Open Access

Berl Münch Tierärztl Wochenschr 129, (2016)
DOI 10.2376/0005-9366-129-15129

ISSN 0005-9366

Korrespondenzadresse:
kdonat@thueringertierseuchenkasse.de

Eingegangen: 30.11.2015
Angenommen: 19.02.2016

Online first: 07.04.2016
http://vetline.de/open-access/158/3216/

Thuringian Animal Diseases Fund (public-law institution), Animal Health Service, Jena, Germany

The Thuringian bovine paratuberculosis control programme – results and experiences

Das Thüringer Programm zur Bekämpfung der Paratuberkulose in Rinderherden – Ergebnisse und Erfahrungen

Karsten Donat

Summary

This study aimed at evaluating the results achieved in voluntary paratuberculosis control in cattle herds in Thuringia, a federal state of Germany, between 2008 and 2014. A total of 76 dairy herds and 29 beef cow-calf herds were involved in the analysis. Cumulative incidence (CI) was used to monitor the control progress; new cases were detected by means of annual testing of the herd's cows by individual faecal culture for *Mycobacterium avium* subsp. *paratuberculosis* (MAP). Herds with at least one positive test result were classified as MAP-positive, while herds without any detection of MAP during three consecutive years were certified as MAP-non suspect. Compared to the MAP-positive ones, herds tested negative at the beginning of the program had a higher chance of achieving this certification by 2014. 13 out of 67 initially MAP-positive herds (19.4%) were certified according to the control programme. In a subset of 25 MAP-positive dairy herds that had been involved since 2008, CI decreased significantly from 14.0 to 5.6% in 2014. Regarding the initial situation in 2008, control progress was significantly higher in herds with CI > 5% compared to herds with CI < 5% as shown by two-way ANOVA. The results support the hypothesis that control of paratuberculosis is feasible at herd level. A herd monitoring based on faecal culture tests and a certification period of three years seem to be adequate to justify the status MAP non-suspect. Once herds achieve a low level of CI, control efforts should be intensified.

Keywords: *Mycobacterium avium* subsp. *paratuberculosis*, herd certification status, MAP-non suspect, faecal culture, cumulative incidence

Zusammenfassung

Zielstellung der vorliegenden Arbeit war es, die Ergebnisse des freiwilligen Programms zur Bekämpfung der Paratuberkulose in Thüringer Rinderherden im Zeitraum 2008 bis 2014 auszuwerten. In die Analyse waren 76 Milchviehherden und 29 Mutterkuhherden einbezogen. Der Sanierungsfortschritt wurde anhand der neu auftretenden Fälle (kumulative Inzidenz, CI) beurteilt, die im Rahmen der jährlichen Untersuchungen der Kühe des Bestandes auf der Basis kultureller Untersuchungen individueller Kotproben festzustellen waren. Herden mit Nachweis von *Mycobacterium avium* subsp. *paratuberculosis* (MAP) in einer Probe galten als MAP-positiv. Herden, in denen drei Jahre in Folge keine neuen Fälle festgestellt werden konnten, erhielten die Anerkennung als Paratuberkulose-unverdächtiger Rinderbestand. Im Vergleich zu MAP-positiven Herden hatten Herden mit MAP-negativer Ausgangssituation eine größere Chance bis 2014 diese Anerkennung zu erreichen. 13 von 67 MAP-positiven Beständen (19,4 %) wurden entsprechend des Kontrollprogramms als Paratuberkulose-unverdächtig anerkannt. In einer Untergruppe von 25 MAP-positiven und seit 2008 teilnehmenden Milchviehherden wurde ein signifikanter Rückgang der CI von 2008 (14,5 %) bis 2014 (5,6 %) nachgewiesen. In Bezug auf die Ausgangssituation war der Bekämpfungsfortschritt in Herden mit CI > 5 % größer als in Herden mit CI < 5 %. Die

U.S. Copyright Clearance Center Code Statement:
0005-9366/2016/129 $ 15.00/0

Ergebnisse unterstützen die Hypothese, dass eine Tilgung der Paratuberkulose auf Bestandsebene möglich ist. Die Herdenuntersuchung mittels Kotkultur und die dreijährige Anerkennungsperiode scheinen für die Anerkennung eines Bestandes als Paratuberkulose-unverdächtig angemessen und ausreichend zu sein. Sobald eine niedrige CI erreicht ist, sollte die Intensität der Bekämpfungsmaßnahmen erhöht werden.

Schlüsselwörter: *Mycobacterium avium* subsp. *paratuberculosis*, Bestandsstatus, Paratuberkulose-unverdächtig, Kotkultur, kumulative Inzidenz

Introduction

Paratuberculosis, a granulomatous enteritis of ruminants, occurs worldwide, is an O.I.E.-listed terrestrial animal disease (O.I.E., 2015), and is caused by *Mycobacterium avium* ssp. *paratuberculosis* (MAP). Animals infected with MAP may suffer from intermittent diarrhoea, excessive weight loss, protein deficiency and oedema after an incubation period of several years, and finally die (Tiwari et al., 2006). Therapy is not available for affected animals. Relevant economic losses already occur in the subclinical stage of the disease as a result of decreases in milk yield (Donat et al., 2014a) and fertility (Marcé et al., 2009). Currently, aims and strategies of monitoring and control of the disease are the subject of some controversy. In Sweden, Japan and Austria paratuberculosis is a notifiable disease subject to official control measures. Culling with compensation is applied to MAP-positive animals with clinical symptoms in Austria, and in Sweden to the whole herd if MAP is detected (Baumgartner und Khol, 2008). Participation is high in nationwide voluntary control programmes in Denmark (Kudahl et al., 2008 and the Netherlands. The Dutch dairy industry bans milk delivery from dairy herds that are not involved in the programme (Weber and Lam, 2012). A nationwide voluntary control programme supported by the dairy industry and the farmers' association exists in Belgium (Mintiens, 2014). The efforts of the dairy industry to take action with regard to paratuberculosis result from the need to maintain and improve the market position of dairy products. Here the assumption is taken into account that the pathogen of paratuberculosis, *Mycobacterium avium* subsp. *paratuberculosis*, could be involved in the aetiology of granulomatous enteritis in humans (Feller et al., 2007). In other countries such as France (Fourichon und Guatteo, 2014) and Canada (Kelton et al., 2014), paratuberculosis control is organized on a regional level and depends on public funding. The approaches differ in terms of diagnostic tools, culling scheme, availability of vaccines and the recommendations to improve hygiene and management within a herd. Despite all this, a broad consensus regarding the main pillars of a paratuberculosis control strategy has existed for many years: Biosecurity and trade control to prevent introduction into a herd, appropriate hygiene and management to prevent new within-herd infections, diagnosis and culling of MAP shedders to reduce the infective pressure, and reduction of the host's susceptibility by vaccination or genetic selection (Sweeney et al., 2012; Bastida and Juste, 2011).

In Germany, paratuberculosis is notifiable if the infectious agent is detected; 484 cases in cattle and 16 cases in sheep and goats were reported in 2014 (Köhler and Möbius, 2014). A nationwide prevalence study using a standardized diagnostic approach has yet to be carried out in Germany. Regional studies were reviewed by Campe el al. (2014), reporting a faecal culture-based MAP shedder prevalence of 5 to 14% and an apparent sero-prevalence of 4 to 45%. Apparent herd-level prevalence was between 11 and 54% in faecal culture-based studies and 2–42% when specific ELISA tests were applied. At the moment, regional voluntary control programmes are effective in North Rhine-Westphalia, Thuringia, Saxony and Brandenburg (Gierke and Köhler, 2009), and under preparation in Mecklenburg-Western Pomerania. The programme in Lower Saxony was abandoned in 2012 due to funding cuts and a continued lack of success in tackling the disease. Recently, the Federal Ministry of Food and Agriculture published nationwide recommendations on hygiene for keeping ruminants, replacing the paratuberculosis guidelines published in 2005 (BMEL, 2014).

The Thuringian control programme has been effective since 2003 and was updated in 2008 (TMSFG, 2008) and 2015 (TMASGFF, 2015). The most recent update accounts for current knowledge about how to control paratuberculosis as well as the federal recommendations (see Material and Methods).

This study aimed at analysing the control success during the second control period in Thuringia, i. e. from 2008 to 2014, and at drawing conclusions for further control measures.

Material and Methods

Thuringian control programme

The "Programme to control paratuberculosis in Thuringian cattle herds", published on 26 March 2008 (TMSFG, 2008), comprised the following principles:
- identification and culling of MAP shedders to reduce infective pressure,
- prevention of new infections by improving hygiene,
- consideration of paratuberculosis status of the herd of origin when introducing new animals into the herd,
- certification of herds as "MAP-non suspect" and control of status at regular intervals.

The programme recommended an annual testing of all animals older than 24 months in each herd, so approximately all cows and breeding bulls, by faecal culture to ensure the detection of a high proportion of MAP shedders in the subclinical stage of the disease. Samples were taken either at one occasion from all cows of the herd or in a continuous manner where the testing schedule is often linked to other herd management measures like e. g. pregnancy checking. This ensures sampling of all cows that are likely to stay in the herd for another lactation.

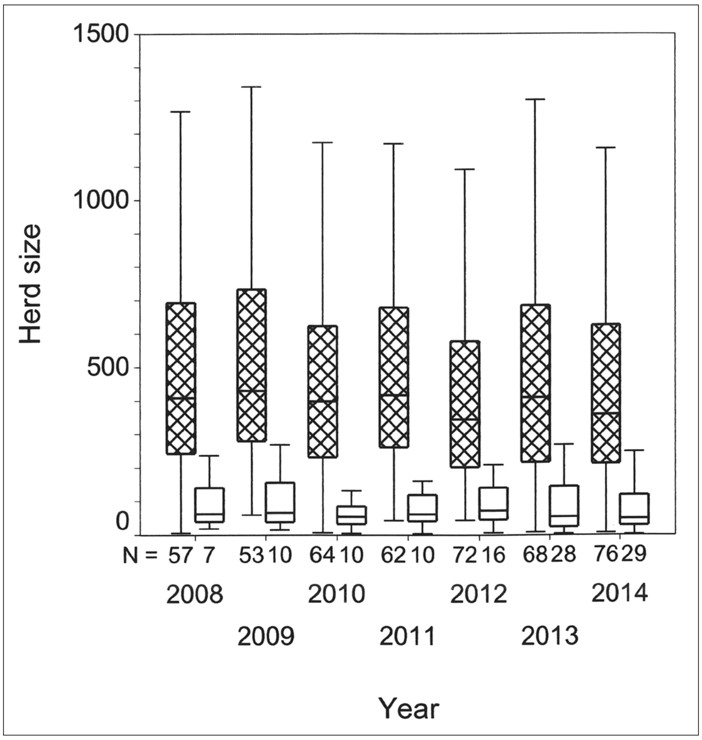

FIGURE 1: *Number of enrolled herds (N) and herd size (number of cattle older than 24 months as reported in the respective year) of the dairy herds (hatched boxes) and beef cow-calf herds (open boxes) participating in the Thuringian Paratuberculosis Control Programme during 2008 and 2014 (bar: median value; boxes: 50% of values, whiskers: 95% of values).*

Animals that tested positive were not retested in the following year. Farmers were advised to cull them as soon as possible. MAP shedders showing clinical signs of paratuberculosis had to be culled immediately. Provided that MAP shedders displayed no clinical symptoms, farms with a high within-herd prevalence were advised not to inseminate their positive cows and to cull them at the end of lactation.

Specialized veterinarians from the Animal Health Service recorded hygiene and management of the herd using a purpose-made questionnaire that serves as a data record and the basis of discussing the herd-specific control measures. Farmers were consulted in order to establish measures for adoption in daily work.

The most recent update of the control programme implemented the recommendations of the German Federal Ministry of Food and Agriculture that established different levels of control according to the diagnostic method used:
– Level 1: environmental sampling,
– Level 2: antibody detection,
– Level 3: detection of the organism.

Improvement of farm management and hygiene as well as immediate testing of cattle showing clinical symptoms of paratuberculosis and prompt culling of MAP shedders are recommended for each level. In addition, the Thuringian control programme introduced level 4 for a low prevalence herd (< 3%) that binds farmers to remove MAP shedders from the herd within one month after detection. Furthermore, the programme update enforces the implementation of management and hygienic measures like trade control, calving hygiene, management of colostrum for first feeding and not saleable milk and staff hygiene in calf and youngstock area within three months after enrolment.

Herds enter the"certification phase'"if no MAP shedder has been identified for twelve months and can be certified as "MAP-non suspect" if no MAP shedders were identified in the annual testing during three consecutive years. These herds were then retested biannually ("maintenance of status").

Due to the limited diagnostic sensitivity of animal-level tests (Collins et al., 2006; Köhler et al., 2008a, Donat et al., 2014b), farmers were advised to focus on the paratuberculosis status of the herd of origin when purchasing animals. The Thuringian Animal Health Service certified a herd as "MAP-non suspect" if no MAP shedders were identified in the annual testing during three consecutive years. These herds were then retested biannually.

Study population

105 farms were enrolled in the programme at the end of the period under review (31 December 2014). Their distribution regarding year and farm type is given in Figure 1. The size of each herd was drawn from the annual report to the Animal Diseases Fund on the reference date, January 3 of each year. As calves and young stock were not included in the testing, the focus was on cattle older than 24 months. Only herds in which 50% or more of cattle older than 24 months were tested annually were considered for further analysis. In 2014, these herds accounted for 33 614 cattle older than 24 months. This involves nearly 20% of the cattle population in Thuringia.

Herds were classified as MAP-negative if no MAP shedders were detected in a herd in 2008. A herd was considered MAP-positive if at least one MAP shedder was detected within the herd and remained MAP-positive until it successfully passed the three-year certification procedure. Due to the triannual certification period, MAP-positive herds without shedders were overrepresented among them. To analyse the progress of control in dairy herds, a subset of 25 MAP-positive herds was selected as follows:
– dairy herds,
– enrolment in 2008 or earlier,
– never reached certification level during 2008–2014 (i. e. CI > 0 in each year).

Only two beef cow-calf herds met these criteria. This analysis was therefore omitted for this herd type.

Collection of faecal samples and MAP testing of faecal cultures

For every cow to be tested, a faecal sample was taken rectally with an unused examination glove and wiped off into a sterile plastic container, supplied by the laboratory and equipped with a bar code, which was then sealed and sent to the examination laboratory within 24 to 48 h by a courier.

The individual faecal cultures were tested on a Herrold's Egg Yolk Medium (HEYM), according to the culture method published in the official Friedrich-Loeffler-Institut (FLI, 2010) collection of methods, as of April 2010, modified for the annual herd tests as follows:

a) All samples were frozen immediately after arrival at the laboratory and stored at –20°C until testing. After initially extracting 3 g of faeces per sample, the samples were frozen again and stored as retained samples until the completion of testing.
b) Due to the high number of samples in the herd test (about 30 000 samples per year) the inoculum was applied to a HEYM tube after decontamination. As soon as rampant growth of undesired accompanying flora was detected, another culture was prepared immediately from the retained sample (repetition sample).
c) A longer decontamination period of 48 h was applied for repetition samples or samples from herds that had shown overgrowth in samples in the past.

From the fifth week of incubation at 37°C, the macroscopic evaluation of the bacteria growth was performed every two weeks. Suspicious colonies were differentiated via Ziehl-Neelsen-staining, subculture (testing the mycobactin-dependent growth) and/or an IS900-PCR (Englund et al., 1999). The results were registered in terms of quality with a SQL database (Fa. Agro Data EDV Service GmbH & Co. KG, Cottbus, GER). Bar code labels were attached to all sample tubes, expendable materials and the culture tubes in order to ensure the correct assignment of the marked animals to the respective samples and results.

Cumulative incidence

The cumulative incidence (CI) based on the following calculations comprises all animals from which samples were sent to the laboratory in the respective year and in which MAP was identified. As determined in the control programme, animals that had been tested positive in the previous year would not be tested and examined again. Therefore, samples from programme farms came from animals that had tested negatively before or animals that were being examined for the first time. The CI for the respective year represents the quotient from the number of new cases per year and the number of animals sampled in the reference year. In rare cases MAP shedders known from previous years were sampled again (altogether ~60 out of ~1200 shedders per year) accounting for a minor overestimation of CI.

Statistical evaluation

The collection and preparation of the data extracted with the lab software of the Thuringian Animal Diseases Fund (Agro Data EDV Service GmbH & Co. KG, Cottbus, GER) as well as the presentation of figures were performed using the spreadsheet software Microsoft Excel (Microsoft Corporation, Redmond, Washington, US). The statistics software SPSS 11.5 for Windows (SPSS Inc., Illinois, Chicago, US) was used for all other calculations. The level of significance was determined to be $p < 0.05$.

The frequency distributions of the farms after achieving the level of control in 2014 and the respective herd status regarding paratuberculosis were tested with reference to the initial status of the herd (MAP-positive or MAP-negative) with Fisher's exact test. The stratified evaluation per farm type (dairy farm or beef cow-calf) was conducted following Mantel and Haenszel, and the homogeneity of the odds ratio of the individual strata was tested with the Breslow-Day test.

TABLE 1: *Initial status and state of control achieved by 2014 in the dairy and beef cow-calf herds participating in the Thuringian Paratuberculosis Control Programme*

Control program phase achieved in 2014[1]	Dairy herd		Beef cow-calf herd		Total	
	MAP-positive[2]	MAP-negative[3]	MAP-positive	MAP-negative	MAP-positive	MAP-negative
Control phase (level 1–3)	24	0	5	0	29	0
Control phase (level 4)	17	1	5	0	22	1
Certification phase	4	3	2	4	6	7
Certified as MAP-non suspected	9	18	1	12	10	30
Total	54	22	13	16	67	38

[1] According to the "Recommendations of the Federal Ministry of Food and Agriculture on hygiene for keeping ruminants" (BMEL, 2014)

[2] Herds with MAP-positive animals in the first faecal culture based herd testing after enrolment in the control program

[3] Herds without MAP-positive animals in the first faecal culture based herd testing after enrolment in the control program

A single factor ANOVA (SPSS package ANOVA with repeated measures data) was applied to analyse the CI between 2008 and 2014 in the 25 selected dairy herds. Since there was no normal distribution of the CI of the herds in the individual years, the values were converted into their decadic logarithm. Normal distribution was examined with one-sample Kolmogorov-Smirnov tests. Because in the Maulchy test sphericity was violated by the principal factor, a Greenhouse-Geisser correction of the degrees of freedom was applied. In order to compare herds with either a good (CI < 0.05) or a bad (CI > 0.05) initial situation, similarly to the procedure described a two-way ANOVA with Bonferroni post hoc test was applied.

Results

From 2008 to 2014 the mean herd size (mean ± standard deviation) of the dairy herds (479 ± 327 vs. 466 ± 336) and the beef cow-calf herds (97 ± 82 vs. 87 ± 94) did not change.

Ten herds (14.9%) of 67 MAP-positive herds in the first year were certified as "MAP-non suspect" and six herds (7.5%) had entered the certification phase at the end of 2014. In these herds, zero to ten shedders had been detected before. 37 of the 38 herds in which MAP had not been detected (MAP-negative) by 2008 or in the year they entered the programme, have now been certified as MAP-non suspect. In three of these herds, evidence of MAP was detected in the meantime, but all of these infections originated from newly purchased animals. Within the observation period, two herds were able to achieve certification or to re-enter the certification phase again. Taken together herds in the certification phase and the herds certified as MAP-non suspect, 53 (50.4%) of 105 farms included in the programme have reached the point where no infectious agent was found in samples from the farms' cows anymore (Tab. 1).

For both farm types it was shown that significantly more herds that had been tested MAP-negative in 2008 achieved the herd status "MAP-non suspect" until 2014 than those that had been tested MAP-positive in 2008

TABLE 2: *Development of the cumulative incidence of the dairy herds (n = 25) included in the ANOVA model*

Herds	Year of control	Mean [%]	Standard-error [%]	Confidence interval of 95% [%]	
				Lower boundary	Upper boundary
All (n = 25)	1	14,50[ab1)]	3.58	7.11	21.88
	2	10.70[abc]	2.74	5.03	16.36
	3	9.27[ab]	1.94	5.28	13.27
	4	9.55[ab]	2.01	5.40	13.70
	5	8.37[abc]	1.96	4.32	12.42
	6	6.41[c]	1.25	3.84	8.99
	7	5.57[c]	1.26	2.98	8.17
CI > 5% (n = 15)	1	22.14	3.99	13.88	30.40
	2	15.51	3.25	8.79	22.24
	3	13.41	2.16	8.94	17.87
	4	13.69	2.27	8.99	18.40
	5	11.96	2.30	7.20	16.72
	6	8.81	1.44	5.82	11.79
	7	7.69	1.50	4.58	10.80
CI < 5% (n = 10)	1	3.03	4.89	Not specified	13.14
	2	3.47	3.98		11.71
	3	3.07	2.64		8.54
	4	3.33	2.79		9.09
	5	2.99	2.82		8.82
	6	2.82	1.77		6.48
	7	2.39	1.84		6.20

[1)] Different letters mark statistically different means; values that are indexed with the same letter are not statistically different

TABLE 3: *Results of the two-way ANOVA of the cumulative incidence of MAP shedders in 25 Thuringian dairy herds regarding year (with repeated measures data) and initial situation with Greenhouse-Geisser correction of the degrees of freedom*

	Degrees of freedom	F value	P value
Year	3.6	5.98	< 0.0001
Initial situation	1	21.87	< 0.0001
Year * initial situation	3.6	1.80	0.142

($p < 0.0001$). The stratified analysis per farm type of the MAP status in 2014 compared to the initial status in 2008 showed this significant correlation in the same way. The joint odds ratio was 32.9 (confidence interval of 95%: 9.8–110.1).

A significant influence of the duration of control measures in years ($p < 0.0001$) was shown among the dairy herds that were included in the ANOVA model (n = 25). The CI mean value (± standard error, SE) of these 25 herds in 2008 (14.50% ± 3.58), when compared with that of 2014 (5.57% ± 1.26), is significantly different ($p = 0.004$). The mean values (± SE) for all years, including the confidence intervals of 95%, are displayed in Table 2.

Comparing herds with either a good (CI < 0.05) or a bad (CI > 0.05) initial situation, the individual factors "year" and "initial situation" have a significant influence in the overall model, although the interaction between the two does not (Tab. 3). In herds with a bad initial situation, the CI mean value of 22.14% ± 1.26 is reduced to 7.69% ± 1.50 in 2014. Due to the small sample size and high variance, this difference is insignificant. In herds with a good initial situation, the mean value hardly changes: from 3.03% ± 4.89 to 2.39 ± 1.84. Due to the high variance in herds with a good initial situation, these have negative lower limits of the confidence intervals at 95% (Tab. 2).

Discussion

This study shows that cumulative incidence can be decreased by the measures which are prescribed by the Thuringian paratuberculosis control programme. This resulted in the certification of ten previously MAP-positive cattle herds within a six years' period. Furthermore, another six herds reached the certification level. If herds that were MAP-negative in 2008 are taken into account as well, it was possible to transfer more than half (50.4%) of the herds involved in the process to a status where no MAP shedder was found during the last herd screening. This verifies that it is possible to reduce prevalence under the detection limit and keep this status for at least three years. The standard applied in Thuringia, so the proof of the absence of MAP shedders over a period of three consecutive years, fulfils the same requirements as the uniform standard for the voluntary bovine Johne's disease control programmes in the US (USAD, 2010) and the regulations in Australia (Jubb and Galvin, 2004). The fact that no further spread of the infection via non-registered shedders was detected in the Thuringian herds beyond the date of certification is further proof of the sustainable character of the certification status assigned in Thuringia. The fact that new MAP shedders were detected in two of the herds was attributable to uncontrolled or rather uncritical acquisitions of new animals or transfers of animals to other herds at the same farm. This small proportion of herds with reinfections shows that the relatively strict certification procedure carried out in Thuringia since 2008 guarantees a high level of security, and that an uncontrolled further development of the infection within the herd or the transmission from one herd to another by undiscovered shedders is avoided to the greatest possible extent. This observation from current control practice corresponds with certain models showing that most herds initially rated as "MAP-negative" become certified as MAP-free if highly specific tests are performed, and that they are also able to keep that status (Ezanno et al., 2005).

Those MAP-positive herds that were certified as MAP-non suspect until 2014 all were herds with a low initial prevalence. The possible lowering of prevalence to zero as demonstrated in this control practice in Thuringia can also be shown in appropriate epidemiological models for herds with an initial prevalence of 5%, provided the hygiene and management measures established in the herd are suitable to effectively break the chain of infection between the MAP shedder and the receiving animal (Kudahl et al., 2008). The successful minimization of MAP-shedders in herds with low prevalence shows that early detection of MAP infections in cattle herds is of great importance: The lower the prevalence at the time of detection, the faster the control of the infection at herd level. A significant reduction of detected MAP shedders from 14.5% to 5.6% was observed in the remaining MAP-positive herds over the six-year period. This development is in line with the results of other studies. In a six-year study of six dairy herds in Minnesota, US, it was possible to reduce the average apparent within-herd prevalence determined by faecal culture from 10.4% to

5.6% (Ferrouillet et al., 2009) and from 17.0% to 9.5% in young cows from nine dairy herds in Wisconsin (Collins et al., 2010). In a study from North Rhine-Westphalia, Germany, apparent prevalence on farms involved in the control programme was reduced from over 3% to under 1% (vom Schloss, 2000; Luyven et al., 2002). When it comes to prevalence-based data, it is important to keep in mind that these might be subject to distortion because culling, as part of the control measures, directly affects prevalence. To a much lesser degree, this applies to analyses based on the number of new cases, as is the case with this study. Therefore, reduction of CI reflects to a higher degree the effectiveness of control measures over time than changes in prevalence.

The reduction of the amount of MAP shedders within the herd or within the population, be it by lowering the rate of new infections or targeted culling of the affected animals, is a pivotal element of the control progress, because it leads to less environmental contamination of susceptible animals in the cattle barn with infectious agents and it lowers the risk of undetected MAP-infected animals transmitting the disease from one herd to another (Ezanno et al., 2005).

The development of the CI within herds with high initial prevalence in our study was significantly different to that in herds with a low share of infected animals in 2008. While the herds with a high initial prevalence showed a drastic CI reduction from an average of 22.2% to 7.7%, those with a low initial CI stagnated around 3%. There are no findings so far regarding the reasons for this difference, but some assumptions can be made. It is possible that the control measures that had been established before 2008 limited the transmission of the infectious agent to a certain extent resulting in a low percentage of new cases, which had a calming effect on the prevalence within the herd and on the farmer's motivation to consistently apply the control measures. Our own research showed that milk yield depression, one of the main problematic effects in herds with high prevalence, is almost imperceptible in herds with a within-herd prevalence of less than 5% (Donat et al., 2014a). Apart from this it can be assumed that clinical cases either considerably drop in numbers or do not even appear anymore. One possible explanation is that, when the number of shedders is small, the risk of infection decreases, and along with it the severity of lesions of the intestinal mucosa (Mortier et al., 2013), meaning the intestinal infection disseminates at a lower speed. If regular tests are carried out then, MAP shedders are detected at an earlier subclinical stage and do not reach the clinical stage by the time they are culled. The motivation to continue to uphold consistent hygiene and culling practices disappears along with this, and the quality of control stagnates. A cohort study of Saxon and Thuringian programme farms showed that hygiene, e. g. the effective cleaning and disinfection of the calving pen after use by MAP shedders (Donat et al., 2016), as well as the mean holding time of MAP shedders after receiving the laboratory report had an influence on reducing the CI in the herds (Donat et al., 2014c). These findings, however, are contrary to the results of the simulation models by Kudahl et al. (2008) and Weber et al. (2008), who considered rapid culling to be of no additional advantage, but correspond with the findings of Eisenberg et al. (2013, 2015). It could be concluded that the focus of consultations in the next step of the Thuringian paratuberculosis control programme will have to be on herds with a low CI. More inspections regarding the implementation of hygiene measures will have to take place and the mean holding time of shedders per herd will have to be monitored so that the supervising Animal Health Service can intervene in the case of deviations from the prescribed measures. In addition to these measures, a culling subsidy for every single shedder that leaves the herd within one month after receiving the laboratory report (in the case of pregnant cows: within one month from calving) will be paid to all farms which commit in writing to the aim of eradicating the infectious agent from the herd and which achieve a CI of less than 3%. A modelling study on the persistence of the paratuberculosis infection in a herd showed that, contrary to spontaneous fadeout, persistence of paratuberculosis infection within a herd is closely linked to the presence of animals with clinical symptoms in the herd. Therefore, the early detection and removal of affected animals is a critical factor to avoid the persistence of paratuberculosis in dairy herds (Marcé et al., 2011).

A large proportion of German dairy farmers remain hesitant or even reluctant to adopt paratuberculosis control measures. This reluctance is often justified with the argument that existing tests fall short of offering a reliable basis for consistent control and that the available tests still do not offer the necessary sensitivity and specificity. The data presented in this study clearly show that it is possible to take control of the disease even with the existing tests. A control programme that is based only on the improvement of hygiene standards may result in comparably good prevalence reductions, as seen in the simulation by Kudahl et al. (2008), but only under the assumption of an optimum interruption of the infection chain. However, practical experience of paratuberculosis control in Thuringia has shown that perfect hygiene management is hard to keep up in the long run. Therefore, reducing the infective pressure in the cattle barn environment by detection and removal of MAP shedders before they reach the clinical stage (Marcé et al., 2011) is of great importance for successful control.

Multi-stage control programmes exist both in the US (USDA, 2010) and the Netherlands (Franken, 2005); they have been recommended for Canada (McKenna et al., 2006) and Austria (Khol und Baumgartner, 2012). The recommendations issued by the Federal Ministry of Food and Agriculture in 2014 as well as the Thuringian control programme, which is based on these recommendations and has been effective since 1 July 2015, provide for a control period that is divided into various steps. The first step of this programme is the establishment of appropriate hygienic measures in connection with herd monitoring via environmental faecal samples. This is a way to begin control measures in MAP-positive herds and a cost-saving means of maintaining active monitoring efforts. However, the fact that human resources are becoming increasingly scarce in the dairy industry, it doesn't seem particularly promising or even practical to focus only on perfect hygiene. In order to effectively reduce the number of new cases and to eventually eradicate the infectious agent, individual diagnostics will be indispensable. The best results in terms of sensitivity and specificity of the testing procedure so far are guaranteed by direct identification in individual faecal samples via faecal culture testing or molecular biological methods (Köhler et al., 2008b).

Conclusion

The results described herein show that a reduction of prevalence is feasible at herd level. Therefore, the control phase of the Thuringian programme now includes a level four of the control phase aimed at eradicating infection from the herd.

As soon as the control measures in a herd have led to a certain success, in this case 3% new cases per year, the accompanying measures of consulting and hygienic control have to be intensified.

Acknowledgement

The author wishes to thank the cattle farmers involved for their active participation in the programme as well as the colleagues of the Thuringian Animal Diseases Fund for carrying out the technical laboratory screenings, consulting the cattle farmers and helping in evaluating data and compiling the manuscript. Georg Siebert and Matthew Romney are acknowledged for improving the English style.

Conflict of interest

There are no protected financial, professional or other interests in a product or a company that could affect the contents or opinions stated in this publication.

References

Bastida F, Juste RA (2011): Paratuberculosis control: a review with a focus on vaccination. J Immune Based Ther Vaccines 9: 8.

Baumgartner W, Khol JL (2008): Paratuberculosis – potentials and limits of surveillance programs. Magyar Allatorv Lapja 130 Suppl. I: 7–10.

Bundesministerium für Ernährung und Landwirtschaft (BMEL, 2014): Empfehlungen des Bundeslandwirtschaftsministeriums für Ernährung und Landwirtschaft für hygienische Anforderungen an das Halten von Wiederkäuern. Banz. AT 01.08.2014 B1, 1–17.

Campe A, Köhler H, Donat, K (2014): Approaches to Controlling Paratuberculosis in Germany – Conditioned by Voluntariness and Federalism. Proceedings of the 4th ParaTB-Forum, Parma, Italy 2014, 31–39.

Collins MT, Gardner IA, Garry FB, Roussel AJ, Wells SJ (2006): Consensus recommendations on diagnostic testing for the detection of paratuberculosis in cattle in the United States. J Am Vet Med Assoc 229: 1912–1919.

Collins MT, Eggleston V, Manning EJ (2010): Successful control of Johne's disease in nine dairy herds: results of a six-year field trial. J Dairy Sci. 93: 1638–1643.

Donat K, Soschinka A, Erhardt G, Brandt HR (2014a): Paratuberculosis: Decrease in milk production of German Holstein dairy cows shedding *Mycobacterium avium* subsp. *paratuberculosis* depends on within-herd prevalence. Animal 8: 852–858.

Donat K, Schlotter K, Erhardt G, Brandt HR (2014b): Prevalence and control measures within the herd influence the performance of ELISA tests. Vet Rec 174: 5.

Donat K, Schmidt M, Köhler H. (2014c): Paratuberculosis control in Germany – Conclusions from five years' voluntary control in Thuringia and Saxony. In: Proceedings of the 12th International Colloquium on Paratuberculosis. Parma Italy 2014, 219.

Donat K, Schmidt M, Köhler H, Sauter-Louis C (2016): Management of the calving pen is a crucial factor for paratuberculosis control in large dairy herds. J Dairy Sci, doi: 10.3168/jds.2015-10625.

Eisenberg SW, Chuchaisangrat R, Nielen M, Koets AP (2013): Relationship between presence of cows with milk positive for *Mycobacterium avium* subsp. *paratuberculosis*-specific antibody by enzyme-linked immunosorbent assay and viable *M. avium* subsp. *paratuberculosis* in dust in cattle barns. Appl Environ Microbiol 18: 5458–5464.

Eisenberg SW, Rutten VP, Koets AP (2015): Dam *Mycobacterium avium* subspecies *paratuberculosis* (MAP) infection status does not predetermine calves for future shedding when raised in a contaminated environment: a cohort study. Vet Res 46: 70.

Englund S, Ballagi-Pordány A, Bölske G, Johansson KE (1999): Single PCR and nested PCR with a mimic molecule for detection of *Mycobacterium avium* subsp. *paratuberculosis*. Diagn Microbiol Infect Dis 33: 163–171.

Ezanno P, van Schaik G, Weber MF, Heesterbeek JA (2005): A modelling study on the sustainability of a certification-and-monitoring program for paratuberculosis in cattle. Vet Res 36: 811–826.

Feller M, Huwiler K, Stephan R, Altpeter E, Shang A, Furrer H, Pfyffer GE, Jemmi T, Baumgartner A, Egger M (2007): *Mycobacterium avium* subspecies *paratuberculosis* and Crohn's disease: a systematic review and meta-analysis. Lancet Infect Dis 9: 607–613.

Ferrouillet C, Wells SJ, Hartmann WL, Godden SM, Carrier J (2009): Decrease of Johne's disease prevalence and incidence in six Minnesota, USA, dairy cattle herds on a long-term management program. Prev Vet Med 88: 128–137.

Fourichon C, Guatteo R (2014): Control of Paratuberculosis in Cattle in France: Components of National and Regional Voluntary Programs and their Application. Proceedings of the 4th ParaTB-Forum, Parma Italy 2014, 27–30.

Franken P (2005): Paratuberculosis control in the Netherlands – The target and an overview of the activities. In: Proceedings of the 8th International Colloquium on Paratuberculosis, Copenhagen, Denmark: August 14–17, 2005. Providence: International Association for Paratuberculosis, 2006, 16–19. [quoted on 24 August, 2015]: http://www.paratuberculosis.info/images/stories/pdfs/119.

Friedrich-Loeffler-Institut (FLI), Bundesforschungsanstalt für Tiergesundheit (2010): Amtliche Methodensammlung, as of April 2010, Paratuberkulose [quoted on 4 May, 2011]: 277–208, <www.fli.bund.de/de/startseite/publikationen/amtliche-methodensammlung.html>

Gierke F, Köhler H (2009): Tenazität, Ausscheidung und Übertragungswege des Paratuberkuloseerregers – Konsequenzen für die Bekämpfung der Paratuberkulose in Rinderbeständen. Praktischer Tierarzt 90: 1169–1178.

Jubb TF, Galvin JW (2004): Effect of a test and control program for bovine Johne's disease in Victorian dairy herds 1992–2002. Aust Vet J 82: 228–232.

Kelton DF, Barkema HW, Keefe GP, Fecteau G, Barker RA (2014): Johne's Disease Control in Canada – 2010–2013 – Successes and Challenges. Proceedings of the 4th ParaTB-Forum, Parma Italy 2014, 115–122.

Khol JL, Baumgartner W (2012): Examples and suggestions for the control of paratuberculosis in European cattle. Jpn J Vet Res 60 Suppl: 1–7.

Köhler H, Burkert B, Pavlik I, Diller R, Geue L, Conraths FJ, Martin G. (2008a): Evaluation of five ELISA test kits for the measurement of antibodies against *Mycobacterium avium* subspecies *paratuberculosis* in bovine serum. Berl Münch Tierärztl Wochenschr 121: 203–210.

Köhler H, Gierke F, Möbius P (2008b): Paratuberculosis – current concepts and future of the diagnosis. Magyar Allatorv Lapja 130 Suppl. I: 67–69.

Köhler H, Möbius P (2014): Paratuberkulose In: Friedrich-Loeffler-Institut, Bundesinstitut für Tiergesundheit (Hrsg.), Tiergesundheitsjahresbericht 2013. Friedrich-Loeffler-Institut, Greifswald Insel Riems, 92–95.

Kudahl AB, Nielsen SS, Østergaard S (2008): Economy, efficacy, and feasibility of a risk-based control program against paratuberculosis. J Dairy Sci 91: 4599–4609.

Luyven G, vom Schloß A, Saßerath M (2002): Paratuberkulosesanierung in Nordrhein-Westfalen. Dtsch Tierärztl Wschr 109: 524–527.

Marcé C, Ezanno P, Seegers H, Pfeiffer DU, Fourichon C (2011): Predicting fadeout versus persistence of paratuberculosis in a dairy cattle herd for management and control purposes: a modelling study. Vet Res 42: 36.

Marcé C, Beaudeau F, Bareille N, Seegers H, Fourichon C (2009): Higher non-return rate associated with *Mycobacterium avium* subspecies *paratuberculosis* infection at early stage in Holstein dairy cows. Theriogenology. 71: 807–816.

McKenna S, Van Leeuwen JA, Barkema HW, Jansen JT, Hauer G, Hendrick SH, Cote G, Salsberg EB, Empringham RE (2006): Proposed Canadian Voluntary National Johne's Disease Prevention and Control Program. Can Vet J 47: 539–541.

Mintiens K (2014): Paratuberculosis in Belgium. Proceedings of the 4th ParaTB-Forum, Parma Italy 2014, 15–18.

Mortier RA, Barkema HW, Bystrom JM, Illanes O, Orsel K, Wolf R, Atkins G, De Buck J (2013): Evaluation of age-dependent susceptibility in calves infected with two doses of *Mycobacterium avium* subspecies *paratuberculosis* using pathology and tissue culture. Vet Res 44: 94.

O.I.E. – World Organisation for Animal Health (2015): OIE-Listed diseases, infections and infestations in force in 2014 [quoted on 17th March 2015]: http://www.oie.int/animal-health-in-the-world/oie-listed-diseases-2015

Sweeney RW, Collin MT, Koets AP, McGuirk SM, Roussel AJ (2012): Paratuberculosis (Johne's disease) in cattle and other susceptible species. J Vet Intern Med 26: 1239–1250.

Thüringer Ministerium für Soziales, Familie und Gesundheit (TMSFG, 2008): Thüringer Programm zur Bekämpfung der Paratuberkulose in den Rinderbeständen in Thüringen. ThürStAnz. Nr. 16/2008; 556–559.

Thüringer Ministerium für Arbeit, Soziales, Gesundheit, Frauen und Familie (TMASGFF, 2015): Thüringer Programm zur Bekämpfung der Paratuberkulose in den Rinderbeständen in Thüringen. ThürStAnz. Nr. 26/2015; 1087–1093.

Thüringer Landesanstalt für Landwirtschaft (TLL) (2015): Entwicklung der Tierzucht in Thüringen, Berichtsjahr 2014. Thüringer Landesanstalt für Landwirtschaft, Jena, 2015.

Tiwari A, van Leeuwen JA, McKenna LB, Keefe GP, Barkema HW (2006): Johne's disease in Canada Part I: clinical symptoms, pathophysiology, diagnosis, and prevalence in dairy herds. Can Vet J 47: 874–882.

United States Department of Agriculture (USDA). Animal and Plant Health Inspection Service (2010): Uniform Program Standards for the Voluntary Bovine Johne's Disease Control Program, as of 1 September 2010, Herd classification, 19–24 [quoted on 27 February, 2014]: http://www.aphis.usda.gov/animal_health/animal_diseases/johnes/downloads/johnes-ups.pdf

Vom Schloß A (2000): Auswertung eines Sanierungsverfahrens zur Bekämpfung der Paratuberkulose in Rinderbeständen in Nordrhein-Westfalen. Hannover, Tierärztliche Hochschule, Diss.

Weber MF, Lam T (2012): Milk quality assurance for paratuberculosis in The Netherlands: achievements of 2006 to 2011. Abstract book of the XXVIIth World Buiatric Congress, Lissabon Portugal 2012, 25.

Weber MF, Nielen M, Velthuis AG, van Roermund HJ (2008): Milk quality assurance for paratuberculosis: simulation of within-herd infection dynamics and economics. Vet Res 39: 12.

Whitlock RH, Hutchinson LT, Merkal RS (1985): Prevalence and economic consideration of Johne's disease in the north-eastern U.S. Proc US Anim Health Assoc 89: 484–490.

Address for correspondence:
Dr. Karsten Donat
Thüringer Tierseuchenkasse
Victor-Goerttler-Str. 4
07745 Jena
Germany
kdonat@thueringertierseuchenkasse.de