5,530	Reference				<0.001
< 5 years after initial assessment of the herd	10,409	-0.809	0.154	<0.001	**0.45 (0.33, 0.60)**	
≥ 5 and < 10 years after initial assessment of the herd	8,403	-1.788	0.221	<0.001	**0.17 (0.11, 0.26)**	
Herd in which heifer is born[1]						
Closed	7,452	Reference				0.097
Open	15,894	0.177	0.172	0.302	1.19 (0.85, 1.67)	
Elsewhere	996	-0.567	0.45	0.208	0.57 (0.24, 1.37)	
Highest S/P ratio of dam[2]						
S/P < 0.30	18,508	Reference				0.005
0.30 ≤ S/P <1.00	1,545	0.392	0.243	0.106	1.48 (0.92, 2.38)	
S/P ≥ 1.00	1,302	0.761	0.213	<0.001	**2.14 (1.41, 3.25)**	
Not tested or S/P unavailable	2,987	-0.019	0.193	0.922	0.98 (0.67, 1.43)	
Age at testing						
730 – 858 days	6,118	Reference				<0.001
859 – 931 days	6,113	0.501	0.241	0.037	**1.65 (1.03, 2.65)**	
932 – 1002 days	6,066	0.499	0.241	0.038	**1.65 (1.03, 2.64)**	
1003 – 1094 days	6,045	1.283	0.218	<0.001	**3.61 (2.35, 5.53)**	
Proportion of samples with S/P ≥ 1.0 in the herd in year 0 3						
Proportion ≤ 0.026	5,096	Reference				<0.001
0.026 < proportion ≤ 0.038	4,725	0.344	0.271	0.204	1.41 (0.83, 2.40)	
0.038 < proportion ≤ 0.048	4,887	0.468	0.259	0.071	1.60 (0.96, 2.65)	
0.048 < proportion ≤ 0.063	5,031	0.755	0.247	0.002	**2.13 (1.31, 3.45)**	
Proportion > 0.063	4,603	1.093	0.231	<0.001	**2.98 (1.90, 4.69)**	
Region of the Netherlands						**0.010**
North	16,459	Reference				
East	3,854	0.129	0.174	0.459	1.14 (0.81, 1.60)	
South	1,937	-0.167	0.3	0.577	0.85 (0.47, 1.52)	
West	2,092	-0.995	0.369	0.007	**0.37 (0.18, 0.76)**	
Predominant soil type in postal code area						
Sand	8,580	Reference				0.072
Sandy loam	3,894	0.45	0.195	0.021	**1.57 (1.07, 2.30)**	
Clay	4,532	-0.062	0.222	0.781	0.94 (0.61, 1.45)	
Low moor bog	6,862	0.276	0.18	0.124	1.32 (0.93, 1.88)	
Other	474	0.542	0.458	0.237	1.72 (0.71, 4.22)	
Intercept		**-5.494**	**0.335**			

Table 1. Final logistic regression model of the probability of a positive milk ELISA result at the first test of a lactating heifer. Data from 129 dairy herds that were assigned status B or C at the initial assessment whilst at least 2% of samples submitted from the herd in year 0 had an S/P ≥ 1.0.

[1]Closed: the heifer was born in the herd in which it is tested, whilst all cattle present at the time of birth were born in the same herd. Open: the heifer was born in the herd in which it is tested, whilst there were cattle born in another herd present at the time of birth of the heifer. Elsewhere: the heifer was born in another herd.[2] Of any serum or milk samples of the dam of the heifer submitted between Jan 2006 and March 2018.[3] Year 0: 0.5 years prior to 0.5 years after the initial assessment. If the first sample of a heifer was tested in year 0, this sample was left out of consideration in the calculation of the proportion of samples with an S/P ≥ 1.0.

3.7. Age at onset of ELISA positivity

A total number of 103,904 ELISA results of milk and serum samples collected from 42,798 cattle in 136 herds were available for analysis. Of the 103,904 samples, 2,488 samples (2.45%) had an S/P ≥ 1.0 (Figure 6). The observations on the 42,798 cattle consisted of 40,473 right censored observations (i.e., cattle with negative ELISA results only), 739 left censored observations (i.e., cattle that were positive at the first ELISA), 1,583 interval censored observations (i.e., cattle with a negative ELISA result followed by a positive ELISA result) and three uncensored observations (negative and positive ELISA result on the same day).

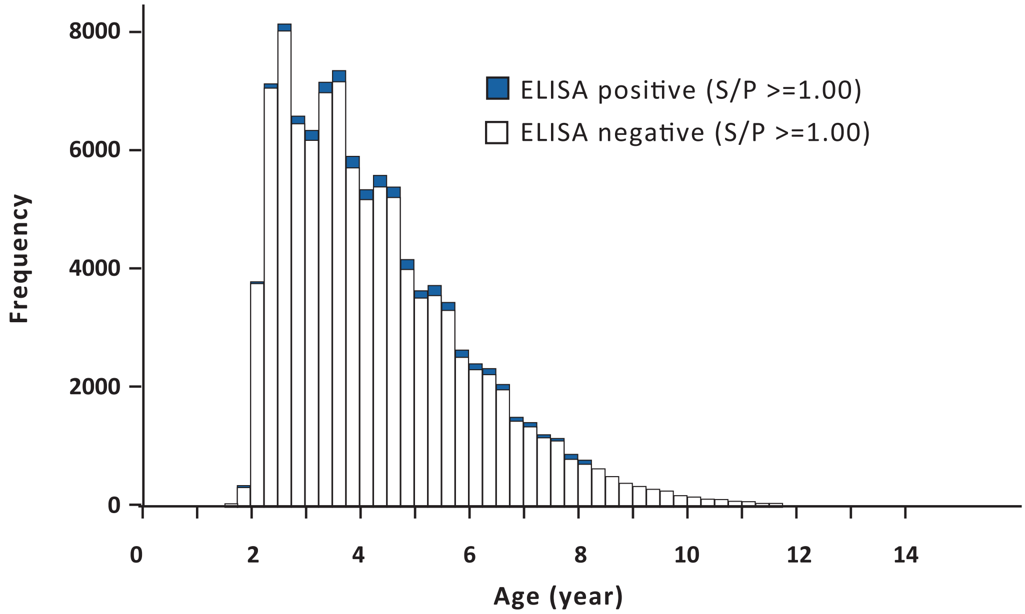

Figure 6. Distribution of age at sampling and ELISA result of 112,773 milk and serum samples collected between Jan 2006 and March 2018 from 47,162 cattle in 136 herds.

The shape parameter of the Weibull model was found to be significantly different for the three groups of cattle (shape ± SE = 1.58 ± 0.05, 1.89 ± 0.06, 3.18 ± 0.18 for cattle born before the initial assessment of the herd, within five years after the initial assessment and more than five years after the initial assessment, respectively). Therefore, no global analysis was possible and separate analyses were performed for the three groups of cattle. Given that the study herds entered the programme in 2006–2007, the maximum follow-up period for cattle born more than five years after the initial assessment was approximately seven years.

The fitted cumulative failure (i.e., the proportion of cattle that became ELISA positive prior to a specific age) of cattle born within five years after the initial assessment was always lower than the fitted cumulative failure of cattle born before the initial assessment (Figure 7). Until 6.5 years of age, the same applied to cattle born more than five years after the initial assessment (Figure 7); at 6.5 years of age, the 95% confidence intervals of the cumulative failure curves started to overlap. Thus, until at least 6.5 years of age, the proportion of cattle remaining ELISA negative was higher in cattle born after the initial assessment of the herd.

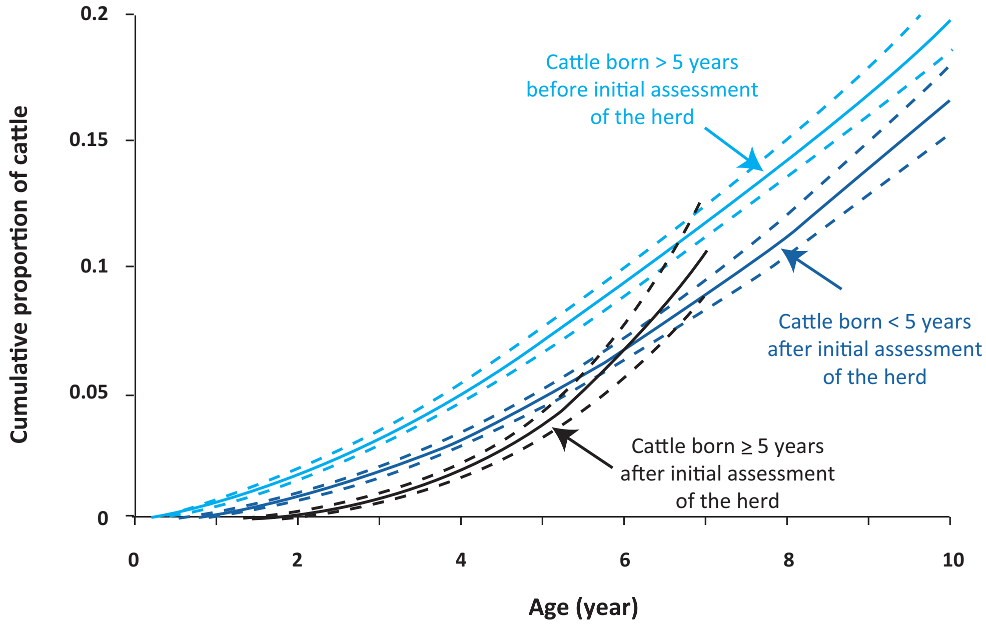

Figure 7. Fitted cumulative failure curves showing the proportion of cattle that became ELISA positive before a specific age. Data of cattle born <5 years before the initial assessment of the herd (14,153 cattle from 136 herds), < 5 years after the initial assessment of the herd (16,173 cattle from 124 herds) and ≥ 5 years after the initial assessment of the herd (12,472 cattle from 113 herds). Dashed lines indicate the 95% confidence interval around the estimates.

4. DISCUSSION

The aim of this paper was to provide an update on the progress obtained over a twelve-year period in the first cohort of herds that voluntarily entered the MQAP in 2006–2007 and to evaluate the effect of control on the age at onset of ELISA positivity in cattle in infected herds.

The results show that, despite frequent transitions between herd statuses (Figures 2 and 3), the overall proportion of herds with status A increased over time and stabilised at around 75% to 80% (Figure 4). This is in line with the proportion of herds with status A in the general population of Dutch dairy herds (Anon, 2018).

The apparent prevalence presents a biennial saw tooth-like pattern. This pattern is related to the different test intervals between herd statuses as only herds with positive test results at the initial assessment were obliged to perform a herd examination in year 1, whereas both positive and initially negative herds were retested in year 2. However, the apparent prevalence in odd years as well as the apparent prevalence in even years decreased over time (Figure 5A). Moreover, the apparent prevalence in heifers, at their first test, decreased over time (Figure 5B), indicating a decreasing rate of transmission of the infection. This decreasing transmission of MAP might be due to culling of infectious cattle as well as preventive management measures taken by farmers.

The decreased probability of heifers becoming ELISA positive was also observed in a subset of 129 dairy herds which were most likely to be infected (Table 1). Heifers born after the initial assessment had substantially lower odds of being ELISA positive at their first test, even after correction for effects of other risk factors for which data were available. The highest S/P ratio of the dam, in serum and milk samples submitted between Jan 2006 and March 2018, was associated with the odds of a heifer being ELISA positive as well (Table 1). Given that the initial apparent prevalence in the herd was included in the model as well, correcting for the fact that in a herd with a high prevalence both dam and daughter are more likely to be test-positive, this observation could be a reflection of vertical transmission of the infection. However, estimates of the effect of time at which the heifer was born, before or after the initial assessment, were identical if the variable 'highest S/P ratio of the dam' was excluded from the analyses (data not shown).

The fitted cumulative failure curves (Figure 7) indicate that the observed decrease of the probability of heifers being ELISA positive corresponded with a delayed onset of ELISA-positivity. Given that the infectious dose is associated with onset of ELISA-positivity (Mortier et al., 2014), the delayed onset of ELISA-positivity is indicative of a reduction of the infection pressure on the cattle that were tested in the subset of herds. Moreover, the delayed onset of ELISA positivity might be indicative of a delayed onset of infectiousness in infected dairy herds after sustained participation in the milk quality assurance programme.

The shape of the cumulative failure curves (Figure 7) differed between the various groups of cattle. At six years of age, the cumulative failure curve of cattle born ≥ 5 years after the initial assessment of the herd crossed the curve of cattle born < 5 years after the initial assessment. Thus, beyond six years of age, a higher proportion of cattle had become ELISA positive (and a lower proportion of cattle remained ELISA negative) in the group of cattle born ≥ 5 years after the initial assessment than in the group of cattle born < 5 years after the initial assessment. However, in comparison to the effect of the delayed onset of ELISA positivity in cattle younger than six years of age, this is likely to have only a minor effect on transmission of the infection because less than 20% of sampled cattle in the participating herds is more than six years old (Figure 6).

In summary, we observed in this first cohort of herds that voluntarily entered the MQAP in 2006–2007, that the proportion of herds with status A steadily and consistently increased over time, that the apparent prevalence decreased over time, that the risk of heifers being ELISA positive decreased with sustained participation in the MQAP, and that the onset of ELISA-positivity was delayed in cattle born after the herd entered the MQAP. These observations are indicative of a reduced transmission of MAP after long-lasting participation in the MQAP and therefore indicate that the MQAP positively contributes to the control of MAP in the Dutch dairy population.

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5

ESTABLISHING A NATIONAL VOLUNTARY CONTROL PROGRAMME FOR JOHNE'S DISEASE IN IRELAND

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1. INTRODUCTION

The purpose of this paper is to present the achievements and lessons learned from the implementation of a three-year pilot Johne's disease control programme (2013–2016), the outline of which was presented to the ParaTB Forum in 2014, (Mullowney & Strain, 2014). Based on the information generated from the Pilot Programme, the subsequent development and implementation of a national voluntary control programme will also be discussed.

Prior to 1990, the occurrence of Johne's disease in Ireland was very uncommon, but a relaxation of strict quarantine and import policies and the subsequent free movement of goods, including cattle, after the introduction of the Single European Market within the European Union is thought to have led to the current situation of an apparent increasing prevalence of disease (Barrett, 2011).

An early Pilot Herd Health Programme which was subsidised by the Department of Agriculture, Food and the Marine (DAFM) was initiated in 2004 and demonstrated that effective control of Johne's disease could be initiated by some farmers but that such an approach required a significant commitment and sufficient levels of resourcing in order to communicate programme goals, the limitations and appropriate use of diagnostic tests, and the importance of biosecurity to differing stakeholder groups, often with diverging priorities and expectations. There were also concerns at the time about the scalability of the approach utilised in the Pilot Herd Health Programme (2004–2009) (Mullowney & Strain, 2014).

Following a period of lengthy consultation between industry stakeholders co-ordinated by Animal Health Ireland through a Johne's disease Implementation Group (JDIG; Mullowney & Strain, 2014) a Johne's Disease Pilot Dairy Control Programme (the Pilot Programme) was launched in October 2013.

The purpose of the Pilot Programme was to:

- Test, evaluate and refine the various programme components, including data handling, diagnostic and on-farm advisory elements which would be required to support a future, extended Johne's disease control programme in Ireland.

- Generate information in relation to the control of Johne's disease on dairy farms in Ireland, including that relating to the economics of the disease and its control, in order to assist the design of a future national or expanded Johne's disease control programme.

2. AIMS AND OBJECTIVES OF THE PILOT PROGRAMME 2013–2016

a. In herds without confirmed evidence of infection:
To identify those herds that test negative for Johne's disease and provide these farmers with the knowledge and professional support to allow them to increase their confidence over time of being free of infection and to protect their herds from the on-going risk of introduction of this disease (bio-exclusion).

b. In known infected herds:
To provide herds identified by the programme, or otherwise, as being infected or having a low confidence of infection freedom, with the knowledge and professional support to allow them to control and reduce the prevalence of the disease over time and ultimately to achieve a high confidence of disease freedom (bio-containment).

c. Market reassurance:
To underpin the quality of Irish dairy and beef produce in the international marketplace.
It was assumed at the beginning of the pilot programme that any future national programme would have similar aims and objectives.

3. STRUCTURE OF THE PILOT PROGRAMME

The Pilot Programme in Ireland drew on international experience (Geraghty, et al., 2014) in the control of Johne's disease and coupled this with the best understanding and advice about the then current situation in Ireland.

Kennedy et al. (2014) and McAloon et al. (2016) have demonstrated that the same risk factors for disease transmission operate in Ireland as in the rest of the world. Thus, the most effective strategy for reducing prevalence and disease control in this country was also likely to require farmers to understand and control the risks associated with inward stock movements along with instituting management measures which reduced within-herd transfer, especially in the calving and pre-weaning areas.

The introduction of cattle was recognised as the main method by which infection was introduced to a herd. Ashe and More (2009) examined the survival and dispersability of a cohort of cattle (dairy and beef) over a period of four years and confirmed that stock movements did occur frequently within Ireland. More et al. (2013) concluded that the number of livestock introductions had the greatest impact on the ability of a test-negative herd to maintain confidence of Johne's disease freedom and that the "results highlight the critical role of on-farm risk assessments and management plans in the management of Johne's disease risk with particular emphasis on bio-exclusion practices in test-negative herds".

In consideration of the available epidemiological evidence, a Pilot Programme was developed which included:

- individual herd risk assessments using standardised risk assessment tools and leading to customised management plans and,
- herd testing for the early identification of disease and to monitor progress in disease control.
- In addition, private veterinary practitioners who had undergone formal training by AHI (Animal Health Ireland) in the conduct of herd risk assessments and the development of management plans (VRAMPS) using standardised risk assessment tools were designated as approved veterinary practitioners, (AVPs). These private veterinary practitioners were the principal contact point for knowledge transfer to herd owners, supported by AHI, through the provision of web-based information and the circulation of regular newsletters. A Technical Working Group developed the tools and testing strategies to underpin the Pilot Programme and also supported the collation of test data which had the dual objectives of early disease detection and monitoring the progress of disease control in herds known to be infected.

4. ACHIEVEMENTS OF THE PILOT PROGRAMME

Over the course of the three years during which the Pilot Programme operated, the data generated considerably enhanced the understanding of Johne's disease and the options for infection control in Irish dairy herds.

The data from the Pilot Programme was used to generate information which would enable producers to control Johne's disease and to protect their herds from the on-going risk of disease introduction. Professional support and advice in interpreting the information available to herd owners was shown to be invaluable in an analysis of a Johne's disease producer survey, which was commissioned as part of the Pilot Programme. Veterinary practitioners were identified as the central point of contact for information for farmers in relation to disease control matters, indicating the importance of this pathway to farmer engagement and compliance with the programme. The analysis also highlighted the elements of the programme in which farmers have engaged and those elements which will require additional interventions and communications (Devitt et al., 2016).

The Pilot Programme concluded in December 2016, by which time negotiations had commenced on the structure and scope of a future national control programme. The key achievements of the Pilot Programme are presented below:

- 1,899 herds enrolled in the Pilot Programme.
- 2,400 risk assessments were carried out by trained vets (Approved Veterinary Practitioners) on the farms of participants.
- Development of Standard Operating Procedures to guide vets carrying out the risk assessments.
- Provision of veterinary training by AHI to 561 private veterinary practitioners, to equip them to carry out VRAMPs and provide Johne's disease advice to farmers on biosecurity and to test interpretation.
- Development of an interactive web-based map to assist producers locate trained veterinarians.
- Development of a network of private laboratories designated to provide testing for the programme according to defined criteria, including accreditation to ISO 17025.
- Development of a programme database housed at the Irish Cattle Breeding Federation (ICBF) to manage test results from designated laboratories and VRAMP information.
- An evidence-based system for allocating herds into risk categories based upon Johne's disease test results and animal movements was developed by a Technical Working Group and will form the basis of a Herd Assurance Score.
- Development and execution of a comprehensive communication plan which included activities such as the production and distribution of a number of evidence-based information leaflets, based on advice provided by the Technical Working Group.
- Communication to farmers, vets and other stakeholders through public meetings, website, social media, bulletins and other channels, with a view to improving understanding of the disease and the control programme.
- Completion of a number of research projects including:
- An economic analysis by Botaro et al. (2016).
- A social research investigation of farmers' attitudes towards disease management by McAloon et al. (2017).
- Completion of two stakeholder surveys (of farmers and approved veterinary practitioners) commissioned by AHI (Devitt et al., 2016) and (Devitt et al., 2018).

5. LESSONS LEARNED

Data collected during the pilot programme (2014–2016) and from the first six months of the Irish Johne's Control Programme (2017–) confirmed that management changes had occurred and that overall there was a consistent reduction in average annual herd scores for all sections of the VRAMP, indicating an improvement in herd risk management in each of these areas (Figure 1). Improvements have been most pronounced in the sections relating to the management of pre-weaned heifers and the calving area. This is seen as positive news for Johne's disease control in Ireland, since lower scores reflect lower risk as a consequence of better management. The careful management of pre-calving and calving cows and young calves is considered crucial, along with effective hygiene practices at these times for reducing the risk of Johne's disease spread within a herd.

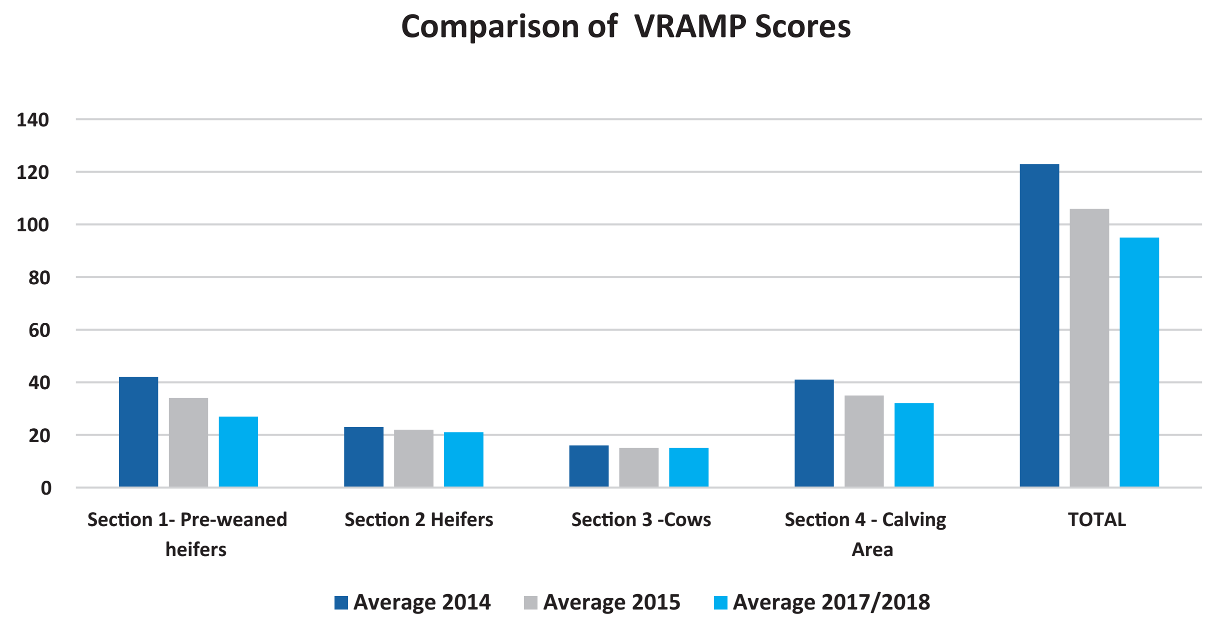

Figure 1. Comparison of paired data 2014–2015 and the current (2017/18) data set.

At the conclusion of the Pilot Programme, approximately one-third of herds were ELISA (milk or blood) test positive to a whole herd test, based on individual animal tests of all animals over the age of two years. In a sample of 616 of these herds containing test positive animals at the end of the Pilot Programme, 86% had five or fewer ELISA-positive animals. Further investigation of individual animals that are ELISA test-positive or test-inconclusive was routinely carried out as part of the Pilot Programme, provided the herds have not previously been identified as infected and this is known as ancillary testing. This practice has also been carried forward into the current Johne's disease control programme. During the Pilot Programme, ancillary testing of the ELISA test-positive animals by either faecal culture or PCR, identified 10% of those ELISA test-positive animals as confirmed infected.

At the commencement of the Pilot Programme it was assumed the national herd level prevalence in Irish Dairy herds was 20.6% (Good et al., 2009). In 2016 national herd level prevalence estimates were calculated again using data from the Pilot Programme. It was estimated that the Johne's disease prevalence amongst dairy herds participating in the AHI pilot Johne's disease programme in 2013–2014 was in the range of 23%–34% (McAloon et al. 2016).

There is considerable uncertainty as to whether these results reflect a genuine increase in Johne's disease prevalence or an apparent increase due to differences in the estimation methods used and in the nature of the populations surveyed in each study. This work has highlighted the challenges of interpreting national prevalence estimates using different statistical methods and data from different sub-populations (McAloon, 2016).

A survey of farmers participating in the Pilot Programme, commissioned by AHI, found the VRAMP helpful in improving calf health generally and in assisting them to develop strategies for managing farm biosecurity. However, support for all elements of the programme was not universal, with a minority of farmers expressing concerns, particularly about the number of changes which were suggested by AVPs and questioning the practicality of implementing some of these.

The irish johne's control programme (IJCP) (2017–)

Consultation with stakeholders on the future of the Johne's disease control in Ireland began in the last quarter of 2016, as the Pilot Programme was drawing to a close. During that time stakeholders:

- Recognised that the presence of Johne's disease in the cattle herd in Ireland presented a risk to both their individual businesses and to the Irish agri-food sector as a whole and were of the view that a structured control programme was required to address these risks.
- Reaffirmed that a future national control programme for Johne's disease should retain both the farm-level and market-level objectives that were being tested in the Pilot Programme. Stakeholders also expressed the view that Johne's disease could not be effectively controlled in the absence of a robust, well designed farm-based intervention.
- Explored options to broaden the remit of Johne's disease control measures, for the Irish programme to reflect an international trend towards broadly based programmes focussed on biosecurity and potentially incorporating other significant animal health conditions into a structured control programme.
- Recognised the difficulties of sustaining Johne's disease control efforts in the longer term and identified the requirement for programme participation to be as broadly based as possible, at farm, processor and State level, to include an equitable sharing of costs between these parties, all of whom benefit to varying degrees.

In late 2017, the national dairy industry took the significant step of implementing a voluntary Johne's Control Programme which provides pathways for test-negative and test-positive herds to demonstrate progress towards an improved confidence of freedom from infection. The IJCP objectives have expanded on those of the previous Pilot Programme to now include a reference to calf health and biosecurity generally, foreshadowing future enhancements to the programme. Currently the IJCP is based on a veterinary risk assessment and management plan (VRAMP), and annual whole herd testing, leading to a herd assurance score. Additional testing of ELISA test positive animals, by PCR or faecal culture, in herds where infection has not previously been identified, as well as a significantly enhanced communication and awareness programme for farmers and their advisers has also been incorporated.

The programme has been restructured to more clearly identify the role different activities contribute to a national control initiative. (Figure 2)

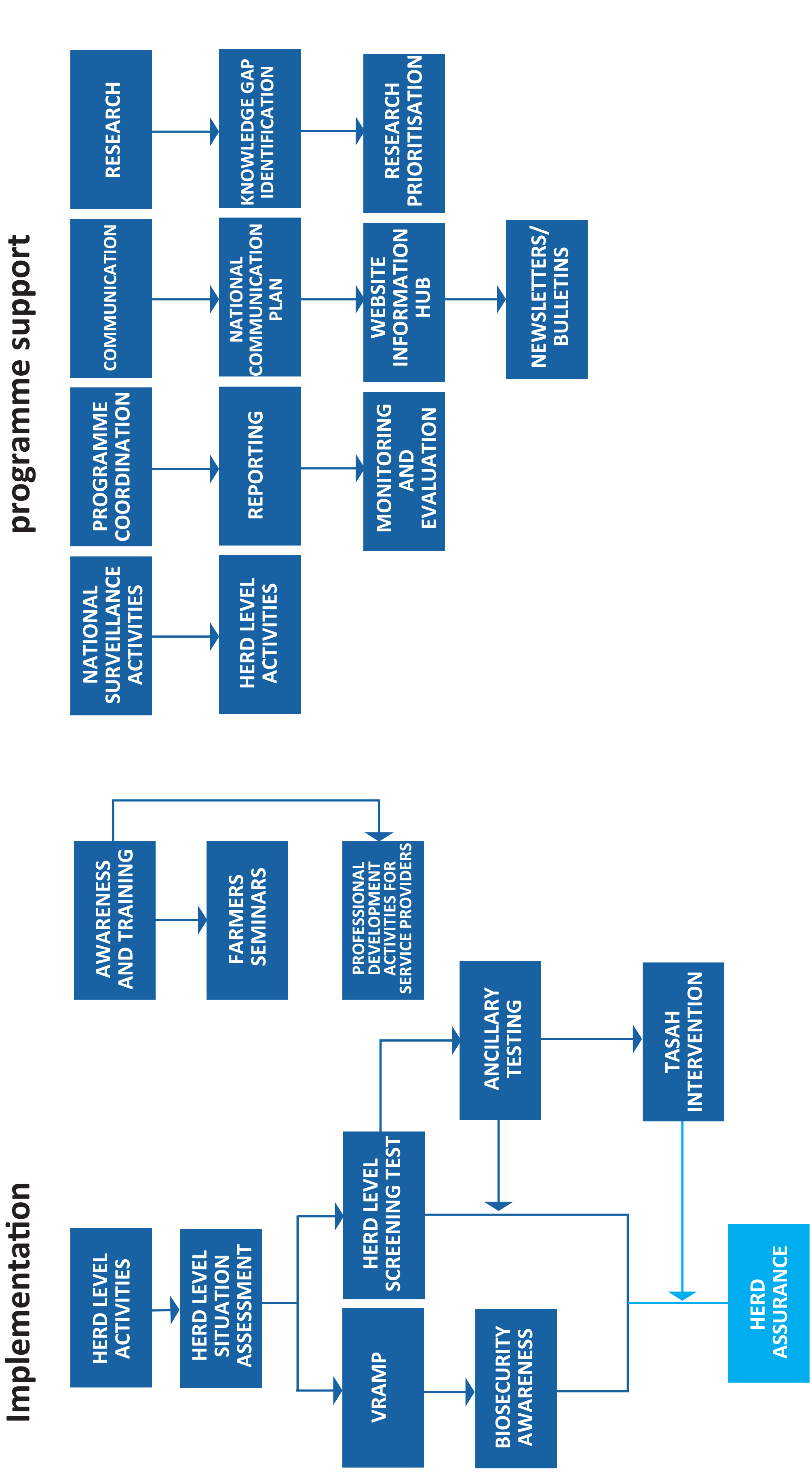

Figure 2. Structure of the Irish Johne's Control Programme.

The herd assurance elements provide the foundations for herd level Johne's disease control, upon which farmers can build individual herd health management plans to minimise the risk of Johne's disease introduction and spread within their herds. The programme support elements contribute to the overall national effort to control Johne's disease.

The IJCP represents a significant step for the Irish dairy industry in developing a long-term approach to control of Johne's disease which recognises the value of effective and on-going disease prevention and containment practices to control the spread of an endemic production disease which, given the characteristics of the bacterium and the present limitations of tests, is not considered eradicable at a national level.

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6

CONTROL OF PARATUBERCULOSIS IN THE CZECH REPUBLIC

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1. INTRODUCTION

Historically, paratuberculosis was included in the Czech Veterinary Law as a notifiable dangerous disease and this was consequently reflected in the measures taken. The first approach to control of paratuberculosis was based on Methodological direction 6/2001. It was based on the ordered control of paratuberculosis only in dairy cattle herds based on culture and was supported by the government (financial compensation for farmers for the laboratory examinations and for the culled animals). The basis of this programme was to test all animals up to 18 months old twice per year by cultivation. All animals found to be positive by culture in faeces (i.e., not only animals in a clinical state of the infection) had to be removed immediately. However, despite the financial compensation for removed animals and culture examination of the herd, there were no significant results. Moreover, the time needed for the control was long and farmers violated the recommendations. The most serious problem with this regulation was the extraordinarily severe measures issued by the veterinary administration. Declaration of an outbreak of a dangerous disease, together with the closure of the breed and the ban on movement and trade of animals, was hugely difficult for the farmer when paratuberculosis was diagnosed in the herd.

In 2008, the paratuberculosis control strategy changed and the new Methodical Manual of the State Veterinary Administration No.5/2008 was issued. This document recommended performing ELISA as the preferred method of testing (and to confirm positive cases by culture or PCR). All positive findings had to be reported and the State Veterinarian had to visit the herd. If there were no clinical signs of paratuberculosis in the herd, no precautions were taken. If clinical signs were detected, the outbreak was declared and all clinical cases had to be confirmed by direct detection method (culture or qPCR). No exceptional and strict preventive measures were taken and even the sale of animals, to a limited extent, was allowed. The infection in the herd was controlled by ELISA and qPCR or culture at defined time intervals. After the elimination of clinical signs of paratuberculosis in the herd, the ban on trade and movement of animals was cancelled.

Because, historically, control of paratuberculosis has not been supported by either state

compensation or by insurance companies, the will of the farmers and owners of the cattle to control paratuberculosis has been rather weak since 2008. Moreover, paratuberculosis was still included in the Veterinary Law and therefore, farmers did not want to complicate their businesses by restrictions connected with the declaration of a paratuberculosis outbreak. These issues led to the reduction in numbers of herds included in some control programmes. Moreover, some interested farmers started to do their own testing mainly based on ELISA and removed positive-testing animals. However, these efforts were not organized and there were no generally accepted and approved recommendations or measures for farmers and veterinarians to take.
In October 2017, an amendment of the Veterinary Law was made and paratuberculosis is no longer a notifiable disease.

2. AIMS AND OBJECTIVES

The objective is to refocus the attention of farmers on their control of paratuberculosis.

3. LESSONS LEARNED

Approaches from the past, relying solely on a single method, either failed or do not have any measurable effect. The problem is also in the continuing lack of inclination by farmers to control paratuberculosis due to the past restrictions arising from the inclusion of paratuberculosis in Veterinary Law. Currently, all the farmers who are predisposed to carry out some control of paratuberculosis believe ELISA is the best method (cheap, however, not specific). The breeder associations just promote this situation.

4. IMPROVEMENTS MADE

We propose to involve farmers more deeply in the control of paratuberculosis. The main motivation for them to abide by the recommendations is through ensuring costs are kept as low as possible. Therefore, it is important to propose a complete solution for control of paratuberculosis to farmers. This package should include cost effective, fast and reliable testing and control methods and clear recommendations for different scenarios. Specifically, it should recommend: which approach should be used to determine the presence of infection in the herd and how prevalent it is within the herd; which methods should be used for the control of young animals entering the herd and at what time intervals; how to effectively remove infected animals from the herd at low cost, and how to keep the herd at very low serological and bacteriological prevalence of paratuberculosis and showing no clinical signs, etc. It would be very advantageous if some internationally advised recommendations could be issued in order to support our efforts. These measures are generally applicable to dairy herds, however, control of paratuberculosis in beef cattle, sheep and goats in the Czech Republic is not covered at all, which represents a sleeping problem for the future.

5. ACKNOWLEDGEMENTS

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7

ADVANCES AND CHALLENGES OF PARATUBERCULOSIS IN BRAZIL

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1. INTRODUCTION

Brazil is the fifth largest country in the world, with 8,515,767 km2, located between latitudes 5°16’19” North and 33°45’07” South, between meridians 34°47’34” and 73°59’26” West. Its coastline is bathed by the Atlantic Ocean and borders almost all South American countries except Chile and Ecuador. With a large territorial area, a diversified climate, regular rainfalls, abundant solar energy and almost 13% of all freshwater available on the planet, Brazil has 388 million hectares of fertile arable land with high productivity, of which 90 million still are not explored. All these factors contribute to making Brazilian agribusiness a prosperous, safe and profitable activity. This scenario is a positive evolution that goes back to the period of Brazil-Colony, with the cultivation of monocultures and animal husbandry.

Despite the economic recession in Brazil, the agricultural sector is one of the few that has sustained positive growth rates in the country. Agricultural activity accounts for 21% of Gross Domestic Product (GDP), 27% of jobs and 43% of exports. Even with good results, it is believed that these figures could be higher in livestock if there was greater commitment from animal health services and government incentives. We also believe that effective communication between government and the researchers could assist in identifying problems that affect livestock and help to solve them together.

Some diseases, such as Foot-and-Mouth Disease, Brucellosis and Tuberculosis, have established National Programs that have made it possible to control and achieve the target of the eradication of some of them. However, in relation to Paratuberculosis, there is still no control program, nor an apparent interest from the competent bodies, although the presence of the disease in the Brazilian herd is known.

The first identification of Paratuberculosis in the country was registered in 1915 by Octávio Dupont in Flemish cattle imported from Belgium to Rio de Janeiro. Since then, it has been thought that the disease was exotic and cases only restricted to imported animals. However, Dacorso Filho et al., in 1960, identified the disease in cattle born and raised in Brazil. Reports of the disease were verified in different Brazilian states demonstrating that the agent is actually spread among the national herds.

The studies regarding the distribution of Paratuberculosis in the Brazilian states is still based on different methodologies and different types of samples, which make it difficult to compare and analyze the results. Therefore, the actual prevalence detection in the country is unknown. In a review by Espeschit et al. (2017), the authors found that among Latin American countries, Brazil is one of the countries that stands out in the number of articles published on Paratuberculosis. The bovine is the most studied species, followed by goats and sheep and there is a discrete production of articles focusing on cheese, milk and water. The authors also observed that microbiological (culture), serological (ELISA) and molecular (PCR) tests prevailed for the following purposes: i) detection of the agent (most articles); ii) epidemiological studies; iii) detecting the frequency of anti-MAP antibodies; iv) preservation of the sequence IS900; v) recombinant protein construction for MAP detection and differentiation of M. bovis; vi) evaluation of different formulations of HEYM medium for faecal culture; vii) identification of risk factors for the disease; viii) typing of MAP strains; ix) study of coinfection in mammary glands and; x) detection of the pass-through phenomenon. The studies come from research groups located almost throughout the entire country, but with greater concentration in the Southeast, Northeast and South regions. Table 1 shows, in greater detail, the studies sampled by Espeschit et al., (2017) and includes more recent papers.

Species	Objective of the study	Techniques used	Authors
Goats and sheep	Reporting digestive disease diagnosed at the Veterinary Hospital	Historical research of clinical cases	Lira et al. (2013)
Cattle, goats, sheep, etc.	Gathering epidemiological data, clinical-pathological and laboratory disease in the country	Culture, ELISA, PCR, etc.	Yamasaki et al. (2013)
Cattle	Reporting the epidemiological aspects of MAP infection in dairy cattle of Garanhuns-PE	Serology (ELISA), investigative questionnaire	Sá et al. (2013)
Buffaloes	Characterizing an outbreak in the southern region	Serology (ELISA), culture, PCR and immunohistochemistry	Dalto et al. (2012)
Goats and sheep	Estimating the frequency of antibodies in animals, in Paraiba	Serology (ELISA) and culture	Medeiros et al. (2012a)
Cattle	Performing serological investigation in herds with and without history of the disease (ELISA)	Serology	Medeiros et al. (2012b)
Commercial milk	Investigating the presence of MAP in commercial milk samples	Culture, nested PCR and genetic sequencing	Carvalho et al. (2012a)
Cattle	Assessing the genetic conservation of sequences of IS900 used for raw milk detection	PCR milk, genetic sequencing and bioinformatics (polymorphism)	Carvalho et al. (2012b)
Cattle	Reporting pathology of three positive animals in Rio de Janeiro-RJ	serology (ELISA) andhistopathology	Rodrigues et al. (2012)
Cattle	Evaluating the efficiency of recombinant protein MAP, serological tests	Cell culture, immunofluorescence, immunohistochemistry and serology (ELISA)	Souza et al. (2011)
Buffaloes	Investigating and characterizing paratuberculosis and its clinical and pathological changes in this species in Pernambuco	Histopathology, Ziehl-Neelsen technique in feces and PCR	Mota et al. (2010)
Goats and sheep	Reporting the occurrence in these species in the Northeast and characterizing the clinical and pathological changes	Intradermal reaction test and histopathology	Oliveira et al. (2010)
Bovine	Evaluating the efficiency of two recombinant proteins of M. bovis (MPB70 and MPB83) in serological tests to differentiate infections caused by MAP from those caused by M. bovis	Serology (ELISA)	Marassi et al. (2010)
Cattle	Investigating the presence of MAP in dairy herds in Viçosa-MG	PCR and gene sequencing	Carvalho et al. (2009)
Cattle	Reporting clinical, anatomical and pathological condition in a dairy herd of Paraíba	Histopathologyandculture.	Mota et al. (2007)
Cattle	Evaluating the effect of tuberculin on ELISA	Serology tests (ELISA) and culture	Varges et al. (2009)

Species	Objective of the study	Techniques used	Authors
Cattle, goats, sheep, etc.	Reviewing the disease	Culture, ELISA, PCR, etc.	Lilenbaum et al. (2007)
Cattle	Reporting the outbreak of the disease	Serology (ELISA and AGID), histopathology and culture	Ristow et al. (2007)
Cattle	Evaluating three different formulations of HEYM in four faecal culture protocols	Culture	Ristow et al. (2006)
Cattle, goats, sheep, etc.	Characterizing a new locus (VNTR-MIRU) in MAP genome isolated from MAC in the South	Culture America, RFLP, PCR and Tandem Repeat	Romano et al. (2005)
Cattle	Standardizing ELISA for use in the country	Serology (ELISA)	Marassi et al. (2005)
Cattle	Evaluating the AGID technique and its applicability in the field	Serology (ELISA and AGID)	Ferreira et al. (2002b)
Cattle	Serologically evaluating the serological tests to detect the disease in herds in Rio de Janeiro	Serology (ELISA)	Ferreira et al. (2002a)
Cattle	Describing the clinical and pathological picture of affected animals	Histopathology and culture	Driemeier et al. (1999)
Cheese	Detecting MAP in curd cheese samples	Cultureand PCR	Faria et al. (2014)
Cattle	Making an agent of the survey conducted in a Brazilian state	ELISA	Costa et al. (2010)
Cattle	Detecting the agent in a Brazilian state	ELISA	Laranja-da-Fonseca et al. (1999)
Buffaloes	Reporting the detection of MAP in uterus and fetus	Histopathology and PCR	Belo-Reis et al. (2016)
Buffaloes	Reporting farms positive for paratuberculosis	Histopathology and PCR	Farias Brito et al. (2016)
Cattle	Determining the prevalence of MAP infection and identifying risk factors associated with herd-level prevalence	Serology	Vilar et al. (2015)
Goats	Identifying and typing MAP	Culture, PCR and REA	Souza et al. (2016)
Water	Identifying and typing MAP	Culture, PCR and REA	Espeschit et al. (2017)
Milk	Identifying MAP in milk samples from positive herds	Real time PCR, and PCR	Albuquerque et al. (2017)
Goats	Passive shedding of MAP	PCR, sequencing	Schwarz et al. (2017)
Cattle	Co-infection with E. coli in MAC-T cells	cell culture and RT-PCR	Schwarz et al. (2018)

Table 1. Indexed studies regarding paratuberculosis in Brazil.

Regarding the study of MAP in humans, it was verified in indexed articles that the country has few studies that seek to evaluate the contribution of bacteria in the inflammatory process in patients with inflammatory bowel diseases, amongst them, Crohn's disease. These studies aimed to detect, quantify and evaluate the risk factors for the presence of MAP in intestinal biopsies and were harvested from scientific databases, as performed by Espeschit et al. (2017) and are available in Table 2.

Species	Objective of the study	Techniques used	Authors
Human biopsies	Detection and quantification of MAP in intestinal biopsies	Real time PCR	Carvalho et al. (2015)
Human and pigs isolates	Identification of Two Novel Mycobacterium avium Allelic Variants in Pig and Human Isolates	PCR-Restriction Enzyme Analysis	Leão et al. (1999)
Human biopsies	Risk factors analysis for the presence of MAP in intestinal biopsies	Uni and bivariated analysis	Espeschit et al. (2017)

Table 2. Indexed studies regarding MAP in humans in Brazil.

2. LESSONS LEARNED

Although studies on Paratuberculosis in Brazil have significantly increased in quantity and quality in the last decades, they are still few when compared to other countries with a tradition in research. In addition, most national surveys present results that do not seek continuity of research or do not present an innovative contribution on the subject addressed. The lack of greater integration among existing working groups, associated with the lack of funding and the lack of incentive of the Brazilian government are factors that contribute to this situation. In this sense, it is evident that the groups that work with Paratuberculosis need to unite, discuss, define the work, set goals and plan a research direction that consolidates the theme and shows the importance of the disease in the national scenario and its implication in the international strategy.

3. IMPROVEMENTS MADE

In Brazil there is no National Program for the disease, but Paratuberculosis is included in the list of diseases of mandatory and immediate notification of any confirmed case to the Official Veterinary Service (IN nº 50 24/09/2013), but within this IN, the test which should be used for confirming the disease is not defined. The practice of vaccination, widely used in several countries that have instituted the Paratuberculosis control program, is still banned in Brazil for interfering with the diagnostic tests for bovine and buffalo tuberculosis provided in the National Program for the Control and Eradication of Brucellosis and Animal Tuberculosis. The lack of knowledge of the real economic and social importance of Paratuberculosis in Brazil portrays the disease as of little relevance for the government to institute control measures. The partnerships of different national and international institutions are fundamental, not only for the elaboration of research with results of real practical importance, but also to impel a more concrete position of the government for the control of Paratuberculosis in Brazil.

The control of Paratuberculosis in Brazil is still hampered by the extensive forest areas that shelter wild animals that can be potential carriers of the etiological agent. There are no indexed studies investigating the importance of these animals in the epidemiological chain of Paratuberculosis in the national territory, nor the risk of transmission to domestic animals.

There are still many challenges to improve the research and control of Paratuberculosis in Brazil, but these challenges could be successfully achieved, depending on the interest and effort of each of the segments of the society involved.

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8

PARATUBERCULOSIS IN COLOMBIA: PAST, PRESENT AND FUTURE

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1. COLOMBIAN STUDIES ON PARATUBERCULOSIS

Twenty-six original studies referring to Johne´s disease (JD) and *Mycobacterium avium* subsp. *paratuberculosis* (MAP) detection have been carried out in Colombia. So far, no study in Colombia has attempted the detection of MAP in food or humans. In addition to the 26 original studies mentioned above, 14 reviews, case reports, case series reports, and editorials were not considered in this document, but they are of great value for the national knowledge on MAP or JD and demonstrate the national concern about MAP and its impact in Colombia through several decades. According to an unavailable document by Plata-Guerrero (1931), the existence of MAP in Colombia was first documented in 1924 by the Cuban veterinarian Ildefonso Pérez Vigueras, in a herd of imported cattle of the municipality of Usme (Province of Cundinamarca; Vega, 1947).

Most studies on MAP or JD have been undertaken during the present decade and most concern animals of the Provinces of Antioquia and Cundinamarca, some in Caldas and Tolima, and a few in Nariño and Boyacá. The original studies on MAP in Colombia have reported results from cattle, sheep, goats, and buffaloes. Research on cattle has been the most common compared to sheep and goats, and buffaloes. Other relevant species in the country (wild mammals or humans) have not been found or cited in any original study reviewed. The most common diagnostic test used to investigate MAP in Colombia is the enzyme-linked immune-assay (ELISA). This is followed by microscopy on Ziehl-Neelsen (ZN)-stained samples (on feces, rectal mucosa scrapings, or tissues), polymerase chain reaction (PCR), intradermal Johnin test (IJT, with bovine and/or avian-purified protein derived), culture (from feces or tissues, and individual or pooled), complement fixation, indirect immuno-fluorescence, and counter immuno-electrophoresis. The studies published so far include cross-sectional designs, diagnostic test comparisons, risk factor analyses, and clinical trials (on treatments). Thus far, no cohort or case and control study has been published in Colombia.

2. WHAT ARE COLOMBIAN STUDIES ON PARATUBERCULOSIS TELLING US?

According to several anecdotal reports and opinions, the national or regional distribution of MAP or JD in cattle and small ruminants in Colombia is not homogeneous or conclusive. Some academics and producers consider JD as a significant problem, while others claim the absence or very low prevalence of MAP in farmed animals. The number of publications reporting original studies on MAP, especially JD, in recent years is relatively low compared to other countries in Latin America (Fernández et al., 2014), but higher than expected for Colombian conditions. The increasing number of publications suggests a growing interest on MAP research in the country, as well as an increasing concern about this microorganism and its negative effects on animal health, animal production, and its zoonotic potential (public health impact) from the academic and producers. Although JD is a notifiable disease in Colombia (ICA, 2015), it is not of major concern to animal health authorities and its control is the responsibility of the farmer. (Anonymous, 2010a; Fedegán, 2010; Fernández et al., 2014). This could explain the low number of initiatives for the research, prevention, and control in animals, as well as for the detection of the microorganism in food, the environment, and humans.

The locations of most Colombian studies do not follow a clear trend but could be related to the high concentrations of cattle in some of the Provinces (i.e., Antioquia and Cundinamarca; ICA, 2017), or to the interests of academics, scientists, or cattle producers. Since the first report in 1924, Cundinamarca has been a Province with common reports of JD (Vega, 1947; Huber, 1954; Isaza, 1978; Mogollón et al., 1983; Góngora & Perea, 1984; Mancipe et al., 2009). This could be explained by the long tradition of the Facultad de Medicina Veterinaria of the Universidad Nacional de Colombia in Bogotá, the oldest faculty of veterinary medicine in the country, where the first studies in the early 20th century were carried out. More recently, the Province of Antioquia has been publishing the majority of original studies, all of them from academics at the Universidad de Antioquia and the Universidad CES. As expected, studies on cattle were the most common, most likely due to the size of the population in the country and to the production systems related to milk and meat. In contrast, studies on sheep populations are less common in the country and could be due to their smaller populations (ICA, 2017).

The common use of ELISA, ZN-staining and IJT is not surprising given their relatively low cost and the availability of materials, qualified personnel, and infrastructure for these types of tests. However, the use of culture and PCR is becoming more common and could be related to the recent development of the diagnostic capacity in universities, compared to national laboratories and to the expansion of the reagents and equipment supplies for such diagnoses in the country. The absence of cohort and case-control studies is common in animal health research in Colombia. These high-profile observational studies, as well as the experimental approaches are more complex, laborious, demanding, and expensive, given the microbiological and pathophysiological characteristics of MAP. Nevertheless, the current MAP situation in Colombia demands additional observational studies in addition to surveys and case reports to enhance our comprehension of the epidemiological situation and to assess the true zoonotic threat.

Definitively, the country needs to cover some knowledge gaps to get to a true understanding of the disease. It is necessary to define the exact status of the disease through well-designed prevalence/incidence studies, considering that there is no national data available. In reference to this, just some local estimates are available so far (Patiño & Estrada, 1999; Ramírez et al., 2001; Fernández et al., 2011a; 2011b; Benavides et al., 2016; Correa-Valencia et al., 2016). The harmonization of diagnostic methods, considering the epidemiologic and biological behavior of MAP under local agro-ecological, productive, and cultural conditions is also needed. In addition, the laboratory infrastructure, mainly developed for foot-and-mouth disease control, should cover other entities with relevance for public health and international trade such as JD (Calderón & Góngora, 2008), improving their testing capacity and access to diagnostic reagents. It is also necessary to improve the training of farmers, making them more aware of the importance of disease control, not only of JD, but also of many others that generate economic losses and are considered a sanitary risk.

Only one study reported the molecular characterization of strains isolated in Colombian territories (Fernández et al., 2011b), and this is insufficient to consider the definition of “indigenous strains” and, ultimately, the design of vaccines. It would be necessary to carry out studies on wider regions, considering infection-assessment on cattle and other-than-cattle susceptible populations (even local wildlife) and, in this way, generate our own prophylactic strategies, according to Colombian MAP molecular and epidemiological diversity. The relationship between MAP and Crohn´s disease (CD) essentially, has not been discussed in academic fields in the country, except for some sporadic reviews (Góngora & Villamil, 1999; Calderón & Góngora, 2008; de Waard, 2010). CD has been known in Colombia since the 1950s and the incidence and prevalence rates are increasing (estimated point prevalence of 77,000 CD cases), but no national consolidated information about the disease is available (Calderón & Góngora, 2008). According to some of these authors, efforts should be made to correlate these two diseases in areas with a high prevalence or incidence of both. In general, important progress has been made on MAP research in the areas of diagnosis and epidemiology as is reported by the studies included in this report. However, many unanswered questions remain and research opportunities in the country are plentiful.

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9

LESSONS LEARNED FROM CANADIAN JOHNE'S DISEASE PROGRAMS

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1. TAKE HOME MESSAGES

- Nearly half of the Canadian dairy herds are infected with MAP
- Participation in regional Johne's disease programs was very high
- The Biosecurity module of the dairy industry's national quality assurance program (proAction) is based on the JD risk assessment
- Involvement of veterinary practitioners is crucial for success of a dairy disease control program
- Concurrent research is important to improve a program, but also to keep participants engaged
- There is a need for a national infectious disease herd status program

2. INTRODUCTION

Johne's Disease (JD) has long been identified as an important production-limiting disease of dairy cattle. In recent years, concern over public scrutiny of *Mycobacterium avium* subspecies *paratuberculosis* (MAP) as a potential zoonotic agent has brought the disease to the forefront among producer groups across Canada. Although programs targeted at JD control have been developed and implemented provincially, coordination of these programs at the national level remains an important issue to ensure some degree of uniformity, as cattle frequently move among provinces.

The Canadian Johne's Disease Initiative (CJDI) has coordinated provincial JD control activities across Canada. Since its inception in July 2009, the CJDI, funded by Dairy Farmers of Canada and the Canadian Cattlemen's Association, has been guided by its Advisory and Technical Committees (each with representation from industry, veterinary schools, and provincial programs). The CJDI priorities were to: 1) increase education about and awareness of JD across Canada among dairy producers, veterinarians and allied industries; 2) encourage development and implementation of control programs in all ten provinces and where possible, to support coordination among programs; and 3) facilitate development and funding of research programs in areas that support the coordinated mission of JD control. The CJDI reached the end of its funded mandate in 2013 and the future of a coordinated national JD initiative is uncertain.

3. AIMS AND OBJECTIVES

Given that JD control is being delivered at a provincial level, the aim of this manuscript is to describe the structure, similarities and differences among these dairy programs and to highlight important lessons learned. Objectives are to:

1. Estimate and compare prevalence of MAP infection in Western Canada, Ontario, Québec, and the Atlantic provinces, as well as among varying herd sizes and housing types;
2. Briefly compare provincial dairy cattle programs (key components, program administration, program delivery, status programs, testing, and participation);
3. Describe lessons already learned.

3.1. Herd-level Prevalence of MAP Infection in Canada

The first Canadian National Dairy Study (NDS) was completed in 2015 with an overarching objective to benchmark the health, productivity and management of the national dairy herd. The study included >1,340 dairy farms (11% of all dairy farms in Canada), of which 46% had participated in a voluntary regional Johne's disease control program. Regional programs in Canada are based on either fecal culture or PCR of environmental samples, or cow/bulk tank milk ELISA tests, thereby limiting ability to compare herd-level prevalence estimates among regions. As part of the NDS, 362 farms in all ten provinces were visited, with environmental fecal and bulk tank milk samples collected for testing. Results of bulk tank milk testing are pending.

A composite manure sample was collected from three areas on each farm: breeding-age heifer (BAH) pen, lactating cow area (alleyways), and manure storage (liquid manure pit or manure pile). Each of these three samples underwent DNA-based (PCR) testing for MAP; based on previous research, resulting in a Se and Sp of 0.38 and 1.0 for the current study, respectively. There was no difference in Se and Sp when including BAH environmental samples. Based on one-time environmental fecal testing, the percentage of test-positive farms was highest in Western Canada and Ontario (20%), moderate in Eastern Canada (12%) and lowest in Quebec (5%).

Test characteristics were applied to environmental culture results from the participating farms in all four regions, resulting in true prevalence estimates of 66% for farms in Western Canada, 54% in Ontario, 24% in Québec, and 47% in Atlantic Canada. Herds housed in tie-stalls had lower prevalence than free-stall housed herds, and herds with 101–150 and >151 cows had higher prevalence than herds with ≤ 100 cows. This was the first time MAP prevalence was determined using one detection method, one laboratory, and within a single year across Canada, enabling direct comparisons of prevalence among regions, housing types, and herd sizes.

3.2. Provincial Programs

Nine of the ten Canadian provinces currently have or have had voluntary JD control programs. In most cases, programs were producer initiated (in Québec the program was initiated by the provincial government, but with strong producer support) and are managed by committees with representation from producer groups, provincial governments, universities, milk recording and veterinary associations. Details are shown in Table 1.

Initiative	Year Initiated / Duration	$ Invested / $ to Invest	Initiative Partners
Quebec Voluntary Paratuberculosis Prevention and Control Program	2007–2016	$1.6 M	Government – Academia - Industry
Ontario Johne's Disease Education and Management Assistance Program	2010–2014	$2.4 M	Industry – Academia - Government
Manitoba Johne's Disease Initiative	2010–2013	$175 K	Government - Industry - Academia
Alberta Johne's Disease Initiative	2010–2013	$730 K	Industry - Academia - Government
Atlantic Johne's Disease Initiative[1]	2011–2014	$1.1 M	Government - Academia - Industry
British Columbia Johne's Disease Initiative	2011–2013	$100 K	Government - Industry - Academia
Saskatchewan Johne's Disease Working Group	Periodic meetings	_	Government - Academia - Industry

Table 1. Canadian Provincial JD Initiatives.

[1] Atlantic Canada includes Nova Scotia, New Brunswick, Prince Edward Island, and Newfoundland and Labrador

All programs have four key elements: 1) education of producers, veterinarians and the public; 2) an on-farm risk assessment administered by a veterinarian; 3) testing (herd and/or cow levels); and 4) applied research. Details are on the following websites:

- Canadian Johne's Disease Initiative:http://www.animalhealth.ca/aspx/public/program_id.aspx?languageid=eng&groupid=4
- Alberta: http://www.albertajohnes.ca
- Atlantic Provinces: http://www.atlanticjohnes.ca/
- Ontario: http://www.johnes.ca/
- Québec: http://www.mapaq.gouv.qc.ca/fr/Productions/santeanimale/maladiesanimales/paratuberculose/

JD control programs have reached >4,700 (>35%) of Canadian dairy farms and >60% of dairy veterinarians across Canada (Table 2). Priority on-farm JD risk areas have been identified (calving management, young calf management, and cattle additions) and targeted herd-management changes were implemented on many farms to reduce JD risks.

Program	Participating farms (%)	Dairy vets trained # (%)	Herd risk change over time (RAMP score out of 300)
Atlantic	459 (69)	49 (60)	POS herds improved 19 points NEG herds improved 6 points
Quebec	1,362 (22)	161 (47*)	N/A
Ontario	2,339 (58)	246 (>95)	ALL herds improved 8 points
Manitoba**	~200 (57)	~20	N/A
Saskatchewan	20 (12)	~10	N/A
Alberta	350 (61)	78 (95)	ALL herds improved 16 points
BC	30 (6)	11 (50)	N/A
CANADA	**4,759 (>35)**	**575 (>60)**	**Reduced risks in herds**

(# number; * 95% if indirect training was included; ** estimated; NA not available; ~ approximate)

Table 2. Impact of Regional – Provincial JD Programs.

3.3. Education of Producers, Veterinarians and the Public

Education about MAP, including spread and control, is central to all provincial initiatives. Delivery included articles in magazines and journals and presentations at conferences and meetings, as well as novel approaches such as small group facilitated self-directed learning (Roche et al., 2015). Clearly, education at both the producer and veterinarian level is a core element critical to the success and long-term viability of these programs.

3.4. On-farm Risk Assessment

The Animal Health Risk Assessment and Management Plan (RAMP) is a questionnaire which guides the herd veterinarian and the producer through a step-by-step assessment of calving, calf raising, and hygiene practices associated with promoting calf and cow health, and excellent milk quality. The goal is to identify risk factors allowing MAP from a shedding cow to infect calves on the farm. After completing the questionnaire (risk assessment), the producer and the veterinarian decide what can and will be done in the next year to mitigate some of the identified risks as part of developing the "management plan". Generally, acceptance of recommendations is good when producers realize that steps taken to reduce new MAP infections will also reduce other calf diseases caused by fecal-orally transmitted pathogens (Barkema et al., 2018).

The RAMP is the most uniform component of the provincial programs, at least in part because there is a national standard for process developed by the CJDI technical committee. Each provincial program has adhered to the standard, although the method of delivery varies. Since private veterinary practitioners are conducting these assessments, training becomes an important component of the overall program. Methods used to train veterinarians range from one-on-one training to group training to on-line web-based methods.

The proAction Biosecurity risk assessment is based on the JD RAMP.

3.5. Testing

Although all Canadian programs have a testing component, the approach and test(s) used vary, as do monetary incentives/subsidies to test. Some programs use environmental testing alone, or in combination with individual-cow testing, whereas others are based solely on individual cow test results. Cow tests in use include milk ELISA, serum ELISA, fecal culture and fecal PCR (e.g., Lavers et al., 2013; Wolf et al., 2014; Laurin et al., 2015; Arango-Sabogal et al., 2017). All testing is done through a provincial or regional diagnostic laboratory or the Dairy Herd Improvement (DHI) milk recording laboratory, all of which are accredited for tests they provide. However, the way these test results are used by the program and by veterinarians/producers varies among provinces (see program websites for details).

Many dairy producers who participate in these voluntary control programs and have therefore demonstrated a desire to control JD in their herd wish to have their efforts recognized. They also want to know how other herds in the country compare, particularly if they want to buy cattle. To meet this demand, most provincial programs have either a status or recognition program. In some cases, the program simply issues a certificate of completion once a herd has met all program requirements, whereas others have a more complex status system which distinguishes among herds and recognizes herds of different JD risk. Given that cows are frequently sold, and that they move within and between provinces, there is a need to harmonize status programs.

The other major concern among dairy producers is disposition of test-positive cattle. Again, programs vary in how they deal with cattle identified as being test-positive with any approved test method. For instance, in Québec, all producers who wish to access their individual cow test results must sign an affidavit stipulating that they will not sell any test-positive cattle. This restricted animal movement is enforced through a provincial animal traceability program, which is currently unique to Québec. Conversely, from 2010 to 2013, Ontario participants who wished to qualify for program funding support had to remove all cows found with high titre (HT) tests (based on the milk ELISA test currently in use, a positive test result is $\geq$0.1 or greater, whereas a High Titre is $\geq$1.0) NOT to another dairy herd or to the food chain, within 90 days after the test date. Producers who removed these HT cows as required by the program received $500 per cow.

3.6. Applied Research

All provincial programs also have research activities focused on JD control. Some programs fund research directly from operating budgets, whereas others make program dollars available to researchers for provincial and federal matching fund applications. These research programs are generally coordinated by faculty at the local/provincial veterinary colleges. These researchers gather annually at a relatively informal research conference where findings are shared and new ideas for collaborative research are developed.

4. LESSONS LEARNED

4.1. Interpretation of Test Results

Many of the challenges posed by JD and its control relate to the prolonged interval between exposure to MAP and development of clinical disease, and the generally poor performance characteristics of tests currently available for identifying infected individuals. As direct consequence, it is imperative that veterinarians and producers understand implications and terminology used in discussing JD control (Ritter et al., 2015; Ritter et al., 2017). For instance, there is generally a poor understanding of the difference between a 'test-negative' herd and a 'Johne's free' herd. Perhaps this is not surprising, given that our previous disease control programs focused on Brucellosis and Tuberculosis (TB), diseases which we have been successful in eradicating with a 'test and cull' strategy. During active stages of these eradication programs, herds were tested annually and designated 'test-negative' herds as 'free' of disease. That we test herds for JD but are not willing to call 'test-negative' herds 'Johne's free' has confused producers and dairy industry advisors (Roche, 2014; Roche et al., 2015). Therefore, there is a clear need to continue to educate all participants regarding this important distinction.

4.2. Involvement of Veterinary Practitioners

Involvement and training of veterinarians to deliver the RAMP were critical. Private practitioners gave the program credibility and were instrumental in recruiting herds. RAMP facilitated discussions between the producer and herd veterinarian focused on areas of the farm (e.g., calf pens and calving area), which are frequently ignored. Deficiencies were often easily corrected, representing strategic control of contagious diseases transmitted fecal-orally, including calf diarrhea. The JD control programs provided examples of successfully implementing targeted biosecurity on Canadian dairies.

4.3. Differences among Provincial Programs

Given the current focus on biosecurity in livestock and poultry, JD control programs are proving to be very effective examples of implementation of targeted biosecurity on dairy farms across the country.

One of the most striking differences among the provincial JD programs is the approach to testing. These differences have been noted (see details on program websites). These differences in testing have prompted many discussions among researchers, veterinarians, and producers. Despite no 'best' approach, dialogue about various strengths and weaknesses has contributed to understanding limitations of testing in general and has prompted further collaborative research evaluating tests and test strategies. Probably the biggest lesson that needs to be learned by most dairy producers is that JD cannot be easily eradicated by solely testing and culling test-positive cows. The notion that false-negative test results are common when testing individual animals with milk or serum ELISA, or fecal culture/PCR, is unsettling at best.

4.4. Movement of MAP-Positive Cows

A key element continuously emphasized by dairy producer representatives on our management committees is the importance of NOT allowing MAP-infected cows to move freely from one herd (region) to another. Although enforcement of movement restriction is currently limited to Québec, the importance of educating dairy producers who must buy replacement cattle to ask about the health status of potential herd additions (Buyer Beware) needs to be a constant message.

4.5. Need for a National Program

Program evaluations demonstrated the extreme importance of a national standardised, simplified, prioritized risk assessment and management practices (i.e., RAMP) process to enable the producer and trained herd veterinarian to effectively change management to control JD.

Additionally, there is a need for a national infectious disease herd status program. It is extremely important that a herd status for a certain disease in one province means the same for all provinces. Leaders of provincial JD Initiatives have started discussions to make this happen for JD. However, it is important that the same happens for other infectious diseases included in a national biosecurity effort. A status program must be national in scope and needs to be developed by farmers for farmers. Leadership from national organizations such as Dairy Farmers of Canada and the national breed associations is needed to move this forward.

4.6. Importance of Research Program

Canada's significant advances related to the control of JD over the past decade have primarily resulted from coordination of integral research – education – program development activities by enthusiastic JD control champions from industry, academia, and the provinces. The CJDI Technical Committee has enabled this forum and the national coordination of JD Program components (research, farm, and laboratory) and standardized approaches for JD program planning/delivery at annual MAP Researchers Meetings since 2008. Results of concurrent research programs have not only led to improvements in JD programs, but due to frequent presentations by graduate students, they have also had an important role in keeping JD as a priority in the mind of Canadian dairy producers and their veterinarians.

4.7. Keeping Producers Motivated

The final lesson and challenge relate to the voluntary participation in the various programs (Sorge et al., 2010; Ritter et al., 2015; Ritter et al., 2017). Given that these programs are producer-initiated, and industry led, initial enthusiasm drives uptake in the first year or two but, with time, many programs suffer from decreases in profile, interest and participation. Canadian JD programs voluntarily attracted up to 70% producer participation. How do we reach the remaining herds, which might include a disproportionate number of JD problem herds?

The support of veterinarians and industry staff (DHI testers in Ontario played a key role in reminding producers about testing opportunities) was very important and effective. However, we need to continuously find new ways to keep the program prominent in the minds of producers and to show program value to not only maintain enrolment but also bring the sceptics and late adopters on board. Extension outreach and focus farm research have been enabled by the JD initiatives and subsequent efforts are needed to maintain the momentum established in motivating on-farm change for JD prevention and control.

5. BUILDING ON JD - TRANSITIONING TO CANADIAN BIOSECURITY INITIATIVES

Recently, Dairy Farmers of Canada (DFC) and the Canadian Food Inspection Agency (CFIA) published two documents on dairy farm biosecurity: Biosecurity for Canadian Dairy Farms: National Standard and Biosecurity for Canadian Dairy Farms: Producer Planning Guide (Canadian Food Inspection Agency, 2018) and (Dairy Farmers of Canada, 2018).

Additionally, biosecurity is one of the six programs which will be implemented as part of DFC's proActionInitiative, an initiative that was accepted by the board of DFC in June 2013 and will be implemented in the coming years. It was decided that the proAction Initiative will be the same in all Canadian provinces.

Applying successful methods and leveraging the JD Initiative lessons learned could optimise the launch of Canadian dairy farm biosecurity. The Technical Committee enabled positive integration of current science into practical field applications and the resultant delivery of effective, standardized provincial/ regional JD control programs. With strong leadership from industry, all veterinary schools, key provincial programs, and the Canadian Food Inspection Agency, substantial progress in JD was achieved within a modest interval. The CJDI Technical Committee strongly supports Dairy Farmers of Canada's proActionand the Biosecurity initiative.

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10 JOHNE'S DISEASE MANAGEMENT IN AUSTRALIA UPDATE

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1. INTRODUCTION

The purpose of a national approach to the management of Johne's disease (JD) in Australia is to facilitate complementary approaches across the affected livestock industries and jurisdictions. The Australian JD programs have been subject to periodic review to ensure they continue to align with national animal health policy direction. Over the last decade JD control has evolved from strongly regulated programs to an industry driven partnership with state governments, based on whole of farm biosecurity.

The National Ovine JD Management Plan (NOJDMP) for 2013–18 has an emphasis on farm biosecurity practices, vaccination, the JD Market Assurance Program for sheep (SheepMAP) and the use of a national Sheep Health Declaration (previously Sheep Health Statement) to certify the risk management practices that are in place on the property. Voluntary regional biosecurity areas were implemented for some areas of low prevalence to reduce the risk of inadvertent disease introductions into these regions.

After extensive consultation with Australian cattle producers and interested stakeholders (including governments and industry peak bodies) on the previous National Bovine JD Strategic Plan, a new strategy was developed: *A fresh approach to the management of Johne's disease in cattle: Management plan for cattle production conditions* (Animal Health Australia, 2016). This strategy, which commenced on 1 July 2016, included deregulation of the disease and a number of tools for producers to use to manage JD or prevent it entering their herd.

JD remains a notifiable disease in all jurisdictions for all species.

2. AIMS AND OBJECTIVES

The objectives for the NOJDMP (2013–18) are to:

- minimise the risk of infection by *Mycobacterium paratuberculosis* spreading to properties and regions which currently appear to be disease free
- reduce the financial impact and adverse animal health and welfare effects of the disease on individual flocks, and on the sheep industry as a whole.
- The objectives for the national management strategy for JD in cattle are to:
- manage and reduce the impact of clinical Johne's disease; and
- provide tools to allow individual producers to manage the spread of *Mycobacterium paratuberculosis* infection in accordance with their business requirements.

The strategy focuses on biosecurity and the reduction and management of the risk of diseases, including JD. Better biosecurity awareness and practices provide a safer environment for producers to operate in, rather than relying primarily on government regulation to reduce on-farm risks.

3. LESSONS LEARNED

New national industry arrangements for managing paratuberculosis depend on biosecurity measures at the farm level driven by individual producers' objectives. This offers greater flexibility to achieve outcomes with reduced costs than the previous model of regulatory protection against spread. Australia's new national programs no longer support government zoning, state entry requirements or regulatory investigation and tracing. These changes were driven by high costs of regulatory control, decreasing effectiveness associated with producer avoidance, and selective and inflexible management of risks.

As the date for implementation of the new strategy approached in mid-2016, Western Australia and the Northern Territory opted to maintain regulation for JD in cattle at the behest of their state-level industry bodies. The two jurisdictions also referenced the voluntary risk profiling tool – the Johne's Beef Assurance Score (J-BAS) – in their entry requirements for cattle. This meant that many cattle producers in Queensland (who send cattle to these two jurisdictions) have been forced to use the tool to access these markets. This initially caused a great deal of concern amongst Queensland producers, but has settled down following the delivery of over 65 workshops around the state (to around 5,000 producers) and communications about what they were required to do.

The development of the cattle-focused J-BAS has led to concern from the national sheep industries as the score is intended to consider cases of JD in any livestock species on the property, not just the cattle. This was partially alleviated by communicating the fact that a biosecurity plan, which includes the sheep on the property, and including vaccination where appropriate, will help minimise any risk. A National JD Project Committee aims to address cross-species issues like this in future.

4. OTHER DEVELOPMENTS

The national red meat farm quality assurance program 'Livestock Production Assurance' (LPA) implemented additional biosecurity and welfare modules on 1 October 2017. This has led to tens of thousands of producers implementing biosecurity plans for their properties that consider a range of animal diseases, pest animals, weeds and chemical contamination risks. Over the next few years, every property that wishes to sell animals under the LPA will need to have a biosecurity plan in place and this is included in program audits. Demand by meat processors drives certification under LPA throughout the production chain. Virtually every cattle, sheep and goat producer in Australia must implement a farm biosecurity plan to remain commercially viable. Cattle producers are encouraged to implement an optional JD section in their biosecurity plans to fulfil J-BAS requirements at the same time.

New Queensland and New South Wales legislation (Biosecurity Acts) provide for a general biosecurity obligation/duty for all people to do what is reasonable and practical to prevent or minimise the likelihood and impacts of biosecurity risks. This law, which requires responsible self-management of risks such as paratuberculosis, complements the new industry framework and tools that have been developed.

The national sheep industry peak bodies (Wool Producers Australia and Sheep Producers Australia) have recently carried out a consultative review on the NOJDMP and the practices and tools that underpin it. Feedback from interested producers and stakeholders will help the national industry bodies to make a decision in the next month or two on what will supersede the current plan.

The national dairy industry bodies (Dairy Australia and Australian Dairy Farmers) have recently reviewed the JD Dairy Assurance Score. This is expected to be completed soon and will continue to be compatible with the J-BAS.

5. COMMUNICATIONS AND EXTENSION

National industry representative bodies have developed and promoted a set of tools for producers and advisors to assess, manage and declare JD risks. These include guidelines, simple risk scores for beef and dairy cattle, health declarations and template biosecurity plans.

The livestock industries remain committed to the management of JD but are seeking to place the responsibility for this management more firmly with producers. Governments remain partners in the new approach, but predominantly as technical advisers, and for notification and certification purposes.

Livestock organisations have also recognised the value of managing several significant endemic diseases through a biosecurity focus using individual and regional risk assessments. In line with this emphasis on farmer-initiated biosecurity, messages about JD control/management are being linked to more general messaging about the importance of farm biosecurity and, in particular, the need for producers to demonstrate animal health assurance through the use of animal health declarations.

Advice on biosecurity and on-farm JD management tools continues to be delivered to beef producers through extension service providers, who assist producers to develop individual farm biosecurity plans. The primary extension service provider model used is funded by the cattle industry through levies raised on producers whenever livestock are sold. Extension service providers employ field staff in most states and territories whose responsibility is to work directly with producers to engage them in implementing biosecurity practices.

Communication messages have been focused for the last year or two on the transition from the previous BJD program to the new JD arrangements for cattle as well as the implementation of the LPA biosecurity requirement. Communications will now focus more on helping producers to understand the tools that are in place to manage or prevent JD in their herds. There will also need to be communications to sheep producers about what the sheep industry peak bodies decide to implement beyond 2018.

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11

PARATUBERCULOSIS IN ARGENTINA: CURRENT STATUS OF DISEASE CONTROL AND APPLICATION OF DIAGNOSTIC TOOL

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1. INTRODUCTION

Johne's disease (JD) or Paratuberculosis continues to be an important production-limiting disease of cattle in Argentina. Additionally, concern over potential public reaction to *Mycobacterium avium* subspecies *paratuberculosis* (MAP) as a potential zoonotic agent stimulated the development, in some regions, of a control herd and, in the future, provincial or regional JD control programs across the country.

Paratuberculosis is endemic in Argentina, it was first described in cattle in the country by Rosenbusch (1935) and in later years, was diagnosed in other species. Beginning in the 90s with the intensification of livestock production - especially cattle for meat, milk and deer - it began to be clinically diagnosed more frequently which generated a demand for laboratory analytical techniques to support clinical diagnosis, early detection of infected animals and improved control and prevention.

The JD is included in the list of notifiable diseases in Argentina (SENASA, 2015) but there is no official national control and/or eradication program. SENASA has a laboratory for the diagnosis of mycobacteria recognized as a reference by the OIE since 2010 and is responsible for the approval of biological products for the diagnosis and control of JD. Official control activities only include general surveillance as part of the differential diagnosis of bovine tuberculosis and other diseases. There are voluntary control programs supported by research organizations such as INTA and universities. These are based on laboratory diagnosis using different techniques, the elimination of positive animals and management measures to reduce horizontal and vertical transmission. Vaccination is not allowed in Argentina.

From the INTA and other institutions in Argentina the priorities are:

1. to increase education about, and awareness of, JD in Argentina among dairy and beef producers, veterinarians and allied industries through national and international courses;
2. to encourage the development and implementation of regional herd control and where possible to support information about JD;
3. to facilitate the development and funding of research to increase the epidemiological information to areas that will support the improvement of JD control;
4. to maintain a formal national working group (Scientific Commission of Mycobacteria, AAVLD), which meets annually to discuss regional activities and issues, including how to keep JD high on the agenda among dairy producers and to promote the implementation of a national JD status program;
5. to understand the immunopathogenic, epidemiological and immune response in cattle, goats, sheep and deer confinement characteristics for disease control;
6. to maintain a national collection of MAP isolated from the milk, feces and tissues of bovine, deer, goat and commercial milk, and typed by molecular methods.

2. AIMS AND OBJECTIVES

The objectives of this review are:

1. to report on the analyzes of regional databases which are based on diagnoses made in veterinary diagnostic laboratories, serological surveys (surveys) at the level of administrative districts and clinical records of rural veterinarians, of the *Mycobacterium avium* subspecies *paratuberculosis* (MAP) infection in Buenos Aires province, and others regions of Argentina;

2. to report an epidemiological analysis from voluntary control programs supported by research organizations such as INTA and universities, over 25 years;

3. to describe some of the communication strategies that have been implemented to keep JD control a current topic of focus for the dairy and meat industry;

4. to present an overview of the ongoing research activities related to JD control.

3. ANALYZES OF REGIONAL DATABASES

In Argentina, there are no prevalence studies at the national level, but there are analyzes of regional databases based on diagnoses made in veterinary diagnostic laboratories, serological surveys at the level of administrative districts and clinical records of rural veterinarians. In one of these studies, the results of 5,520 consultations on health problems in cattle from the Pampa Húmeda region of Argentina were analyzed and sent to the Regional Veterinary Diagnostic Laboratory of INTA in the district of Balcarce for the purpose of obtaining a diagnosis. In the cow category, the most frequent diagnosis in the period 1997 to 2004, was JD. In another study in the same region, a database of diseases in cattle diagnosed by rural veterinarians in 5,000 livestock establishments in the period 2001 to 2007, was analyzed. Of the 3,624 cases/outbreaks in cows for meat production, JD was the 4th most frequent disease (165 cases) with an average morbidity of 1.0% and a mortality rate of 0.3%. In dairy cows, over 690 diagnosed health cases were found, and clinical ParaTB occupied 11th place with a morbidity of 0.9% and a mortality of 0.3%.

3.1. Retrospective serological study of JD

The Laboratory of Bacteriology of the Animal Health Group of the EEA of INTA in Balcarce has performed the serological diagnosis using ELISA since 1991. The *in house* ELISA kit uses protoplasmatic antigen (PPA-3, Allied Monitor, Fayette, MO, USA), the serum samples are preadsorbed with *Mycobacterium phlei* and diluted 1:100. The interpretation is performed calculating the relationship between the optical density (OD) of the negative control and the OD of the sample. OD 1 to 1.4 is negative; 1.5 to 2.0 is suspect, and greater than 2.1 is positive. Since 1991, the technique was systematically carried out by the same technicians and professionals. Data of interest for the diagnosis and subsequent epidemiological analysis were recorded for each sample and establishment. During these 25 years, 1,732 referrals were received and 128,568 adult bovine sera were analyzed:

- Cattle for meat: 79% of the remissions (n = 383) had at least one positive serum and 6.3% of the 65,841 sera analyzed were positive to ELISA. The temporal analysis indicates that there is a declining secular trend in the study period (1991–2015): the percentage of remissions and positive sera was reduced from 85% to 48% and from 10% to 4% respectively.

- Dairy cattle: 81% of the remissions (n = 200) had at least one positive serum and 15.7% of the 27,112 sera analyzed were positive to ELISA. The temporal analysis indicates that there is also a declining secular trend in the study period (1991–2015): the percentage of remissions and positive sera was reduced from 92% to 68% and from 22% to 7% respectively.

The two historical series show a significant decrease in the percentage of referrals (protocols) and positive samples, both in cattle for meat and in dairy cattle. It is possible that, after the emergency, diagnosis and regional characterization of the ParaTB in the first ten years of the period analyzed, voluntary control programs were applied at the establishment level, which resulted in a progressive reduction of the global seroprevalence. Much of the work carried out in cattle for meat and also deer has shown a 0% reduction of seropositives when the criteria of control, identification of animals and elimination to slaughter were systematically applied.

In another UBA study from 2017 to 2018, the focus was on cattle farms located in the Buenos Aires province (a major region for beef cattle production) and on 257 serum samples taken from two herds on beef farms where JD had been proven by culture. Sampling was carried out at random and the number of sera with an expected prevalence of 15% and a confidence interval of 95% from districts of Laprida (n=169/4588) and Chacabuco (n=46/59) was calculated. An *in-house* ELISA (PPA antigen and anti-IgG bovine HRP) test was used. The seroprevalence obtained were 4.3% (Chacabuco) and 5.9% (Laprida). Animals were categorized and the percentage of positive samples was calculated: heifers (10.3%), bulls (3.0%), cows (5.0%), pregnant cows (5.5%) and 0% of calves were detected as positive. The positive results show that there could be differences among animal categories as was described previously. The percentage of seroprevalence obtained for the breeding herds (between 4.3% and 5.9%) shows a difference from the previous data, this could be due to the different management practices on dairy farms in comparison with the extensive breeding in the province of Buenos Aires. The seroprevalence evaluated here might change the number of animals that should be studied in order to complete the survey in Argentina.

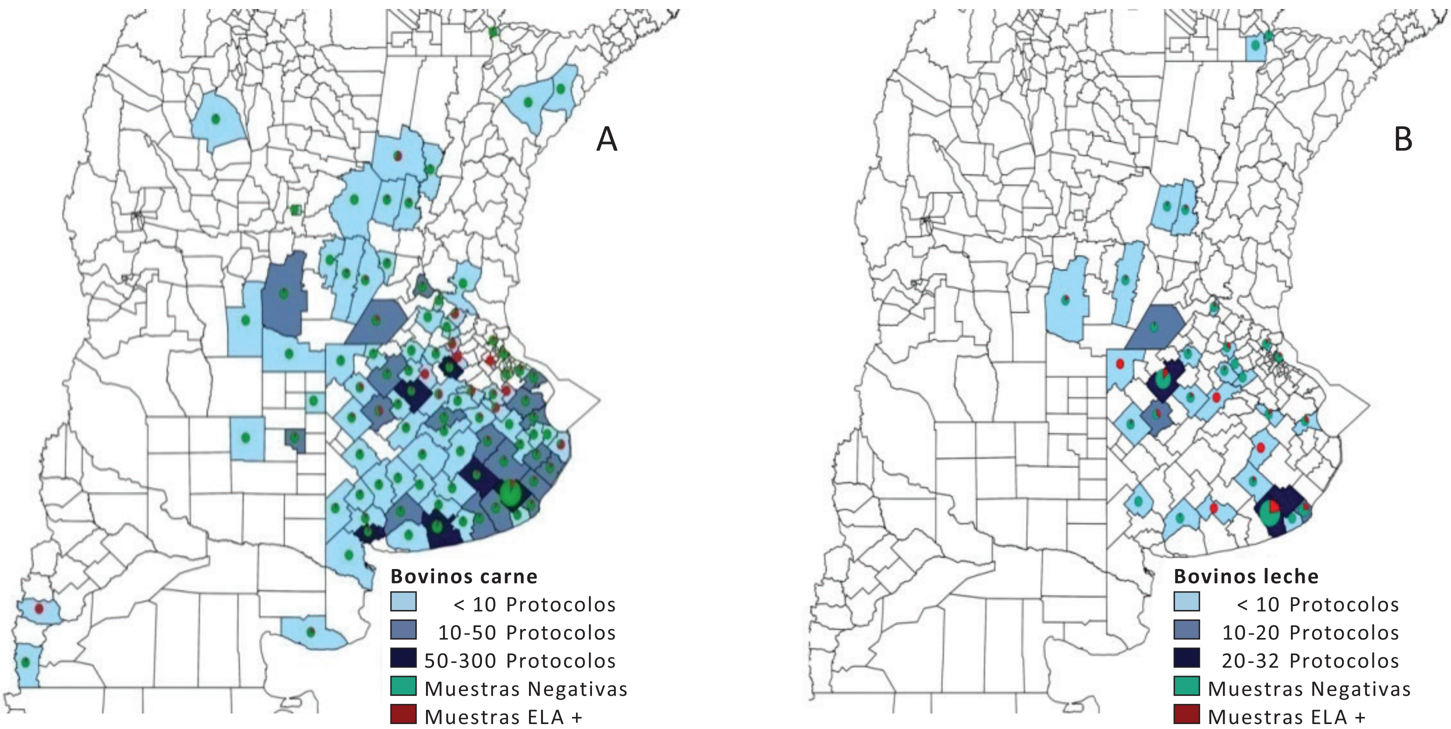

Figure 1. Geographical origin of samples of meat (A) and dairy (B) cattle in Argentina. The color intensity of the department indicates the number of protocols and the graph indicates the percentage of positive samples (red) on the total analyzed (green).

3.2. Risk factors of JD

A case-control study, using the data previously described (Späth & Becker, 2012), enabled the evaluation of the relationship between the laboratory diagnoses of JD and the type of production from which the samples came (meat or milk). Cases were considered from all those establishments where there was a diagnosis of JD (MAP isolation, positive PCR, intestinal and lymph node lesions with positive Ziehl-Neelsen bacteria, typical histopathology, positive serum samples with a herd criterion (Cameron & Baldock, 1998). These were cases referred for the diagnosis of JD which did not meet the previously described criteria. Of a total of 243 herds, 179 were from cattle for meat and 64 were from cattle for milk production. The dairy herds had an almost four times greater risk of being positive to JD compared to meat herds (OR 3.98, 95% CI 1.9-8.3, X2 p <0.05). A second case-control study using the database described in the previous section, that is, only serological results by *in house* ELISA, showed a similar association. A herd criterion (vide supra) was also used to classify herds as positive (cases) and negative (controls). Data were available from 321 herds (230 meat production and 91 dairy farmers). Dairy herds also had a significantly higher risk of being JD positive than meat production (OR 10.6, 95% CI 4.7-23.9, X2 p <0.0001).

Recently, a voluntary program was implemented in one region of Buenos Aires province. The objective of the program is to control the movement of bovines from certified herds and identify positive animals in order to avoid contamination to other free areas. In Argentina, mandatory implementation and voluntary adoption of herd control programs is believed necessary. Farmers with infected herds can voluntarily apply a control program to gradually reduce within-herd prevalence by adopting biosecurity (preventing the introduction of MAP infection into the herd) and biocontainment measures (preventing the spread of MAP among animals within the herd) and an appropriate testing scheme.

4. RESEARCH TOWARDS IDENTIFICATION IN MILK AND THE RISKS TO PUBLIC HEALTH

The first isolation of MAP from commercial pasteurized milk in Argentina was reported by Paolicchi et al., in 2012. From all the milk samples analyzed, 2.86% were culture positive and the colonies cultured on HMP and HMPA presented the typical morphology and ZN-positive stains. MAP was identified after eight weeks in one sample of pasteurized milk and in one sample of milk which was ultra-pasteurized at high temperature that was further processed. In the primary isolation, a few colonies (n=5–10) were recovered but the colonies were positive for the IS*900* PCR. The genetic identification in the MAP strains isolated here from commercial milk revealed that they belong to pattern “A” (RC17 European type), which is the most prevalent one in Argentina, as we have demonstrated previously. This finding is the first reported of its kind in Argentina from commercial milk and indicates a risk of population infection from those usually consuming this product. On the other hand, one possible explanation for the presence of MAP in commercial milk could be post-pasteurization contamination. If raw milk containing MAP is brought into a processing plant and sanitation methods are not adequate, MAP could be present in the environment and contaminate the final product. The results obtained in this work emphasize the importance of improving controls in dairy farms and in the milk industry with the aim of protecting consumers from the risk of infection with MAP.

It was also proposed that the viability of MAP during preparation and refrigerated storage of yogurt should be determined. Yogurts were used which had been prepared using pasteurized commercial milk and the yogurt was artificially contaminated with MAP and *E. coli* and *S.* Enteritidis. Samples were taken during and after the fermentation process until day 20 after inoculation. MAP was not detected during their preparation and short-term storage but was found 180 min after inoculation and storage. Live bacterial counts of *E. coli*, and *S.* Enteritidis increased during the first 24 hours, followed by a slight decrease towards the end of the study. In this study it was shown how MAP, *E. coli* and *S.* Enteritidis resisted the acidic conditions generated during the preparation of yogurt and low storage temperature and emphasizes the need to improve hygiene measures to ensure the absence of these pathogenic microorganisms in dairy products.

Standardizing a diagnosis procedure to detect MAP DNA in raw cow milk samples under field conditions was also proposed. A procedure that combines both immunomagnetic separation and IS*900*-PCR detection (IMS-IS1 PCR) was employed on milk samples from 265 lactating Holstein cows from MAP infected and uninfected herds in Argentina. IMS-IS1 PCR results were analyzed and compared with those obtained from milk and fecal culture and serum ELISA *in house*. IMS-IS1 PCR showed a detection limit of 10^1 CFU of MAP/mL of milk, when 50:50 mix of monoclonal and polyclonal antibodies were used to coat magnetic beads. All of the 118 samples from the MAP uninfected herds were negative for the set of the tests. In MAP infected herds, 80 out of 147 cows tested positive by milk IMS-IS1 PCR (55%), of which two (1.4%) were also positive by milk culture, 15 (10%) by fecal culture, and 20 (14%) by serum ELISA, showing a slight agreement between the different tests (<0.20), and the proportions of agreement were ≤0.55. The IMS-IS1 PCR method detected MAP in milk of the cows that were not positive through other techniques.

5. RESEARCH ACTIVITIES TO IMPROVE THE CONTROL OF JD IN ARGENTINA

To investigate the immunopathogenic, epidemiological and immune response characteristics in cattle, sheep, deer and goats in confinement for the control of the disease, work is being carried out on:

- Characterization and sequencing of MAP strains with differential virulence for pathogenesis studies and the development of diagnostic strategies.
- Maintenance of a national collection of MAP (n=360) and other mycobacteria typed by molecular methods (PCR and MIRUs/VNTR).
- Tests of antigenicity and virulence of environmental mycobacteria and their interference with JD and tuberculosis in cattle.

- Virulence assays of MAP strains in the bovine model for studies of cellular, humoral and excretion dynamics in bovines.
- Host-pathogen relationship in infections caused by MAP.
- Production of attenuated mutants of MAP.
- Study of virulence genes.
- Development of methods to characterize the mycobacteria affecting pigs in Argentina.
- Identification of immunoreactive lipids in local strains of M. bovis and the MAC complex.
- Prepare liquid culture media to shorten the time for MAP incubation.
- Development of immunodiagnostic-based technology for JD.
- Studies of the immunopathogenic characteristics and the pathogen host relationship.
- Undergraduate and Postgraduate Teaching.

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12
HOW TO CONTROL PARATUBERCULOSIS IN CATTLE HERDS IN SLOVENIA

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1. INTRODUCTION

Paratuberculosis is a common disease of ruminants in Slovenia. The disease is mostly spread among Black-and-white and Limousin breeds. The last seroprevalence study was conducted in 2008. Animals older than two years in 20% of randomly selected cattle herds were ELISA-tested for the presence of antibodies against *Mycobacterium avium* subsp. *paratuberculosis* (MAP). The study showed that true prevalence at the herd level was fairly low (18.49%) compared to many European countries (Kušar et al., 2011). However, later studies in larger dairy cattle herds (average herd size 184 heads) showed the presence of antibodies against MAP in every herd with 50% of herds being also culture-positive (Starič et al., 2011). In addition, PCR-based investigation of subclinically infected animals of different age groups from a herd with a history of paratuberculosis revealed that the vast majority (up to 89%) of the animals within a herd might be infected with MAP (Logar et al., 2012). These facts call for immediate implementation of measures necessary to control the disease.

2. HOW TO START?

Primarily, all stakeholders must agree that paratuberculosis is a problem worth solving. In order to secure the long-term success of measures, we have to reach a required compliance of key stakeholders. Thus, they must believe that it is worthwhile to invest time and resources to reduce the problem. In parallel with the preparation of the draft plan of measures, articles have been published about paratuberculosis in most popular expert and scientific journals and meetings have been held with the leaders of farmer associations and the veterinary chamber. The second stage is preparation of the preliminary plan of measures for the control of the disease together with a financial analysis. At this stage, support is also required, or at least agreement, from the Veterinary Administration (Administration of the Republic of Slovenia for Food Safety, Veterinary and Plant Protection) and the Ministry of Agriculture, Forestry and Food.

3. MEASURES

The first step is to establish the current prevalence of the disease. The results of the faculty's previous work on paratuberculosis suggested that the specificity of the ELISA kits is questionable because of the cross-reactivity with other subspecies of *M. avium* which are evidently widespread in the environment in Slovenia. Therefore, pooled faecal PCR (culture) will most likely be the method of choice. One pooled faecal sample for every 50 animals should be taken. It would be optimal if we could test all the herds, but because of financial constraints the prevalence study will include all of Black-and-white and Limousin cattle herds with more than 100 animals.

In addition to testing, analysis of the spread of MAP in a typical Slovenian dairy herd using stochastic modelling in the R programme will be carried out. The model will be based on the Susceptible-Infectious-Recovered (SIR) model, which will be adjusted according to the epidemiological features of MAP. Data specific to this area will be obtained from previous research conducted at the faculty and from other sources. Information on the epidemiological characteristics of the agent and of the disease spread will be supplemented with the data from published literature. The latter is usually estimated for the area the study refers to; therefore, it will be adjusted, and the weight of certain values will be calculated, using expert opinions.

It was shown that between-farm transmission of MAP is significantly associated with animal movement. Network analysis of cattle movement will be performed, which will enable the systematic investigation of animal trade and allow the evaluation of risk potential for the transmission of MAP. Detailed data on cattle movement will be obtained from the Veterinary Administration. The network will be generated in the Pajek program. Centrality measures will be used to detect premises that pose a higher risk for disease introduction or spread and premises that are connected to MAP-positive herds identified in the first stage. In the second stage, those herds will also be tested for the presence of MAP. In herds which test negative at this stage, the epidemiological investigation of connected premises will be conducted.

In infected herds, the proposal is that technological measures to control the disease based on the individual rate of infection and farming method will be implemented. The effectiveness of the proposed measures will be monitored. The results will contribute to the development of the general control measures for the spread of paratuberculosis in Slovenia.

The fourth and final step of this programme will be the implementation of the voluntary national certification system. The certification system will enable farmers to purchase healthy animals from MAP-negative herds. At the same time, the value of animals from negative herds will increase in domestic and international trade. The effective control of paratuberculosis will reduce the presence of the agent in the food chain and the environment, which will be beneficial for the protection of human and animal health.

4. FUTURE STEPS

The implemented control measures and related results will be compared to control measures and results from other countries as part of the COST Action titled Standardizing OUtput-based surveillance to control Non-regulated Diseases of cattle in the EU (SOUND-control). The main objective of the project is to harmonise the outputs from different EU countries surveillance, control or eradication programmes for non-regulated cattle diseases. Outputs will be assessed based on available data and different statistical and mathematical methods in terms of the confidence of freedom of the disease and cost-effectiveness of measures. This will provide common ground for comparison of epidemiological and economic equivalence of different control efforts.

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13 THE UK NATIONAL JOHNE'S MANAGEMENT PLAN - PHASE 2

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1. INTRODUCTION

The National Johne's Management Plan in the UK has entered a new phase of delivery after a prolonged period of farmer and veterinarian engagement. The plan has been developed based on the experiences of practical application of Johne's (JD) control at farm level in the UK in dairy herds of variable size and prevalence

The UK Johne's programme has been built using commercial solutions and synergisms to deliver a practical National Johne's Management Plan (NJMP) which allows any dairy farmer to select an appropriate control strategy for their herd. Six possible control strategies designed to meet the aspiration and resources of the farmer are offered to participating farmers, providing practical and affordable prevention and control rather than a rigid centralised surveillance programme. Minimal external funding has been utilised throughout the process with the focus kept firmly on vet and farmer engagement and robust control of JD spread within and between herds. This paper seeks to explain the progress since 2010 with JD control in the UK dairy sector with the aim to manage and then reduce the incidence of Johne's disease in dairy cattle and engage 80% of dairy farmers in Great Britain in credible and robust Johne's management activities.

Particular focus in this paper will be on the most recent two years' work since the last Paratuberculosis Forum and the lessons learned during this phase of the programme. Please refer to previous proceedings for the experiences and lessons learnt from the early phase of the Plan from 2010–2016.

The objectives of the NJMP are to extend the participation to 95% of the UK milk supply by 2020.

2. AIMS AND OBJECTIVES

2.1. Background 2010–2015

In 2010, Dairy UK (the milk processor representative body) formed a Johne's Action Group to tackle Johne's Disease (JD) in the UK dairy sector. The group has representation from the British Cattle Veterinary Association, Farming unions, milk processors, milk recording organisations, Myhealthyherd, farmers and practising vets. A collaborative JD engagement Plan was developed to engage dairy farmers, milk processors, regional monitoring organisations and veterinary surgeons with the effective control of Johne's disease. The early outputs of the group were focused on establishing coherent messages for inclusion in vet and farmer training.

Practical standardised surveillance was encouraged to determine herd status and estimate prevalence using targeted sampling through milk ELISA tests from 30 high risk cows (aged 3–6 years of age, high cell count, clinical suspects, weight/milk loss and history of poor health). The use of the targeted sampling approach was shown to deliver a similar sensitivity as a random sample of 60 cows and correctly identified known positive herds in 95% of samples (Hanks, 2013). This low-cost approach was delivered through the Milk Recording Organisations (MRO). A number of milk processors chose to further subsidise initial surveillance for the farmers removing financial barriers for engagement. The adoption of the targeted 30-cow screen was central to the success of the Plan as this allowed a simple methodology to engage both vets and farmers with JD control.

Risk assessments were undertaken to help farmers appreciate the risk of disease introduction (biosecurity risks) and spread within the herd (biocontainment risks) (Rossiter, 1998; Orpin, 2009). Based on the results of surveillance and risk assessments trained vets were well placed to select an appropriate strategic control plan for the herd.

As a parallel development, a commercial web-based health planning tool (Myhealthyheard, 2018) was designed linking the farmers, vets, monitoring organisations and labs. A specific Infectious Disease manager module within the program facilitated structured risk assessments of disease entry and spread to be completed with a graphical traffic light illustration of relative risks. A prevalence prediction graph was created using a combination of a targeted milk ELISA sampling of 30 high risk cows and risk assessment results to prompt action from the farmers. The Myhealthyherd tool was used to underpin two large regional funded Johne's programmes (South West Healthy Livestock and North West England) and a processor driven education programme - Operation Johne's (Orpin, 2011)

In 2013, the national certification body CHeCS (Statham, 2011) introduced a risk-based categorisation model for Johne's herd classification. The NJMP signposts farmers towards the CHeCS programme and those who identify a commercial benefit from membership are encouraged to join the scheme (Figure 1).

Status	Definition
Risk Level 1:	This is directly equivalent to the previous CHeCS Accredited status. Level 1 status is associated with th lowest risk when buying in stock. Health plan required.
Risk Level 2:	Herds with Level 2 status have had one or two clear consecutive herd tests. Health plan required.
Risk Level 3:	Herds with Level 3 status have reactors identified at the annual herd test, but these are no more than 3% of the animals tested. Health plan required.
Risk Level 4:	Herds with Level 4 status have reactors identified at the annual herd test, and these amount to more than 3% of the animals tested. Health plan required.
Risk Level 5:	Any herd that is not carrying out the required testing or does not have a suitable health plan in place automatically falls into this category. Level 5 status is considered to be the greatest risk with respect to Johne's Disease when buying in stock.

Figure 1. Risk levels for the CHeCS JD programme.

The uptake within the CHeCS programme remains low. The majority of UK dairy farmers appear to be focused on protecting the herd through effective biosecurity and controls rather than risk-based trading.

Certification of herds remains an obsession amongst vets worldwide but has proven to be a much lower priority for farmers who are broadly concerned with cost effective control.

In 2014, consistent messages and materials were prepared by Dairy UK and delivered through participating veterinary practices across England.

2.2. Development of the National Johne's Management Plan Phase 1

In 2015, the engagement plan was enhanced by the development of a more formalised National Johne's Management Plan, which aimed to further engage the processors to encourage their contracted farmers to commit to effective JD control. The first phase of the Plan, running from 1 April 2015 to 30 September 2016, focused on farmers carrying out a risk assessment to assess the risks of entry, presence and spread of MAP infection in their herd, determining their Johne's risk and status and choosing one of the six control strategy options for their farm. (Figure 2).

Strategy	Target Farm Type
Biosecurity protect and monitor	test negative farms with very low or zero prevalence to control risks of entry only.
Improved farm management (IFM)	Low prevalence farms with low risk of entry and low risk of spread
IFM and strategic testing	Infected farms with significant prevalence, controllable risks of entry and manageable risk of spread
IFM Test and Cull	Low prevalence farms with low risk of entry and low risk of manageable risk of spread hoping for accreditation
Breed to terminal sire	High risk of entry and high risk of spread farms with significant prevalence and limited resources
Firebreak vaccination	High risk farms with high prevalence that need lead in to alternative strategies

Figure 2. Strategy options of the NJMP.

The Action Group developed a more structured framework using financial contributions from milk processors and matched funding from the AHDB (Agriculture and Horticulture Development Board) to finance a delivery team and website hosting. (Action Johne's, 2018; Orpin, 2017; Sibley, 2016).

2.3. Development of the National Johne's Management Plan Phase 2

The British Cattle Veterinary Association developed an online training portal for veterinary surgeons wishing to partake in the NJMP with an accreditation process to develop a standardised approach to JD control. Over 800 vets have undertaken the training. 82% of the UK milk supply via their processors have now signed up to the NJMP which commits farmers to engage with a BCVA accredited JD vet to conduct a risk assessment and create a written control plan with the selection of an appropriate strategy and related tasks to deliver effective JD prevention and control. The target for completion of Phase 2, where every participating farm has a robust JD Management plan, is 31 October, 2018. (Orpin, 2017)

The NJMP is commercial and is driven by the processor with the farmer paying for planning, advice and controls. The success of the NJMP in the UK will be dependent upon financially viable and beneficial prevention and control programmes being introduced and maintained in participating farms: this requires flexibility and specificity.

The process which must be applied by each participating farmer is as follows:

- *An on-farm Johne's disease risk assessment* – carried out by the BCVA Johne's Certified Veterinary Advisor, ideally using a formal risk assessment tool. Risk of disease entry and spread will be identified to help determine the correct control plan for the herd.

- *An assessment of herd Johne's disease status* – this requires a screening test. Generally the minimum testing requirement to determine status for the NJMP is either a 30-cow screen or a cull-cow screen. A bulk milk test is not sufficiently sensitive to identify infection at a prevalence less than 5%, and therefore is not acceptable for the NJMP.

- *A written Johne's management plan* – this will be based on the findings from steps 1 and 2 and must be agreed between the farmer and the vet. One of the six NJMP control strategies will be selected, and a bespoke written management plan created for the herd, which fulfils the objectives of the NJMP and sets out the management tasks required.

Every farm is unique and as such no one strategy is appropriate to every farm. Clinical judgement is required to choose the most suitable approach for the farm. The choice of strategy will be driven by the predicted or actual prevalence as determined by structured risk assessment and testing and combining this with farmer aspiration and resources. (See Figure 3)

The objective is to help engage the farmer and improve the farmer aspiration to taking positive action by adoption of the appropriate strategy or, in some cases, to moderate an unrealistic aspiration or expectation. For example, implementing an aggressive test and cull programme in a herd with high incidence might not be appropriate unless there are substantive numbers as replacements. It could be more appropriate to start with a test and manage approach and then shift over time to test and cull if appropriate.

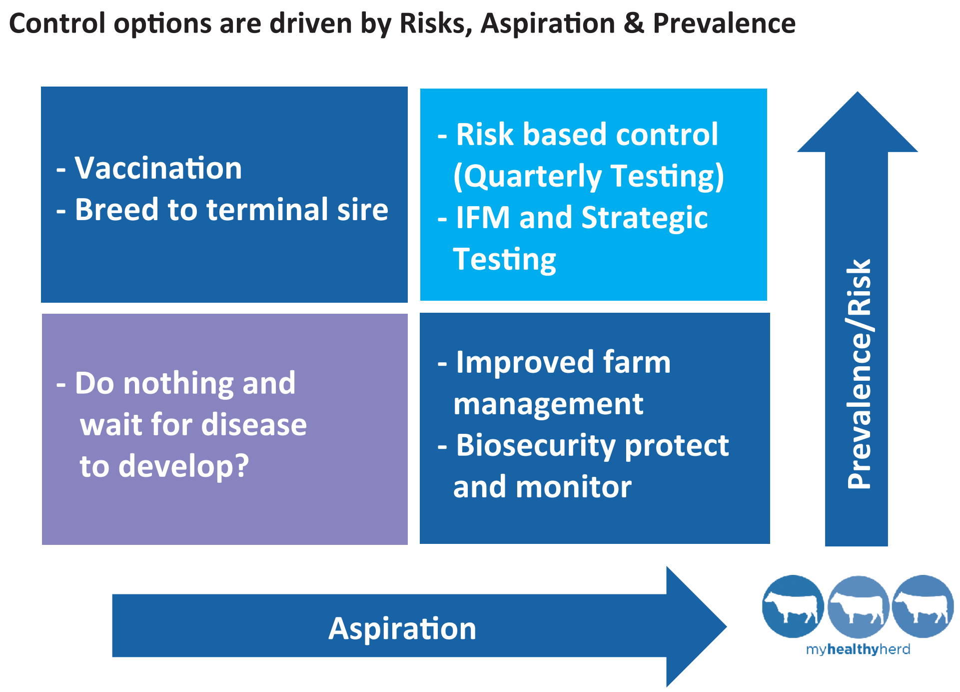


Figure 3. How risks, prevalence and aspiration impact on the chosen control strategy.

The Veterinary Declaration was agreed by the Veterinary Defence Society (specialist veterinary indemnity insurer):

"I, as a Certified BCVA Johne's Veterinary Advisor, can confirm that an assessment of Johne's disease risk and status has been undertaken on this farm in the last 12 months and that there is a written Johne's management plan in place which has been agreed with the herd owner. In my opinion, the plan complies with the objectives of the National Johne's Management Plan."

The farmer is expected to sign a parallel declaration to ensure that the veterinarian was not able to sign the certificate and also be compliant the RCVS guidance on veterinary certification:

"I can confirm that an assessment of Johne's risk and status has been undertaken on this farm in the last 12 months in conjunction with a NJMP trained vet and I undertake to adopt the written Johne's disease management plan." (See Appendix)

2.4. Progress with Phase 2

Engagement with the different processors has been variable. Some processors have included a clause in their milk supply contract which states that the farmer has to be compliant with the NJMP. These are typically smaller processors with export contracts of premium products.

The larger processors during 2017 were focusing on the Antimicrobial Resistance and internal resources were focussed on non-Johne's matters. As the October deadline gets closer, increased activity is anticipated. Arla and Muller (which process circa 40% of UK milk supply) have arranged eight regional veterinary training meetings for June/July 2018 to help further explain how the veterinarians can help with their NJMP initiatives with an expected push to encourage farmers to participate during 2018.

The National Red Tractor farm assurance standard has included a reference encouraging the vet delivering the annual health plan to tick a box to state that he/she has discussed the benefits of the NJMP and BVD control programmes with the farmer. Over time this may extend to compulsion once the adoption of the programme exceeds 80% or more.

2.5. Farmers' attitudes to JD control

The Myhealthyherd team commissioned a survey of dairy farmers to establish their attitudes to Johne's control in order to define what more could be done to make the programme a success and they presented this data at the Dairy UK conference in 2017. (Orpin, 2017).

A convenience sample of 394 non-randomly selected dairy farmers completed a survey. Of those surveyed, 71% had developed a robust JD control plan in conjunction with their vet and a further 22% have created a plan based on their own research and talks. A further 4% planned to start JD control soon. None of the farmers surveyed believed there was no need to control the risks of JD. However, the objective of the survey was to capture the views of the non-participating farmers which it spectacularly failed to do: they failed to respond.

47% of responding farmers were happy with their control plan and had no concerns. The major concerns cited were insufficient buildings to segregate high risk cattle, confusion with the tests and concerns regarding the costs of culling.

The key problems with JD control related to segregation of high risk animals (53%), TB testing interfering with results (40%) and uncertainty on when to cull test positive cows (38%).

The major benefits of effective JD control were better overall health of the herd (82%), reduced forced culling (63%) and improved fertility (49%).

When asked how they felt about JD control, 78% classified themselves as firm believers and would recommend it to other farmers. A further 13% were practising control for the benefit of their processor. The Net Promoter score for Johne's control was positive with 51% rated as promoters (score 8–10) and only 21% detractors (score 0–6).
An assessment of the NJMP was conducted by Dairy UK through a survey of processor attitudes and a qualitative assessment of progress with the JD framework using the RESET model as described by Lam (2017).

The RESET model of behaviour change describes the use of five drivers to change: Rules; Education; Social Pressure; Economics and Tools (Figure 4). Each component must be successfully addressed for behavioural change to be achieved. In the delivery of the NJMP, over an eight year period, it is clearly apparent that the elements have been delivered, albeit over an extended timescale whereupon the "need" for JD control was developed through Education and Economics, the Social Pressure and Tools developed before the element of Rules have been developed by Phase 2 of the NJMP, and individual processor contractors enforcing compliance with the scheme (Figure 5). This staged approach has been central to the success of the programme and has delivered high levels of engagement.

The engagement process, in the early years of the Engagement Programme (2010–2015), secured a Social Norm and understanding amongst the whole industry on the importance of JD control. The economic benefits have always been incorporated in messaging to farmers. The areas that caused some difficulty were the variable timescales and rules adopted by the processors. Each processor has defined their own emphasis and timescales and this has created some time slippage with the programme. For maximum benefit, all elements of the RESET model should be applied synchronously (Lam, 2017) albeit given the extended timescale for progress with JD control, a longer more protracted process has proved to be beneficial.

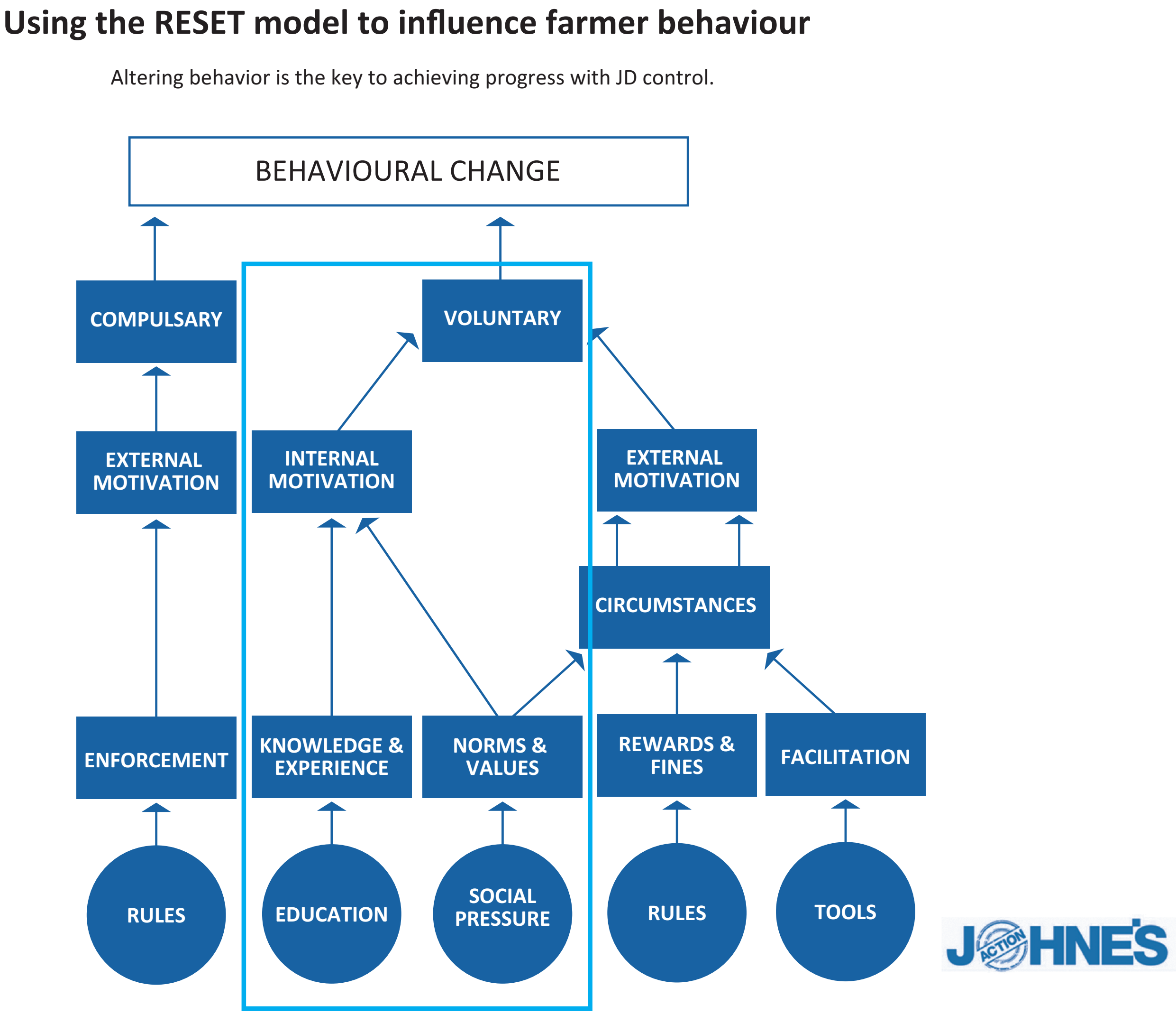


Figure 4. The RESET model and key elements to achieve behavioural change. (Lam, 2017)

Figure 5. Progress of the UK NJMP over an eight-year period from 2010–2018.

3. LESSONS LEARNED

The programme of JD control in the UK has always focussed on reducing infection within infected herds and preventing infections in low or zero prevalence herds.

Early on in the programme, it was identified that there were currently no obvious drivers to incorporate a numerical assessment of within-herd prevalence. Attempts to capture this information appeared to create a culture of suspicion amongst the farmers and vets that the system would be used to segregate milk supply based on JD prevalence to the detriment of those who took part and honestly described their status.

The confounding impact of TB testing and the cross reaction with the JD ELISA within 2–3 months of the TB test also has created challenges in terms of interpretation of tests. It is interesting to note the Irish Johne's programme has recommended a three-month time period after a TB test to ensure the correct specificity of the test to demonstrate absence of disease. A significant proportion of UK dairy herds enter a period of TB restriction with repeated 60-day Tb tests and this does create challenges for interpretation.

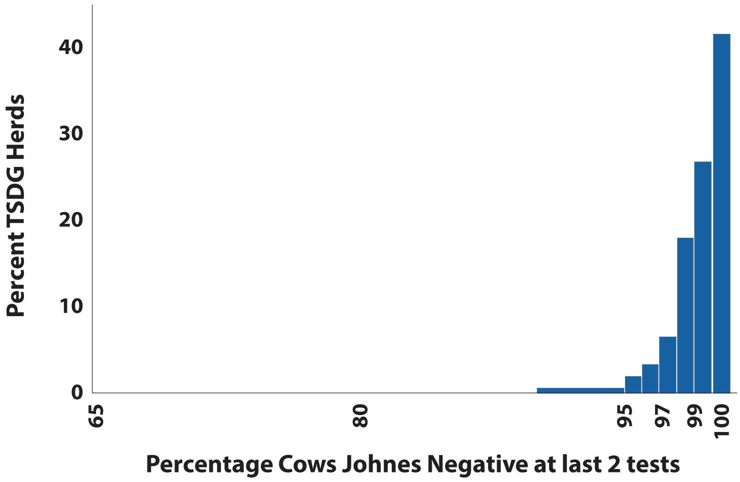
Purchase of replacement dairy cows remains a necessity for a proportion of dairy farmers and it became obvious that this is a high-risk policy: animals purchased from herds in the UK and non-UK herds revealed that the disease was widely prevalent in vendors' herds irrespective of assurances provided to the contrary. Certification of disease prevalence appeared to be expensive and might not deliver the benefits promised as the disease control process is not dependent on the level of surveillance alone. Farmer engagement with control and biosecurity combined with the selection of the most appropriate JD control plan is crucial for success.

The advent of sexed semen in dairy herds has allowed for reduced demand for purchased stock and allowed farmers to increase herd size without the need for the purchase of livestock further depressing the commercial attraction of more complex risk-based trading programmes.

What has become apparent, are the marked differences between individual processors and the markets they supply as to their attitudes to the NJMP. This has created difficulties creating momentum amongst the processor pool as a whole. Niche suppliers with export contracts appear to be more robust in their application of NJMP with some notably demanding 100% compliance for the farmer's contract to be retained.

Tesco is one of the largest retail groups with direct supply contracts with 650+ farmers and has made quarterly testing and centralised collation of test positive animals a key part of their animal health and welfare assurance programme. During this period the overall incidence of JD has declined from 24% of the herds with 99% test negative status to 84% of herds with 99% test negative status (Figure 6). The next steps for the Tesco group is to create a risk-based trading opportunity to allow low risk replacements to be sourced from test negative herds within the Tesco supply group and continued use of risk assessment to ensure full compliance with controls.

Within hear prevalence herds supplying TESCO supermarket 2012-2016



	2012	2014	2016
% negative cows	95.4%	97.0%	98.5%
% farms 99% negative cows	26%	58%	84%

Test positive defined as 2 consecutive INDEXX milk ELISA above 0.3 SP, 3 months apart

- TESCO supplied by 650 herds who are contracted to test every quarter by milk ELISA
- Results submitted to retailer every quarter and scored as part of farmer's HEALTH INDEX

Figure 6. Graphic illustrating progress with JD control within the Tesco Supply group of farmers 2012–2016.

Progress at a national level might therefore, be more processor or retailer dependent rather than driven centrally by the actions of the NJMP, which broadly defines the logical framework for those to progress JD control within their own specific processor pools. On reflection the National Johne's Management Plan is a framework for progress rather than a more typically described Disease Programme and this has contributed to the success of the Plan.

The conspicuous absence of a cheap, reliable screening programme remains a challenge: for example, there is a need for a quantitative JD Pathogen test which cannot be influenced by husbandry, TB testing or selection bias and which can be performed on cheaply available samples such as bulk milk. The 30-cow targeted screen has been highly successful in engaging farmers with control but, can easily be skewed if a lower prevalence is to be demonstrated. Whole herd screening using ELISA milk tests to demonstrate the absence of disease (as opposed to use for disease control in infected herds) has not been universally adopted due to the obvious lack of commercial drivers.

The progress of a commercially driven JD programme centres on retaining commercial benefit for those who contribute to the programme. For the programme to be sustainable, it has to deliver an economic benefit for the farmer whilst also providing a commercial opportunity for those involved in the Johne's Action Group. It is crucial to avoid the situation of developing a programme which primarily benefits a laboratory or academic institution rather than the farmers and processors. JD is effectively controlled by husbandry and risk management and in low prevalence herds, the most effective controls rely on application of improved farm management: testing might not be required.

National Milk Laboratories (NML), the largest provider of quarterly milk testing for JD control, has recently adopted the Myhealthyherd risk management program for use by its Herdwise customers and groups of farmers. This has provided a system where farmers using the testing programme can ensure that the Herdwise JD program will work by the production of a Prevalence report based on the algorithms provided within the Myhealthyherd program. Preliminary work on over 520 herds by NML has shown that up to 40% of herds might not be doing enough to effectively control disease entry and spread within herds (unpublished data). Testing is not enough. Risk management and disease control has to be the key focus.

4. IMPROVEMENTS MADE AND FUTURE FOCUS

The key focus for the NJMP will be based on:

- Providing a standardised approach for all processors based on the lessons learned by the early adopters. To maximise engagement "the need to control JD has to be created before the solution is provided". The typical build up to delivery would involve farmer educational meetings combined with newsletters profiling farmers who have controlled the disease. Providing a clear road map for delivery is essential.

- Parallel news dissemination of progress with the NJMP to the BCVA JD vets so that they themselves can update and remind their clients to get involved.

- An improved method of tracking progress with processors to demonstrate progress with the NJMP programme. This is a combination of qualitative (not yet started, farmer's awareness, delivery in process of completion) and quantitive data assessing engagement (% farmers within the processor pool with completed declarations).

- An assessment of the uptake with the NJMP in June 2018 demonstrated steady progress towards the 31 October 2018 deadline. 33% of the processors had progressed to the delivery phase with veterinary declarations being collected and 41% were progressing through the marketing phase of the programme. (Figure 7)

NJMP Phase 2Progress - June 18

Stage of Delivery	% of NJMP member processors
Delivery phase: Program ongoing and completed declarations are being collected	33%
Marketing phase: Farmer awareness and communications to farmers via processor communication routes	41%
Planning phase: Internal organisation ongoing and little/no farmer communication carried out	0%
Not yet started	0%
No recent update received	0%
No engagement to date	26%

74% of NJMP processors engaged, uptake processor and retailer dependent

Figure 7. Progress of the NJMP delivery through the 26 milk processors committed to the NJMP.

The maintenance of an annual NJMP JD conference to report and brief industry on progress with the programme.

The acceptance that, despite centralised deadlines for measurement of success, each processor will develop their own timelines based on their own priorities and resources.

To continue to work collaboratively within the UK and with other international schemes to help perfect the programme and overall reduction of JD.

5. ACKNOWLEDGEMENTS

The success of the UK NJMP has been dependent on the continued support of Dairy UK and member processors who have committed wholeheartedly to the support of the programme. Special thanks must go to BCVA for arranging and financially supporting the development of the Accredited Veterinary Training module created by Karen Bond. The support for the Action Group Johne's by veterinary groups and academic institutions (Myhealthyherd, RVC, Liverpool) and the energy of Ben Bartlett's team from National Milk Records, which has supported substantive parts of the delivery of the programme, has been central to the success of the Plan.

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ANNEXES

National Johne's Management Plan

NJMP Declaration

This declaration should be completed with support from your BCVA Accredited Johne's Veterinary Adviser (BAJVA). A copy of this certificate must be shared with your milk processor by 31st October 2018.

Farm Details	
Farm name	
Address and postcode	

Determine Herd Status
Please indicate which tests were used to determine your herd status

☐ Cull cow screen ☐ Targeted 30 cow screen	☐ Whole herd screen ☐ Other (please specify) ______________________

Control Strategy
Please indicate by ticking the appropriate box which strategy you have agreed to adopt within your written Johne's disease management plan. **Please note that only ONE strategy should be selected.**

☐ 1. Biosecurity protect and monitor ☐ 2. Improved farm management ☐ 3. Improved farm management and strategic testing	☐ 4. Improved farm management test and cull ☐ 5. Breed to terminal sire ☐ 6. Firebreak vaccination

Veterinary Declaration
I, as a Certified BCVA Johne's Veterinary Advisor, can confirm that an assessment of Johne's disease risk and status has been undertaken on this farm in the last 12 months and that there is a written Johne's management plan in place which has been agreed with the herd owner. In my opinion, the plan complies with the objectives of the National Johne's Management Plan.

Signed:	MRCVS		
Print name:		Date:	
Vet practice name and address:			

Farmer Declaration
I can confirm that an assessment of Johne's disease risk and status has been undertaken on this farm in the last 12 months in conjunction with a Certified BCVA Johne's Veterinary Advisor and I undertake to adopt the written Johne's disease management plan.

Signed:			
Print name:		Date:	

www.actionjohnesuk.org

NJMP Declaration_May 2018

National Johne's Management Plan

JOHNE'S ACTION

Declaration

National Johne's Management Plan – Phase 2

The operational requirements of Phase II are:

- Members of the NJMP would require their associated farmers to submit annually beginning 1st October 2017 an NJMP declaration signed by the farmer and by a BCVA Johne's Certified Veterinary Adviser that they will be implementing one of the six control strategies specified by the National Johne's Management Plan.
- By 31st October 2018 the NJMP declaration would confirm that the farmer had:
  - undertaken to assess their risks and herd status and,
  - put in place the necessary management information, equipment, husbandry and resources to implement the agreed control strategy.
- By 31st October 2019 the NJMP declaration would confirm that the farmer at the time of the consultation:
  - had reassessed their risk and status and,
  - was correctly utilising the management information, equipment, husbandry and resources to implement the strategy effectively.
- Only vets that have undergone the BCVA training programme would be permitted to sign the declaration.
- The BCVA Accredited Johne's veterinary adviser would issue a declaration that in their opinion, the farm's Johne's management plan was appropriate and compliant with the objectives of the NJMP.
- If deficiencies are identified by the vet then these would have to be rectified by the farmer before the declaration could be signed. Alternatively, the chosen strategy could be changed to enable compliance with the resources available.
- The farmer would make a copy of the signed declaration available to their milk purchaser if they were requested to do so.
- Purchasers would provide the Delivery Team annually with information on the number of farmers submitting NJMP declarations using a standard questionnaire developed by Action Group on Johne's.
- Membership of CHeCS would be recognised as giving equivalence to the requirements of Phase II.
- In respect of the use of risk assessment tools it would be at the judgement of vets which tool was most appropriate to use for an individual farm.

National Johne's Management Plan – control strategies

1. **Biosecurity Protect and Monitor**
2. **Improved Farm Management**
3. **Improved Farm Management and Strategic Testing**
4. **Improved Farm Management Test and Cull**
5. **Breed to Terminal Sire**
6. **Firebreak Vaccination**

For further detail on the control strategies, please visit www.actionjohnesuk.org or consult the BCVA Accredited Johne's veterinary advisor training manual.

www.actionjohnesuk.org

NJMP Declaration_May 2018

Copy of the NJMP Declaration

Programme

6th PARATUBERCULOSIS FORUM

9:00am to 12:50pm. Monday, 4 June 2018

International Convention Centre, Riviera Maya, Mexico

TIME	COUNTRY	TOPIC	SPEAKERS	AFFILIATION
9:00am	Welcome and Introductions		David Kelton	University of Guelph
9:10am	Germany	Paratuberculosis in Germany: Next step forward to control in cattle herds	Karsten Donat; Suzanne Eisenberg	Eisenberg Animal Health Service, Thuringian Animal Diseases Fund; Animal Diseases Fund of Lower Saxony
9:25am	Italy	Bovine paratuberculosis in Italy: Results after four years of application of the National Guidelines	Norma Arrigoni	Istituto Zooprofilattico Lombardia Emilia Romagna
9:40am	Spain	Long-term results of an experimental vaccination trial in dairy cattle in the Basque Country. Did we reach eradication?	Joseba Garrido	NEIKER-tecnalia, Basque Institute for Agriculture Research & Development
9:55am	The Netherlands	Results of milk quality assurance programme for paratuberculosis in Dutch dairy herds indicate reduced transmission of the infection	Maarten Weber	GD Animal Health
10:10am	Ireland	Establishing a national voluntary control programme for Johne's disease in Ireland	Lorna Citer	Animal Health Ireland
10:25am	Czech Republic	Control of paratuberculosis in the Czech Republic	Petr Kralik	Veterinary Research Institute
10:40am	Brazil	Advances and challenges of paratuberculosis in Brazil	Maria Aparecida Moreira	Universidade Federal de Viçosa
10:55am	Break			

TIME	COUNTRY	TOPIC	SPEAKERS	AFFILIATION
11:15am	Columbia	Paratuberculosis in Colombia: Past, present and future	Jorge Fernández-Silva	Universidad de Antioquia
11:30am	Canada	Lessons learned from Canadian Johne's disease programs	Herman Barkema	University of Calgary
11:45am	Australia	Johne's disease management in Australia update	Robert Barwell	Animal Health Australia
12:00pm	Argentina	Paratuberculosis in Argentina: Current status of disease control and application of diagnostic tools	Gabriel Traveria	Instituto Nacional de Tecnologia Agropecuaria; Universidad Nacional de Mar del Plata
12:15pm	Slovenia	How to control paratuberculosis in cattle herds	Matjaz Ocepek	University of Ljubljana
12:30pm	United Kingdom	The UK National Johne's Management Plan-Phase 2	Peter Orpin	UK Dairy
12:45pm	Closing remarks			

International Dairy Federation

INSTRUCTIONS TO AUTHORS

Submission of papers

Submission of a manuscript (whether in the framework of an IDF subject on the programme of work or an IDF event) implies that it is not being considered contemporaneously for publication elsewhere. Submission of a multi-authored paper implies the consent of all authors.

Types of contribution

Monographs; separate chapters of monographs; review articles; technical and or scientific papers presented at IDF events; communications; reports on subjects on the IDF programme of work.

Language

All papers should be written in English.

Manuscripts

- Files to be sent electronically by e-mail or via our FTP site. Login details will be sent upon request.
- Final document in Word 2003 or 2007
- All tables/figures included in final document to be sent also in separate Word, Excel or PowerPoint files, in black-and-white or colour format.
- All files to be named with author's surname plus title of paper/tables/figures.

References

- References in the document to be numbered and placed between square brackets.
- Reference lists at the end of the document to contain the following:
  - Names and initials of all authors;
  - Title of paper (or chapter, if the publication is a book);
  - If the publication is a journal, title of journal (abbreviated according to 'Bibliographic Guide for Editors and Authors', published by The American Chemical Society, Washington, DC), and volume number;
  - If the publication is a book, names of the publishers, city or town, and the names and initials of the editors;
  - If the publication is a thesis, name of the university and city or town;
  - Page number or number of pages, and date.

Example: 1 Singh, H. & Creamer, L.K. Aggregation & dissociation of milk protein complexes in heated reconstituted skim milks. J. Food Sci. 56:238-246 (1991).

Example: 2 Walstra, P. The role of proteins in the stabilization of emulsions. In: G.O. Phillips, D.J. Wedlock & P.A. William (Editors), Gums & Stabilizers in the Food Industry - 4. IRL Press, Oxford (1988).

Abstracts

An abstract not exceeding 150 words must be provided for each paper/chapter to be published..

Address

Authors & co-authors must indicate their full address (including e-mail address).

Conventions on spelling and editing

IDF's conventions on spelling and editing should be observed. See Annex 1.

ANNEX 1

IDF CONVENTIONS ON SPELLING AND EDITING

In the case of native English speakers the author's national conventions (British, American etc.) are respected for spelling, grammar etc. but errors will be corrected and explanation given where confusion might arise, for example, in the case of units with differing values (gallon) or words with significantly different meanings (billion).

"	Usually double quotes and not single quotes
? !	Half-space before and after question marks, and exclamation marks
±	Half-space before and after
micr<u>oo</u>rganisms	Without a hyphen
Infra-red	With a hyphen
et al.	Not underlined nor italic
e.g., i.e.,...	Spelled out in English - for example, that is
lit<u>re</u>	Not liter unless the author is American
ml, mg,...	Space between number and ml, mg,...
skimmilk	One word if adjective, two words if substantive
sulfuric, sulfite, sulfate	Not sulphuric, sulphite, sulphate (as agreed by IUPAC)
AOAC <u>INTERNATIONAL</u>	Not AOAC<u>I</u>
progra<u>mme</u>	Not program unless a) author is American or b) computer program
milk and milk product	rather than "milk and dairy product" - Normally some latitude can be allowed in non scientific texts
-ize, -ization	Not -ise, -isation with a few exceptions
Decimal comma	in Standards (only) in both languages (as agreed by ISO)

No space between figure and % - i.e. 6%, etc.

Milkfat	One word
USA, UK, GB	No stops
Figure	To be written out in full
1000-9000	No comma
10 000, etc.	No comma, but space
hours	Ø h
second	Ø s
litre	Ø l

<u>t</u>he Netherlands

Where two or more authors are involved with a text, both names are given on one line, followed by their affiliations, as footnotes

for example A.A. Uthar[1] & B. Prof[2]
[1] University of ....
[2] Danish Dairy Board .....

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