Simulating economic impact of paratuberculosis in Dutch dairy herds

Dieuwke Twisk

BEC-80424
Henk Hogeveen
2nd supervisor Tariq Halasa (DTU)
Wageningen University

Introduction

Paratuberculosis (PTB), also called Johne's disease, is a chronic and contagious infection, which is slowly caused by *Mycobacterium avium* subsp. Paratuberculosis (MAP). The infection is highly prevalent in dairy cattle, but also capable of affecting other ruminants such as sheep, goats and deer. It takes years to develop in the animal and they can be subclinically infected for a long time before showing clinical symptoms as diarrhea, malnutrition and death (Gonda et al., 2007). This not only causes a reduced animal welfare, but also economic damage in the dairy industry. Since the first of January 2011, Dutch dairy farms are required by milk processors to participate in the "Paratuberculose Programma Nederland" (PPN). This programme contains annual milk testing with ELISA on individual animals, and classification. These classifications provide certification of fully paratuberculosis negative tested herds called "Status A", and herds with positive tested animals classified as "Status C". After culling all the positive tested animals, a farm becomes "Status B". The requirements made by dairy producers is to be at least classified as "Status B". When a producer is "Status C", processers will not buy and collect the milk (Weber 2012). The test used in this programme is the commercial Pourquier ELISA kit (Sensitivity 40%, Specificity 100%), the test is only carried outin the animals that are being milked at the moment of testing (van Weering et al., 2007). The dried off animals and heifers are not tested. Before culling a positive animal a fecal PCR can be carried out to confirm the infection of the animal (confirmation testing). This test has a higher sensitivity on heavy shedding cows, but is more expensive (€38.04 per cow ; GD 2015), compared to ELISA (€2.53 per cow GD 2015). To keep an ELISA test positive cow on the farm while still remaining "Status B", it is mandatory to be tested negative with PCR. But because of the high price of PCR, the relative old age of the positive tested animals and high specificity of the test most farmers prefer to cull the cow without checking with PCR. This current method might be working, but there are no studies done on the true cost PTB and this system in the Netherlands. Besides in other countries like Denmark a different test is used, so maybe using a different test or test frequency our system can become more efficient.

The objective of this study is to estimate the monetary costs of MAP-infection under the currently used test-and-cull strategy. Besides, possible changes in the strategy will be evaluated.. This is done by applying the the Danish PTB-ICull model to the Dutch MAP situation. The PTB-iCull model is a stochastic, mechanistic and dynamic discrete event simulation model that deals with the spread of MAP within a dairy herd in Denmark. Different test-and-cull strategies will be compared in the model adjusted to the Dutch situation. The model not only takes into account the epidemiological factors, but also the economic aspects on the farm that are impacted by the infection. On basis of the results, producers and processors can become more aware of the cost-effectivity of current and alternative test and cull strategies to reduce the spread of paratuberculosis.

Literature

In this chapter appropriate literature about Paratuberculosis and related circumstances to our study are described.

Transmission - The intensity of transmission is highly correlated with the density of the animals. If there is high density of animals, there is more contact between the cows and hence more transmission is likely to occur. In the clinical stage, cows can infect potentially 25 other cows in their environment (Whitlock and Buergelt, 1996). The most important transmission method of MAP is the oral-fecal route, but different pathways of transmission are possible (Eisenberg et al., 2010). Bulls that are infected with the MAP bacteria can excrete it via semen and feces (Larsen et al., 1981). Cows on their term can emit the MAP bacteria in colostrum, milk and feces (Streeter et al., 1995). The fact that MAP bacteria can occur in milk has consequences for humans. The bacteria can occur in raw milk, but also have a chance of surviving the process of pasteurization and then transmit to humans (Gao et al., 2002). When the bacteria are transferred to human, it could potentially cause Crohn’s disease. Crohn's disease is a chronic inflammatory disease, primarily affecting the gastrointestinal tract, the clinical signs are abdominal pain of bowel obstruction or diarrhoea with passage of blood or mucus, or both (Baumgart and Sandborn, 2012). Although there is no specific prove that MAP is linked to Crohn’s disease, the bacteria is frequently detected in infected patients (Bull et al., 2003; Naser et al., 2004).

Economics - The costs of paratuberculosis as mentioned before are not only due to welfare impairment of affected animals, but also due to economic losses to the dairy industry. The damage of PTB is mainly caused by a reduced milk yield, higher replacement costs, decreased feed conversion ratio (FCR) and potential consequences for trade (Ott et al., 1999; Rideout et al., 2003; Weber, 2006; Richardson and More, 2009). The monetary costs of MAP contamination can be challenging to evaluate due to the lack of exact data associated with disease prevalence and that most MAP infections are subclinical (Johnson-Ifearulundu and Kaneene, 1997; National Research Council, 2003). In the UK, the average consequences of PTB per cow per year have been calculated as £26 for dairy cattle and £17 for beef cattle (Gunn et al., 2004). In the United States, the average costs of MAP infection were estimated at $22 to $27 per cow per year (Ott et al., 1999). Nevertheless, the cost of the infection in positive herds was calculated to be $100 per cow, and clinical cases within the herd could cause losses that may exceed $200 per animal (Ott et al., 1999). But, the total net economic impact in the whole US dairy industry caused MAP infection is estimated to vary from $200 million to $1,500 million annually (Losinger., 2005; Ott et al., 1999). French researchers estimated PTB related costs to be €461 for a subclinical case and €1,940 for a clinical case, in view of a usual dairy farm in France (Dufour et al., 2004). In Ireland, one case study over a 10-yr period showed that the yearly margin in this herd decreased between €130 and €155 per cow during the study period (AHI, 2012), while Barrett et al. (2006) described decrease in margin between €168 and €253 per animal in an average Irish herd that test positive to PTB. The economic losses originate from different levels, first of all the losses due to a decrease in milk production. Previous studies have shown that the value of this decrease can be 500 up to 1,400 kg/cow/year (Beaudeau et al., 2007; Gonda et al., 2007). Another way paratuberculosis decreases profit is because of the reduction in slaughter weight. The weight loss is estimated up to 31% and slaughter value decreases up to 48% compared to cows that are not infected with MAP bacteria (Kudahl and Nielsen., 2009). The weight loss is caused by an inflammation of the gastrointestinal tract which is linked to a reduced feed efficiency.

When the dairy cow becomes less efficient this leads to premature culling or natural death. Previous studies have shown that the mortality rate of a positive tested herd could be up to 3% higher, which will lead to extra losses (Johnson-Ifearulundu et al. 1999).

Paratuberculosis situation in the Netherlands - Paratuberculosis has first been detected in the Netherlands in 1919. These first detections were done by the culture of feces from clinically infected and suspected animals. Later, different methods were used to prevent and detect PTB. For example in 1922, the infection was traced with injecting avian tuberculin in the cattle. By identification of the reaction MAP bacteria could be traced, but this was very time consuming. So after 1931, a new test was developed called Johnin allergy test. The test was quicker than avian tuberculin, so animals could be traced in an early infected stage. In the year 1942, a more organised control began and animals were tested every year with the Johnin allergy test. In the beginning of the 1950s a system of subsidies was implemented. It became illegal to sell positive tested cattle and farmers were compensated for culling suspected animals. Afters some years, in 1954, the complement fixation test (CFT) came on the market. CFT can detect the presence of either specific antibody or specific antigen in serum. Starting from 1958, all cattle older than 18 months were tested using the CFT. A new plan was developed, because no significant change was noticed. In 1979 animals at ages from 4 to 18 months were tested with the Johnin test. If the animals were older than 18 months, the CFT was used. Still the results were not as expected, and the costs were high (Benedictus et al., 2000). Because a higher significant change was desired, the focus of the programme changed in 1983 to the vaccination of calves with a killed vaccine. The results of this strategy were higher in reducing clinical paratuberculosis, nevertheless the infection was not gone (Dijkhuizen et al., 1994). Through the year a lot of different strategies were created to decrease PTB within herds. The latest programmes are the 'Intensive Paratuberculosis Programme' (IPP), the 'Bulk Milk Quality Assurance Programme' (BMQAP) and the current used 'Paratuberculose Programma Nederland' (PPN). IPP was introduced in 1998, targeting at eradication of PTB from infected herds and low-risk trade of cattle from certified paratuberculosis-free herds (Weber.,2006). But, participation from farmers to this programme has remained low, since the programme has relatively high costs. For that reason, a new much cheaper bulk milk quality assurance programme (BMQAP) was started in January 2006 (Weber., 2012). This programme is an addition to the IPP. The BMQAP aims at reducing human exposure to MAP bacteria through dairy products (Weber et al., 2007).

Since the first of January 2011 Dutch dairy farms are required by milk processors to participate in the 'Paratuberculose Programma Nederland' (PPN). This programme contains annual milk testing with ELISA on individual animals, and classification. These classifications provide certification of fully paratuberculosis negative tested herds called “Status A”, and herds with positive tested animals classified as “Status C”. After culling all the positive tested animals a farm becomes “Status B”. The requirement made by dairy producers is to be at least classified as “Status B”, when a producer is “Status C” processers will not buy and collect the milk (Weber 2012). The test used in this programme is the commercial Pourquier ELISA kit (Se 40%, Sp 100%), the test is only done on the animals that are being milked at the moment of testing (van Weering et al., 2007). Only the animals that give milk at the moment of testing are tested, so highly pregnant called dried off animals and heifers are not tested. Before culling a positive animal fecal, PCR can be carried out to double check infection of the animal. This test has a higher sensitivity on heavy shedding cows, but is more expensive. It costs €38.04 per cow to test with PCR (GD 2015), compared to ELISA which is €2.53 per cow (GD 2015). To keep an ELISA test positive cow on the farm while still remaining “Status B”, it is mandatory to be tested negative with PCR. But because the high price of PCR and the chances to be tested positive again, most farmers prefer to cull the cow without checking with PCR (expert opinion).

The within-herd prevalence of MAP infection was tested with ELISA in milk in 12,077 Dutch herds. These participating herd revealed that 6,438 herds had no test-positive animals and 1,712 herds contained 1 test-positive animal. Two or more test-positive animals were identified in 2,153 herds, but the within-herd prevalence in these herds stayed below 5%. From the 12,077 ELISA tested herds 1,232 herds had a within-herd prevalence between 5 and 10%, and 542 herds had a within-herd prevalence of minimum 10% (van Hulzen et al., 2011). Higher within-herd prevalence were also shown in the Netherlands with scenarios that go up to 70% (Muskens.,2000; Weber.,2005). But the most relevant high prevalence, this is estimated at 40% (Expert opinion).

Modelling on PTB - Several PTB simulation models have been have been developed and have shown confirmed to be useful in evaluating the impact of MAP spread whit in herds and efficiency of different measures on reducing the prevalence (Groenendaal., 2003; Kudahl et al., 2007; Mitchell et al., 2008; Marcé., 2010). An example is the Dutch JohneSSim model. In this study the control strategies test and cull, calf-hygiene management and vaccination were simulated and compared using a stochastic simulation model. Test-and-cull strategies and vaccination appeared not effective in decreasing the true prevalence, within the 20 years of simulation only calf-hygiene management tools could reach a low mean true prevalence(Groenendaal., 2003). Another example of a PTB simulation model is the PTB-Simherd (Kudahl et al., 2007) .This is a dynamic, stochastic, and mechanistic Monte-Carlo model simulating a dairy herd including young stock. Findings were that breaking the infection routes was the best strategy to reduce the true prevalence of PTB in a herd, using test-&-cull alone was found to just delay the increase in prevalence (Kudahl et al., 2007). R.M. Mitchell built several models upon previous work. The models developed were used to test three hypotheses. The first one, infectious transmission following intervention is relatively insensitive to the presence of high-shedding animals. The second hypotheses tested, vertical and pseudo vertical transmission increases prevalence of disease but is insufficient to explain persistence following intervention. And the last assumption verified is transiently shedding young animals might aid persistence. The result indicated that using the test-and-cull intervention caused elimination of infection in herds. Since contagiousness of high-shedding adult animals in combination with vertical transmission were serving as the predominant contributor to transmission (Mitchell et al., 2008). These examples show that it is essential to evaluate PTB control strategies on a longer period in order to define the ideal programme. During a longer time phase the most optimal strategy for a herd might change due to transformations in the herd, for example change in prevalence or yield.

Very recently the PTB-iCull model has been developed. It is a stochastic, mechanistic and dynamic discrete event simulation model that deals with the spread of MAP within a dairy herd in Denmark. This is a new model and it has some striking differences compared previous published simulation models.

1) The shedding to the environment and the build-up of MAP in infected farm sections is included in the model. This model approach was previous only included beef-herd simulation (Humphry et al., 2006). 2) Section to section contamination with MAP is included, resembling machinery or human cross over trough the different parts of the farm. 3) Like in reality age-dependence sensitivities in the

tests are used(Nielsen et al., 2013; Nielssen., 2013). The age-dependence is also modelled in the susceptibility function so that older animals had a lower probability of infection, unlike previous models were animals become resistant after a maximum age of 1 year(Marcé et al., 2010). 4) The culling of cows was linked with the replacement. This was modelled by voluntarily and involuntarily culling for reasons as injuries and disease. 5) A time step of 1 day is used, so every management decisions occur on daily basis. For example infection events, insemination and voluntary and involuntary culling, in current modern systems it is also the case that these decisions are made daily(Nielssen., 2011). Former PTB models have used larger time steps as one week up to one year, these steps can be unrealistically large (Pouillot et al., 2004). 6) The model used not only takes into account the epidemiological factors, but also the economic aspects on the farm that are impacted by the infection.

Materials and methods

The model used in this study is the new PTB-iCull model from Denmark (Kirkeby et al., 2015). This model is a stochastic, mechanistic and dynamic discrete event simulation, which simulates the spread of MAP within a herd. The model and its use to study impact on herds and surveillance programmes have been described in detail in The iCull Model Part II by Kirkeby et al., 2015. When testing the scenarios the model runs them for 16 years using the first six years as a burning period to calibrate the model. Each of the scenarios are simulated 500 times in the model. In all simulations a burn-in period of 6 years was implemented to stabilize the herd. During this period, the baseline scenario was simulated. This ensures that all scenarios have the same starting point before implementing different test or culling strategies. Previously, the model was run with a 3-year burn-in period(Kirkeby et al., 2015), but when applying Dutch parameters the herd size was reduced more than half. So the model became less stable and a longer period is needed. These six years are not shown or discussed, the ten years after this are used to observe the results and shown in the study.

Herd

The model simulates a closed herd, with animals in all life stages. To start the model, an initial herd has to be generated. Therefore the fraction of heifers, lactating cows, dry cows and young stock is calculated to calibrate the herd. For some parts standard Danish herd parameters were used, as they do not differ much from the Dutch system. These values are shown in Table 1. First of all the same breed is used which will also have similar heritability of characteristics. But to keep the model running, the animals can only spend a certain amount of days in a life step. The different life steps that cows in the model can go through are represented in Figure 1. For example when a cow becomes pregnant it still gives milk for 224 days, until the farmer stops milking the cow for 56 days to prepare for the new birth (dried off). Heifers do not give milk yet so they just stay in the life step pregnant heifer for 280 days (Table 1 and Figure 1) (Kirkeby et al., 2015). Further changes that were made to adapt the herd are mentioned.

Table 1.
Unchanged parameters used in creating herds in the model.

Parameter used	Value
Breed	Holstein
Milk heredity	0.13
Max age for cow	3650 days
Time spend as calf	365 days
Time spend as heifer	110 days
Time spend as inseminated heifer	41 days
Time spend as pregnant heifer	280 days
Time spend as inseminated cow	41 days
Time spend as pregnant cow	224 days
Time spend as dried off cow	56 days

The stable herd is aimed at 91 cows in this study because it is the average number of cows on a farm in the Netherlands (LEI 2013). When simulating, the model keeps track of the life state of all animals. Every cow is assigned to an individual production level. This level is modelled with a daily variation and specific parameters using the Wood lactation curve (Wood., 1967). To go to another life state, average Dutch data are used, for example to become pregnant a cow has to succeed at a 47% change of heat detection (Heres et al., 2000) and an insemination rate of 42% (Inchaisri et al., 2011).

To model the prevalence of MAP two initial situations are used. The first one is the average percentage of 5% found in the Netherlands representing a normal hygiene herd. This estimation comes from previous Dutch studies in which 1,232 herds found to have a within-herd test prevalence between 5 and 10%. The true prevalence should be lower than the tested prevalence, so an average of 5% is assumed (van Hulzen., 2011). But there are also 542 herds found that had a within-herd test prevalence of at least 10% (van Hulzen., 2011), and even scenarios that go up to 70% (Muskens.,2000; Weber.,2005). For comparison with the average-hygiene herd, a second scenario is simulated to represent “a low hygiene herd” with the most relevant high prevalence, this is estimated at 40% (Expert opinion). To calibrate the level of infection force, a series of simulations were run. These simulations characterise the baseline with a 5% initial prevalence and a 40% initial prevalence, keeping the median prevalence stable for 10 years. For both scenarios, 40 varying infection force levels were set and then ran with 500 repetitions and a 6-year burn-in period. The repetitions were visualized in a boxplot, and the set of parameters that came closest to keeping the true prevalence stable at 5% or 40% is chosen, based on visual inspection. Based on this method the infection force for the normal hygiene herd is set to 0.0000775 per cow/day, and for the low hygiene herd the infection force is 0.00008 per cow/day.

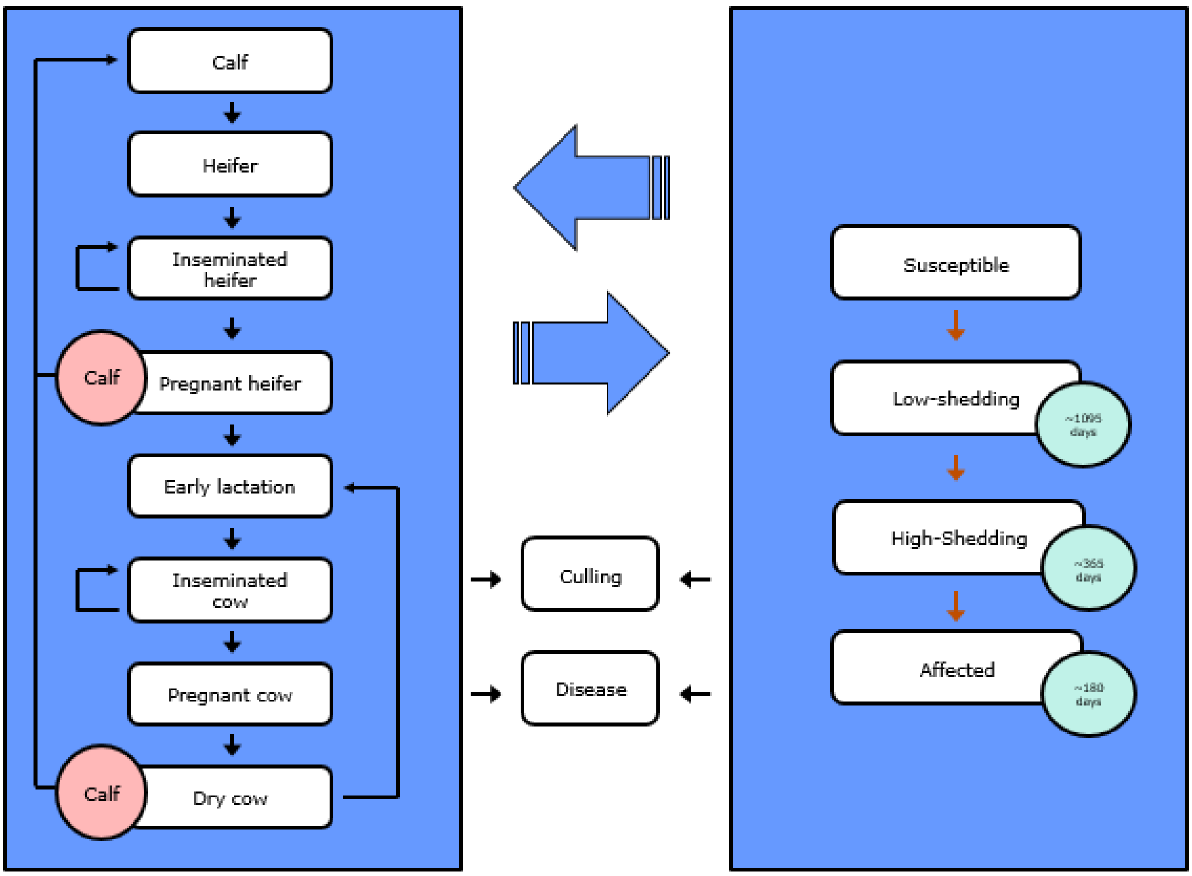

Figure 1: Systematic representation of life steps that can occur to animals in the model

Feed description

The initial iCull model was not very detailed in the costs of feeding. For this reason the costs of feeding were changed in the model used in this study. In the model, feed costs are calculated by using the energy requirements at the different live stages of the cow, for example requirements followed from milk production and maintenance (including gestation), requirements of dairy cows and from growth and maintenance requirements of young stock. The following assumptions were used (CVB, 2007;Berentsen et al., 2011). The energy unit used was mega joules (MJ) of net energy (NE). NE is the amount of energy in feed potentially utilisable by animals (metabolisable energy) minus the heat loss.

- The energy requirements per day for the maintenance of a dairy cow are 36.6 MJ of NE.
- For milk production a dairy cow uses 3.17 MJ of NE/kg of energy corrected milk.
- Additional daily requirements for gestation are 3.11, 6.56, 10.4, and 18.6 MJ of NE for month 6, 7, 8, and 9 of gestation, respectively (Assuming a 1-yr calving interval). Between these values the numbers are linear interpolated.
- Daily requirement for young stock differs from 17.2 MJ of NE in the first months and goes up to 51.8 MJ of NEL in the last months before calving.

The costs of these energy requirements are dependent on the source of energy. The average diet of a Dutch dairy cow consists of around 70% silage and 30% concentrates (Tamminga et al. 2004; CBS, 2009; Bannink., 2011). The costs for silage were calculated using 15,000 kg or 63,066 MJ produced per hectare and (Peeters & Kopec., 1996;Berentsen et al., 2011), divided by the costs of 110 euro per 1000 kg of silage (efarm.nl., 2015). This gives a costs of 0.026 euro per MJ of NE. The same calculation is done for the price of concentrate. The energy of concentrate is assumed to be 4.4 MJ per kg (Sevenster et al., 2007). This is then divided by the costs of concentrates, which are 1,809 per 10,000 kg (LEI., 2012). This gives an average costs for concentrate of 0.04 euro per MJ of NE. Using the proportions of the Dutch diet (70% silage-30% concentrate), this gives an average of 0.0302 euro per MJ of NE, this is the general costs used for calculating the total costs of feed for heifers, lactating cows and dry cows.

Economics

As we simulate a Dutch herd, Dutch prices were used to estimate the economic performance of the herd. For example, the price for energy corrected milk (ECM) is assumed to be €0.34 per kg (Cijfers die spreken 2009-2014). Other prices used in the model are labour cost of €16 per hour (Cijfers die spreken 2009-2014), insemination price €27 per insemination (CRV 2009-2014), destruction price €34.62 per cow (Rendac 2010-2015) and the price per heifer is €1021.5 (Cijfers die spreken 2009-2014). These prices are not stable and can go up and down during the year therefore the average price paid during 2009 until 2014 is used. For cost of testing and subscription, the most recent prices were used because these prices are less variable. The Dutch ELISA test cost, €2.53 per cow, but €9.80 has to be added for sending all the tests and a quarterly subscription on the PPN programme of €14.45 (GD 2015). The model does not include costs for housing, machines and other fixed assets, these costs and depreciations can vary a lot between different farmers but will not be effected by the state of disease.

PTB impact on economics - In the original ICull model, the slaughter value is reduced due to weight losses. If cows has tested positive in at least one of the last three tests (Kudahl and

Nielsen., 2009). But in the currently used, changed model, all cows are culled when tested positive. To adapt the model, the Danish ELISA was still used in every scenario, only in the background. Which means. these test outcomes are solitary used to adapt the slaughter values and the relative energy corrected milk yield level (rECM). Cows with fluctuating responses lose 12.9% of their slaughter value, those with only the latest test positive lose 7.9% of the slaughter value, and those with repeated positive ELISA tests lose 16.6% of their slaughter value. The rECM in infected cows is reduced according to the latest ELISA value. An increase in MAP S/P ratio is correlated with a decrease in milk production relative to the average cow on each farm. At a MAP S/P-value of 1, the rECM was 0.94, while it was 0.78 at the MAP ELISA value of 5 (Græsbøll et al., 2015).

Definitions in results - The expenses shown in the table are calculated by cumulating costs of feed, testing, destruction and insemination. Fixed costs for example housing, labourers and machineries are not included. The definition used to compute the return is the gain or loss in a particular period and consists the income gains relative on an investment. This means all incomes from products produced on the farm are added together to calculate the return. The margin is the difference between all incomes of the farm and the production cost made during production not taking the fixed cost into account. Also the costs of feed, testing and destruction are presented in the results. The cost of feed are accumulated from concentrate and silage as mentioned above. The testing cost are different for the tests used per scenario, this can be Dutch ELISA, Danish ELISA or Dutch ELISA plus PCR. Destruction costs are the costs for both voluntary plus involuntary culling together. So in these destruction costs not only the costs for culling caused by PCR are cumulated, but the costs for all cows culled in a simulation.

Scenarios

After the parameterisation of the Dutch data in the ICull model. Six different scenarios were created to compare the effect of the infection. 1) The baseline for these scenarios is the most common way of paratuberculosis control in the Netherlands. This consists of testing once a year with the commercial Pourquier ELISA kit and culling all positive cows. 2) This scenario is the same as the first one. Except that those cows that are positive to an initial test are tested again. This way of handling paratuberculosis on the farm, is called serial testing, and is also allowed in the Netherlands, but used less because of the high price of PCR testing. By including this scenario, in the model, we can see if this higher expense in the long run is actually true. 3) The third scenario is the same as the baseline except that testing is done with the ELISA currently used in Denmark. This test has a higher sensitivity (Nielsen et al., 2013). This scenario will show if using a better test will have a benefit. 4) In the fourth scenario, no testing or other measurements against PTB are done. 5) In the fifth scenario the commercial Pourquier ELISA kit will be used as in the baseline, except that now the test will be done twice a year and positive cows will be culled, to find out if testing more frequently will be an advantage. 6) The last scenario is using the same test frequency as scenario five. Only here the indirect ELISA screening kit is used as in Denmark. All scenarios were tested with the normal prevalence of 5% and with the high prevalence of 40%.

Culling - Cows are culled when the number of cattle exceeds the maximum amount of places in the herd, ensuring a stable number of cows in the herd at all times. This maximal amount is set to 91 cows in the model; this includes the lactating and the dry cows. Cows can be culled voluntary or involuntary. Involuntary culling includes animals that are injured or subject to other diseases and are therefore sent to slaughter, these cows are randomly selected. Voluntary culling is carried out by a strategic decision; this is based on milk production, reproduction status and the ELISA or PCR test results. In our study a test-and-cull strategy is used, so for the different scenarios the cows chosen for culling are adapted. 1) According to the Dutch system cows should be culled after tested positive, so in the baseline, animals found positive by the used commercial Pourquier ELISA kit are culled. 2) The second scenario uses a serial test. Therefore, only cows that test positive with both ELISA and PCR are considered to be affected, and only the effected cows are culled. 3) For the third scenario the same culling strategy is used as in the baseline. The only difference is that the Danish ELISA test is used to find the positive cows instead of the previous used Dutch ELISA test. 4) This scenario does not include any measurements for reducing PTB, so no cows are tested nor culled. 5) For the fifth scenario the exact same culling strategy is applied also with the same tests, but in a higher frequency. Because in this situation test-and-cull is done twice a year as an alternative to the once a year in the baseline. 6) This is the same for the last scenario, again test-and-cull is done twice a year, but this time with the Danish ELISA.

In the model positive tested cows are assigned the highest priority for culling. This means when there are PTB positive tested cows these are always culled. But when there are no cows tested positive for PTB and the number of cattle exceeds the maximal limit. Cows with low scores on milk production or reproduction status are chosen to be culled.

<u>Sensitivity</u>

The sensitivity of the ELISA test incorporated in the model is based on previously published data from Denmark, where ELISA performance was adapted according to the age of the cow (Nielsen et al., 2013). The cut-off of the ELISA used in the Dutch control system is enhanced from 0.2 S/P was 1.0 S/P, leading to an expected increase in specificity and decrease in sensitivity. Assuming that disease dynamics in Dutch cows are similar to dynamics in Danish cows, the Danish dataset, which was used to estimate the age dependent ELISA sensitivity for the Danish control program, was used to estimate the performance of the Dutch ELISA taking the enhanced cut-off into account (Table 2. and Fig. 2). The sensitivity level is in equation (1) so that the sensitivity matched the Dutch test. In order to create a maximum sensitivity of 40% equation(1) should be 0.518. The incorporated ELISA sensitivity is a logarithmic function dependent on the age of the tested animal, resulting in an ELISA test value indicating if the animal is infected.

$$sens(age) = sensitivity.level \cdot \left(\frac{exp(\beta_0 + \beta_2 \cdot exp(\beta_1 \cdot age))}{1 + exp(\beta_0 + \beta_2 \cdot exp(\beta_1 \cdot age))} \right) \quad (1)$$

Table 2.

Parameters to create Dutch ELISA sensitivity

Parameter	**Value**
β_0	1.32
β_1	-0.70
β_2	-9.38
Sensitivity level	0.518

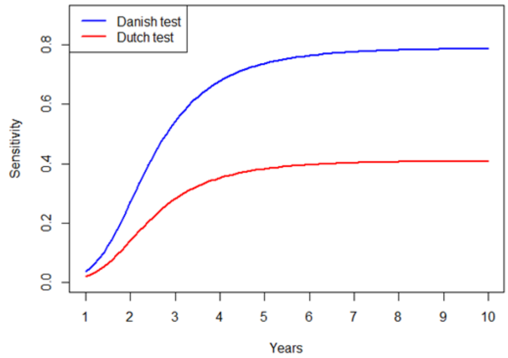

Figure 2: the different sensitivities of the Danish and Dutch test during the years of a cows lifetime

Table 3.

Sensitivity PCR test

Parameter	**Percentage**
Low shedding	40%
Heavy shedding	95%
Clinical disease	90%
Specificity	100%

The current Danish test (the commercial ID Screen Paratuberculosis Indirect ELISA screening kit) has a sensitivity that during the age of the cow goes up to about 80% (Nielsen et al., 2013). This sensitivity level is then adjusted to the Dutch test (commercial Pourquier ELISA kit), which goes up to 40% van (Weering et al., 2007). In the second scenario the cows that are tested positive with Dutch ELISA are tested again with fecal PCR. The sensitivity of this test differs with the state of the disease (Table 3), these numbers are also taken into account in the model (Weber., 2008).

Results

Average-herd.

In Table 4, the results of the average-hygiene-herd and low-hygiene-herd are shown, the low-hygiene-herd is discussed later. The true prevalence shown in Table 4 is the end prevalence after 10 years of simulation. This true prevalence is shown over time in Figure 3.

Table 4.
True prevalence of the scenarios on an average-hygiene herd and an low-hygiene herd. The numbers are median results with 5% and 95% confidence limits. Numbers are shown in % is the resulting prevalence at the end of the simulations.

Scenario	**Average-hygiene True prevalence**		**Low-hygiene True prevalence**	
	50%	**(5% ; 95%)**	**50%**	**(5% ; 95%)**
1. Dutch ELISA	3.30	(0 ; 29.67)	39.56	(19.78 ; 59.34)
2. Dutch ELISA + PCR	8.79	(0 ; 34.50)	43.48	(24.16 ; 59.34)
3. Danish ELISA	0	(0 ; 13.24)	28.57	(5.49 ; 56.06)
4. No control	20.88	(0 ; 50.00)	50.55	(34.07 ; 64.85)
5. Dutch ELISA 2/year	0	(0 ; 12.14)	26.23	(5.49 ; 57.14)
6. Danish ELISA 2/year	0	(0 ; 1.10)	18.68	(0 ; 57.15)

Table 4 presents three scenarios that resulted a median true prevalence of zero. So when executing the right test, it is possible to completely eradicate PTB. But when you look at the confidence limits some farms will still have PTB when using this strategy. When testing once a year with the Danish ELISA, the true prevalence was reduced to a median level of 0%. This also occurred when testing twice a year with either Dutch or Danish ELISA. The scenario of no control also showed the highest true prevalence at the end of 10 years of simulating. But the confidence limits are big so the situation can end up to be a lot different on different farms, for example when no control is executed prevalence can still eradicate but also go up to 50%.

Table 5.
Economic results of the scenarios on an average-hygiene herd. Average values of expenses, return and margin per year from the 10 year model simulation. The numbers are median results with 5% and 95% confidence limits. Economic values are shown in thousands.

	Expenses		**Return**		**Margin**	
Scenario	**50%**	**(5% ; 95%)**	**50%**	**(5% ; 95%)**	**50%**	**(5% ; 95%)**
1. Dutch ELISA	181	(177 ; 185)	310	(305 ; 315)	129	(123 ; 135)
2. Dutch ELISA + PCR	181	(176 ; 184)	309	(304 ; 315)	129	(123 ; 134)
3. Danish ELISA	181	(177 ; 185)	310	(305 ; 315)	129	(123 ; 135)
4. No control	180	(176 ; 184)	308	(302 ; 314)	128	(123 ; 134)
5. Dutch ELISA 2/year	181	(176 ; 185)	310	(305 ; 316)	129	(124 ; 135)
6. Danish ELISA 2/year	181	(177 ; 185)	311	(305 ; 316)	130	(124 ; 135)

The scenario where Danish testing was used twice a year generated the highest margin, all the other scenarios had a lower margin but did not differ much from each other. The lowest margin was found when no control was performed (Table 5). The highest return was also found in using Danish ELISA twice a year. And the expenses for all scenarios stayed similar, except for the no control scenario which was lower.

Table 6.
Results of the scenarios on an average-hygiene herd. Expenses made in feeding, testing and destruction of animals. The numbers are median results with 5% and 95% confidence limits. Economic values are shown in thousands, and are average values per year from the 10 year model simulation.

	Feed		**Test**		**Destruction**	
Scenario	**50%**	**(5% ; 95%)**	**50%**	**(5% ; 95%)**	**50%**	**(5% ; 95%)**
1. Dutch ELISA	1710	(1665 ; 1756)	2.72	(2.66 ; 2.78)	3.76	(3.09 ; 4.47)
2. Dutch ELISA+ PCR	1709	(1664 ; 1752)	3.73	(2.70 ; 5.95)	3.75	(3.05 ; 4.43)
3. Danish ELISA	1711	(1665 ; 1755)	3.75	(3.66 ; 3.83)	3.77	(2.98 ; 4.54)
4. No control	1707	(1662 ; 1751)	0	(0 ; 0)	3.79	(2.98 ; 4.57)
5. Dutch ELISA 2/year	1708	(1662 ; 1750)	4.88	(4.80 ; 4.94)	3.73	(3.03 ; 4.41)
6. Danish ELISA 2/year	1709	(1665 ; 1752)	6.92	(6.82 ; 7.02)	3.75	(3.05 ; 4.50)

The feed expenses are the same over the different scenarios. Except in Danish ELISA it is higher and the lowest value is in the no control scenario. It is clear that when no control is used there are no costs for testing, but the costs for using Danish tests twice a year are by far the highest. Using PCR and implementing a Danish test a single time has a similar price. In all scenarios the costs of destructions does not change that much, but it is highest in the no control scenario (Table 6).

In Figure 3, the median, lower and upper quartile of the true prevalence is shown. Figure 3A presents the three different ways of testing, using Dutch ELISA, Dutch ELISA followed up with PCR and Danish ELISA, are shown. These tests are all done once a year in an average-hygiene situation. In Figure 3B the same situation is used but then testing twice a year or using no control measurements at all. It is clear that when no control is implemented or when PCR is used to confirm, the prevalence in the herd increases. And with using Danish ELISA twice a year the prevalence goes down steepest. In Figure 4 the margin per cow is shown. In this Figure the scenario of testing once a year with Dutch ELISA is still used as the baseline, and the other scenarios are shown as a comparison to the baseline. In the beginning of implementation using Danish ELISA twice a year results in the highest margin per cow. When the action is implemented for a longer period the three scenarios with the highest margin per cow come closer together. But in the end of the 10 year simulation testing with Dutch ELISA two times a year comes out with the highest value. The lowest margin per cow takes more distance ??during the years, and is represented by the no control scenario.

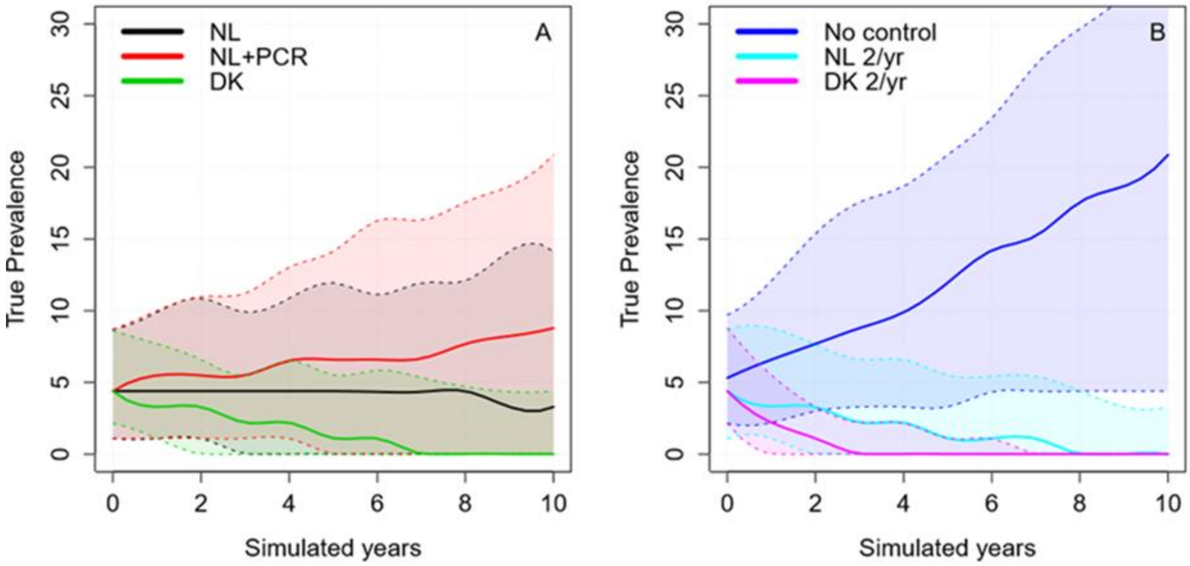

Figure 3: True prevalence: 50% simulation envelope over 10 simulated years for the tested scenarios in the average-hygiene herd

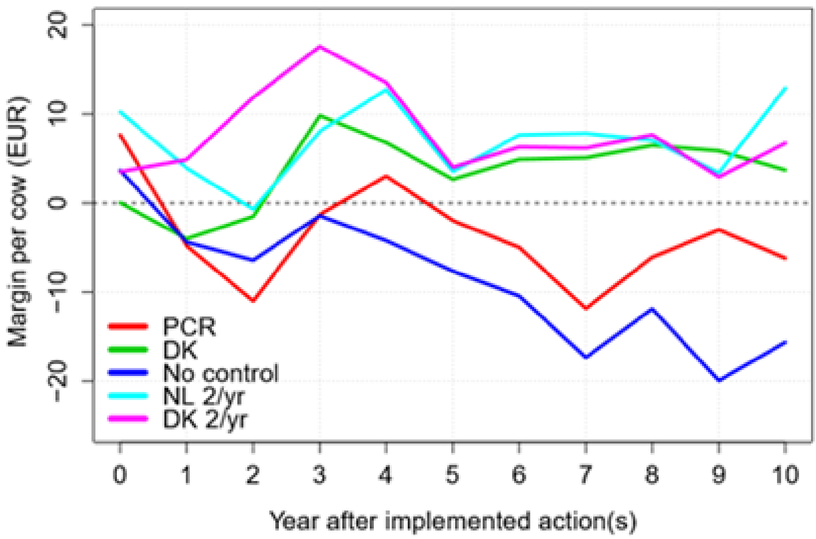

Figure 4: Margin over time for the normal prevalence scenarios. The difference in margin per cow for each scenario compared to the baseline is shown.

High-prevalence-herd.

In the high-prevalence-herd, no scenarios are expected to eradicate PTB completely, because the mean prevalence is always higher than 0. Only when using Danish testing twice a year it is possible to get a true prevalence of 0%. When checking up with PCR and doing no control the prevalence again goes up, just as shown in to average-prevalence herd(Table 4).

Table 7.
Economic results of the scenarios on an low-hygiene herd. Average values of expenses, return and margin per year from the 10 year model simulation. The numbers are median results with 5% and 95% confidence limits. Economic values are shown in thousands.

	Expenses		**Return**		**Margin**	
Scenario	**50%**	**(5% ; 95%)**	**50%**	**(5% ; 95%)**	**50%**	**(5% ; 95%)**
1. Dutch ELISA	179	(175 ; 182)	307	(301 ; 313)	128	(123 ; 134)
2. Dutch ELISA + PCR	179	(175 ; 183)	306	(300 ; 312)	128	(122 ; 134)
3. Danish ELISA	179	(175 ; 183)	308	(302 ; 313)	128	(123 ; 134)
4. No control	178	(175 ; 183)	304	(298 ; 310)	126	(120 ; 131)
5. Dutch ELISA 2/year	179	(175 ; 183)	309	(303 ; 315)	130	(124 ; 135)
6. Danish ELISA 2/year	179	(175 ; 183)	308	(302 ; 315)	129	(123 ; 135)

To calculate the values the same method is used as explained with Table5. The scenario a with the highest margin is testing with a Dutch ELISA twice a year, closely followed by testing with Danish ELISA twice a year, the return was also highest in this scenario. The lowest margin was observed in the no control scenario, in here the expense was the lowest but the return was also lower than all other scenario's (Table 7).

Table 8.
Results of the scenarios on an low-hygiene herd. Expenses made in feeding, testing and destruction of animals. The numbers are median results with 5% and 95% confidence limits. Economic values are shown in thousands.

	Feed		**Test**		**Destruction**	
Scenario	**50%**	**(5% ; 95%)**	**50%**	**(5% ; 95%)**	**50%**	**(5% ; 95%)**
1. Dutch ELISA	1689	(1643 ; 1734)	2.72	(2.66 ; 2.78)	3.69	(2.92 ; 4.43)
2. Dutch ELISA + PCR	1690	(1640 ; 1737)	7.41	(5.42 ; 9.28)	3.71	(3.03 ; 4.39)
3. Danish ELISA	1690	(1646 ; 1735)	3.74	(3.65; 3.84)	3.71	(3.02 ; 4.43)
4. No control	1691	(1649 ; 1742)	0	(0 ; 0)	3.78	(3.05 ; 4.50)
5. Dutch ELISA 2/year	1691	(1647 ; 1734)	4.88	(4.82 ; 4.95)	3.69	(2.94 ; 4.47)
6. Danish ELISA 2/year	1692	(1647 ; 1739)	6.92	(6.83 ; 7.04)	3.73	(3.03 ; 4.39)

In the Dutch ELISA, once a year testing scenario, the feeding costs are the lowest. The costs for feeding the highest in the Danish ELISA twice a year, although the differences are small. Again there are no costs in no control and the highest costs in Danish ELISA twice a year scenario. The destruction costs scenario, in general, seem to be lower in a high-prevalence-herd. But again the lowest costs are in Dutch ELISA and the highest cost in the no-control scenario.

In Figure 5 it is shown in the first years of the simulation, testing twice a year both Dutch or Danish ELISA reduces the prevalence in a likewise. After a few years of implementation the Danish ELISA pushes down the prevalence better than the Dutch ELISA.

In Figure 6 the margin per cow is presented. As the years of implementation add up the margin per cow of the different scenarios drift apart. Making the margin per cow of Dutch ELISA used twice a year come out on top. The margin per cow revenue comes out higher in the average-prevalence-herd, this due to the bigger changes situation in this situation. But again, the no control scenario leads to the lowest margin per cow. Implementing PCR tests also scores a negative margin per cow, because of the increase in prevalence.

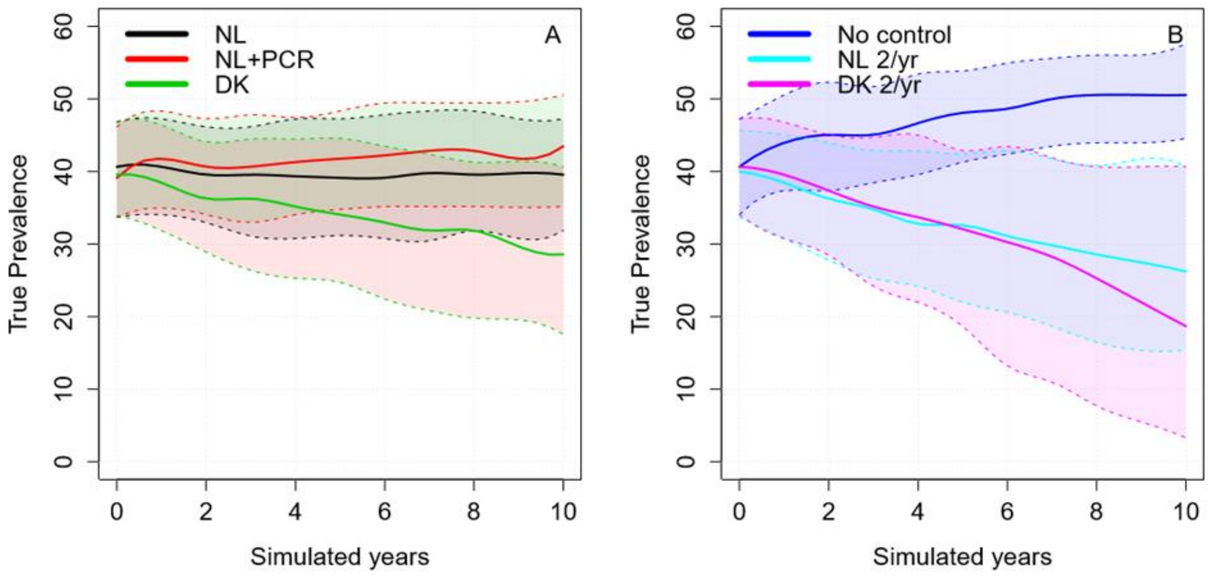

Figure 5: True prevalence: 50% simulation envelope over 10 simulated years for the tested scenarios in the low-hygiene herd.

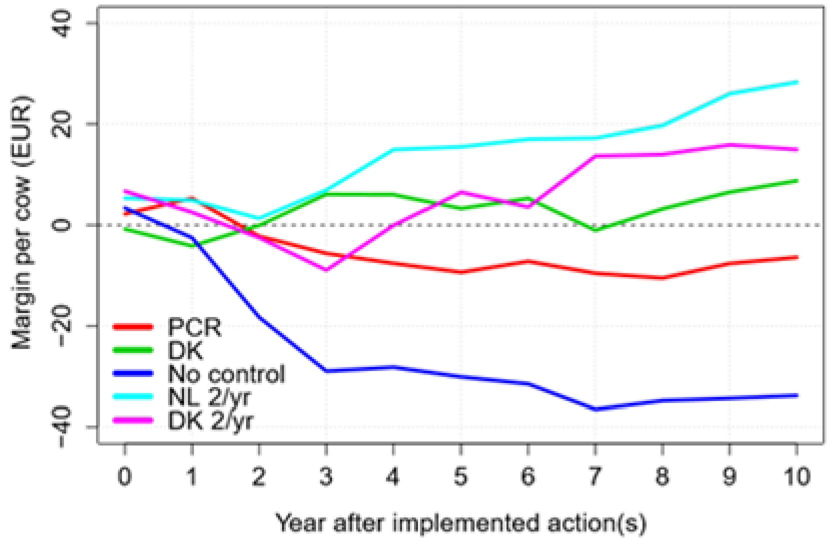

Figure 6: Margin over time for the high prevalence scenarios. The difference in margin per cow for each scenario compared to the baseline is shown.

Discussion

Within the 10-year simulation the model shows that the system that is currently used in the Netherlands is functioning well. Under the used assumptions, the prevalence in the baseline fits a stable median prevalence level of 5% and 40% over 10 years simulation. There is a noticeable difference between the no control scenario and the baseline. The no control scenario goes steeply up, in the same situation that the baseline stays stable. Based on this observation, it seems that the current Dutch testing scheme helps keeping the prevalence down. This is a good finding because it means that the testing scheme is effective. Nevertheless, comparing the baseline with other strategies shows average results in economics and prevalence, which means there is still room for improvement. There are scenarios that at the same time reduce the prevalence and have a higher margin than the baseline. This was the case for both the low- and the high-prevalence-herd, but the most cost-effective scenario was not the same for both situations.

In the Danish ELISA once and twice a year and the twice a year Dutch ELISA strategies for the average-prevalence-herd, the mean prevalence goes down to zero already after a few years of simulation. When this occurs the prevalence will not go up anymore because there is no infection with in herd and the herd is closed. At this point the frequency of testing could be reduced or even stopped. Doing this will likely affect the efficiency of the strategy, because costs will be saved in the long run so a better, more expensive test can become more cost efficient. In such scenario's, the time preference of money needs to be taken into account. The value of money decreases of time and money spent earlier has a higher value than money saved later. To correct for this effect, discounting should also be applied in the economic calculations. There is no discounting done in this study but this does not affect the results a lot. Because the costs and benefits through the years stay similar in the different scenarios.

This model simulates a closed herd. Many farmers in major milk-producing countries and most Dutch dairy farmers have a fully closed farm, which means they rear their own young stock to provide replacement heifers (Gabler et al., 2000; Tozer and Heinrichs, 2001; Mohd Nor., 2015). Other herds were found to be working on breeding their own replacement to become more closed, or keeping completely open to be able to trade. Open herds are more likely to be exposed to MAP from outside, because new entries in the herd could carry MAP inside the herd. When cows are more exposed to the infection, the infection pressure increases. This can result in a higher prevalence. But also every cow newly introduced in the herd brings a risk. Cows can be tested false negative and when introduced they will infect the herd. This means it becomes harder to stabilize the prevalence in the herd due to the higher risk of infection. When a lot of farms decide to open their herd this could negatively affect the economy of dairy producers ,because the change of infection becomes higher.

Another assumption made in the model is a stable milk price, but in reality this price is highly fluctuating. When the price would drop in the model, it will result in a lower return in milk. This pressure on the margin of the farm can increase the importance to have healthy cows. Because the infection reduces the milk yield without a reduction in feed intake so the cost per ECM becomes higher. Keeping the costs as low as possible is then critical for the farm so the need for healthier cows becomes higher.

Each scenario as previously indicated, is iterated 500 times to make obtain stable results. The study of Kirkeby et al., 2015 also used 500 iterations in one model run, but in that study 200 cows per herd were used. In our study, the herd seize was 91 cows. This can influence the stability of the model. As visually noticeable in Figure 3 and 5, the graphs are not completely stable. With more than 500 iterations, the graphs could become more stable but there was not enough time to test this. To study how the uncertainty in the output of our changed ICull-model can be allocated to various sources of uncertainty in its inputs, a sensitivity analysis should be carried out. This is a technique that defines how changed values of an independent variable will affect the particular dependent variable under the assumptions given. Unfortunately, in the short time frame of this study we were not able to perform this analysis, but if possible this would still be a meaningful analysis to do.

In the average prevalence scenario, applying the Danish ELISA twice a year reduced the prevalence the most, and also gavethe highest margin. Whereas in the high-prevalence-herd, applying the Danish ELISA twice a year still came out with the lowest prevalence, but the Dutch ELISA twice a year had the highest margin (Tables 4 & 5). Previous studies show that the economic effects are associated to the prevalence in a herd (Ott et al., 1999; Weber, 2006; Richardson and More, 2009). Keeping this association in mind, it would be expected that the scenario ending in the lowest prevalence would end in the highest margin, due to an increased return from a higher milk yield and the higher slaughter value of healthy cows (Kudahl and Nielsen., 2009; Græsbøll et al., 2015). As earlier described, this is not the case in the high-prevalence-herd. This can be caused due to the higher price of the Danish ELISA and the smaller difference in prevalence. The difference in prevalence is then too small to compensate for the higher price of the Danish test. Hence, using a test at a different price point can reduce the prevalence more, but reduction of the prevalence should be done at a reasonable price. The best test in performance is not the most cost efficient test. So the Dutch farmers can still use the Dutch test, only it can be done more efficiently reducing the prevalence and making a higher margin at the same time. However, when looking at the confidence intervals in both economics and prevalence, it can be noticed that the difference of the impact can be large. So it should be taken into account that, when using for example the Dutch ELISA twice a year strategy on different farms, it could resultin complete eradication but there is also a chance of still keeping some positive animals on the farm.

Using no control creates the worst situation in economics as well as in prevalence. But in the Netherlands, when doing this under the current regulations, the farm is not allowed to sell the milk to a processor. Only 17 farms in the Netherlands process and sell all the milk that is produced on the farm (Boerderijzuivel.nl). So using no control on PTB is only an option for these 17 farms.

But using a serial test as in scenario two, where the PCR is used to confirm the initial ELISA positive tested animals, is legal for all farms in the Netherlands. As discussed before, when this system is used, the MAP prevalence of the herd increases (Figure 2 and 4). This herd level effect can be explained by the effect of a serial test. Serial testing maximizes the specificity and the predictive value of a positive test result, but lowers the sensitivity and negative predictive value (Thrusfield., 1997). When using the Dutch ELISA with the current high threshold (1.0 S/P), the specificity of the test is 100%. This means no false positives are found when using this test. Then using the PCR test on the positive animals only reduces the sensitivity, because the specificity cannot increase any further already 100%. As a consequence, less cows are culled and the chance of keeping positive cows in the herd increases. This is why in this particular scenario the prevalence increases and the margins decreases, both because of higher test costs and higher prevalence (Table 4 and Table 5). If a farmer really wants to use the PCR, it can be an idea to use the Dutch ELISA with the previous cut-off (0.2 S/P). The ELISA test than used will have a lower specificity and a higher sensitivity, compared to the test used with a higher cut-off. By doing this, more positive animals will be detected, and using a serial PCR test the false-positive can be removed, which gives overall better test results. This way you will make better use of the serial testing characteristics and might have a better chance in finding the true positive animals.

In the normal-hygiene-herd, the farmer only makes, 1,000 Euro less per year in the no control scenario than with the current Dutch testing system and only 2,000 Euro less per year in the low-hygiene scenario (Table 4 and Table 5). This number divided by the 91 cows per herd, gives an average cost of 22 Euro per cow per year. Supporting the result is of previously estimated values at $22 to $27 per cow per year in the United States (Ott et al., 1999). These differences might be not big enough for the farmer to care much about compared to other inefficiencies on the farm. The motivation to still care about the prevalence of PTB in the herd can of course be the welfare of the animals. In herds with a high prevalence of MAP (low hygiene) it is possible to, on average, earn 2,000 Euro per year more just by testing twice a year instead of once a year (Table 5). So the motivation to control MAP might differ between farms. So to create a more efficient programme a farm-specific approach can be worthwhile.

In the results, slight variations were noticed in the feeding expenses (Table 6 and Table 8) cumulated over the 10 years. Because the numbers are cumulated over ten years, they can vary considerably. So this can explain the slight variations. Nevertheless, there were some reoccurring variations as the higher expense in destruction costs for the no control scenario. Also expenses for feeding and destruction could be noticed in the high-prevalence-herd, compared to the average-prevalence-herd. The first effect is due to the fact that more cows will be infected in a high shedding or clinical state. If these animals are not culled by coincidence, they will have to be culled due to the Dutch control programme. This costs more money than just culling. The second effect were the lower expenses observed in high-prevalence-herds. This effect can be explained because the cows might not become very old. This means there will be more heifers and younger cows in the herd who eat less. The change in age distribution in the-low-hygiene herd, also explains the different costs for culling and destruction.

We found that it is possible to decrease the prevalence within the herd, using just the milk ELISA test to detect positive cows for culling, supporting the results of Kudahl et al(2007). In both the low and average-hygiene herd it was observed that the test-and-cull method used was reducing the prevalence. This finding is supported by Nielsen & Toft (2011). On the other hand, this opposes the conclusions from the JohneSSim and SimHerd models, where it was found that test-and-cull strategies are not economic attractive and could not lower the prevalence (Groenendaal H.,2003; Kudahl.,2007). Nevertheless, in the SimHerd model, an ELISA-positive cow must be confirmed by a fecal culture. This is the same structure of testing as was used in our second scenario where all initial positive animals are tested again with PCR. In this scenario the test-and-cull strategy was also not observed to be cost-effective or reducing the prevalence. With the simHerd model they explained this observation with being more time consuming and expensive than just ELISA testing (Kudahl.,2007), this is supporting our results. Also, the JohneSSim model was based on a low sensitivity ELISA test to test the disease state of the animal, This is in contrast to the age-dependent and disease state sensitivity used in the iCull model.

Conclusion

To conclude, we used current knowledge and recent literature of the PTB system and constructed a new model for simulating PTB infection within a Dutch herd. Different types of test-and-cull strategies were simulated taking epidemiological and economic effects into account. The developed scenarios were run in a normal and a low-hygiene herd. The current Dutch testing system was actually found as an efficient strategy. The prevalence of MAP is not increasing and the costs are reasonable. But improvements to this strategy are found to be possible. The most cost-effective scenario over 10 years in the average-hygiene herd was using the same test twice a year. This scenario also reduced the prevalence more. In the low-hygiene herd, testing twice a year with the Dutch ELISA was again the most cost-effective strategy. But using the Danish test twice a year would reduce the prevalence the most. So to create the most efficient system, the strategy should be adapted to the farm situation and not just one strategy for all Dutch farms. The results of this study can help Dutch farmers and the Dutch dairy chain stakeholders to improve the PTB control strategies, and thus lower the prevalence within a herd and in the Netherlands overall.

Acknowledgement

I would like to thank both Tariq Halasa and Henk Hogeveen for giving me the opportunity to work on this project and travel to Copenhagen. It was a great experience to travel, meet new people and learn the ICull-model. I also want to thank them for the useful comments, remarks and engagement through the learning process of this thesis. Finally, I would like to thank Carsten Kirkeby. Without him teaching me how to work with the model this would not have been possible, furthermore his great support along the way was very helpful for me.

References

AHI (Animal Health Ireland). 2012. Johne's Disease: Frequently Asked Questions. Accessed Jul. 27, 2014. http://www.animalhealthireland.ie/ckfinder/userfiles/files/20120704%20JD%20FAQ.pdf.

Bannink, A., van Schijndel, M.W., Dijkstra, J., 2011.A model of enteric fermentation in dairy cows to estimate methane emission for the Dutch National Inventory Report using the IPCC Tier 3 approach.Animal Feed Science and Technology, 166–167, 603–618

Barrett, D., M. Good, M. Hayes, and S. J. More. 2006. The economic impact of Johne's disease in an Irish dairy herd: A case study. Ir. Vet. J. 59:301–305.

Baumgart, D.C., Sandborn, W.J. 2012. Crohn's disease. The Lancet 380(9853), 1590-1605

Berentsen, P.B.M., Kovacs, K., van Asseldonk, M.A.P.M.,2012. Comparing risk in conventional and organic dairy farming in the Netherlands: An empirical analysis J. Dairy Sci., 95, 3803–3811.

Boerderijzuivel.nl (only in Dutch) De Bond van Boerderij-Zuivelbereiders (BBZ) is de belangenbehartigingsorganisatie voor de boerderijzuivelbereider in Nederland. Accessed dec. 2015. http://www.boerderijzuivel.nl/boeren/

CRV, CRV Jaarstatistieken Nederland (in Dutch), CRV Holding BV, Arnhem, Accessed sep. 2015. https://www.crv4all.nl/wp-content/uploads/2015/02/CRV-Jaarstatistieken-2014-NL.pdf

CVB (Central Bureau for Livestock Feeding). 2007. Tabellenboek veevoeding 2007, voedernormen landbouwhuisdieren en voederwaarde veevoeders. Central Bureau for Livestock Feeding, The Hague, the Netherlands.

Dufour, B., R. Pouillot, and B. Durand. 2004. A cost/benefit study of paratuberculosis certification in French cattle herds. Vet. Res. 35:69–81.

Efarm.nl (only in Dutch). Markt voor de moderne veehouder. Accessed sep. 2015. http://www.efarm.nl/aanbod/index.php

Gabler, M. T., P. R. Tozer, and A. J. Heinrichs. 2000. Development of a cost analysis spreadsheet for calculating the costs to raise a replacement dairy heifer. J. Dairy Sci. 83:1104–1109.

GD. 2015. De Gezondheidsdienst voor Dieren. Actuele Prijslijst Laboratoriumbepalingen. Accessed nov. 2015. http://www.gddiergezondheid.nl/producten%20en%20diensten/product-overzicht/producten-rund

Græsbøll K, Nielsen SS, Halasa TH, Christiansen LE, Kirkeby C, Toft N. Association of MAP specific ELISA-responses and productive parameters in 367 Danish dairy farms. In: Proceedings of the 12th International Colloquium on Paratuberculosis, Parma, Italy, June 22-26, 2015; 2014. p. 139–141.

Groenendaal H, Nielen M, Hesselink JW. 2003.Development of the Dutch Johne's disease control program supported by a simulation model. Preventive Veterinary Medicine. 60(1):69–90.

Groenendaal, H., Controlling Johne's disease: Results of computer modelling. Cattle Practice. 2003;11:219–225.

Gunn, G. J., R. W. Humphry, and A. W. Stott. 2004. Comparison of the modeled effects and consequential losses due to Johne's disease outbreaks for beef and dairy herds in Great Britain. Cattle Pract. 12:1.

Heres, L., Dieleman, S.J., van Eerdenburg, F.J.C.M., 2000. Validation of a new method of visual estrus detection on the farm.. Vet. Q. 22:50–55.

Humphry, R., Stot,t A, Adams, C., Gunn, G. A model of the relationship between the epidemiology of Johne's disease and the environment in suckler-beef herds. The Veterinary Journal. 2006;172(3):432–445.

Inchaisri, C., Jorritsma, R., Vernooij, J.C.M., Vos, P.L.A.M., van der Weijden, G.C., Hogeveen, H., 2011. Cow Effects and Estimation of Success of First and Following Inseminations in Dutch Dairy Cows. Reprod Dom Anim 46, 1043–1049

Irenge, L.M., Walravens, K., Govaerts, M., Godfroid, J., Rosseels, V., Huygen, K., Gala, J. Development and validation of a triplex real-time PCR for rapid detection and specific identification of M. avium sub sp. Paratuberculosis in faecal samples. Veterinary Microbiology. 136, 166-172

Johnson-Ifearulundu, Y. J., and J. B. Kaneene. 1997. Epidemiology and economic impact of subclinical Johne's disease: A review. Vet. Bull. 67:437–447

Kirkeby, C., Græsbøll, K., Nielsen, S.S., Christiansen, L.E., Toft, N., Rattenborg, E., Halasa, T., 2015. The iCull Model Part II - Simulating the epidemiological and economic impact of paratuberculosis *(submitted to Journal of Dairy Science, November 2015)*

Kudahl A.B, Nielsen S.S., 2009. Effect of paratuberculosis on slaughter weight and slaughter value of dairy cows. Journal of Dairy Science. 92(9):4340–4346.

Kudahl AB, Sørensen JT, Nielsen SS, Østergaard S. 2007. Simulated economic effects of improving the sensitivity of a diagnostic test in paratuberculosis control. Preventive Veterinary Medicine. 78(2):118–129.

Kudahl, A.B., Østergaard, S., Sørensen, J.T., Nielsen, S.S., 2007. A stochastic model simulating paratuberculosis in a dairy herd. Preventive Veterinary Medicine. 78(2):97–117.

LEI (Agricultural Economics Research Institute). 2012. Farm Information Network. Accessed Sep. 2015. http://www3.lei.wur.nl/ BIN_ASP/show.exe.

LEI. 2013. Agricultural Economics Research Institute. Agricultural statistics. Accessed Sep. 2015.2014.http://www3.lei.wur.nl/BIN_ASP/show.exe?aktie=vindtoon&bj=2010&ej=2013&language=NL&Valuta=2&publicatieID=3&kiestabel=2.02&Database=LTC

Losinger WC. Economic impact of reduced milk production associated with Johne's disease on dairy operations in the USA. Journal of Dairy Research. 2005;72(04):425–432.

Marcé, C., Ezanno, P., Seegers, H., Pfeiffer, D., Fourichon C. Modelling the spread of Mycobacterium avium subsp. paratuberculosis towards fadeout or persistent infection in a dairy cattle herd. PhD Thesis, Universit´e de Rennes 1 – University of London. 2010.

Marcé, C., Ezanno, P., Weber, M., Seegers, H., Pfeiffer, D., Fourichon C. 2010. Invited review: Modeling within-herd transmission of Mycobacterium avium subspecies paratuberculosis in dairy cattle: A review. Journal of dairy science.93(10):4455–4470.

Mohd Nor. N., Steeneveld, W., Mourits, M.C.M., Hogeveen, H., 2015. The optimal number of heifer calves to be reared as dairy replacements. J. Dairy Sci. 98:861-871.

Mitchell, R., Whitlock, R., Stehman, S., Benedictus A, Chapagain, P., Grohn. Y., Simulation modeling to evaluate the persistence of Mycobacterium avium subsp. paratuberculosis (MAP) on commercial dairy farms in the United States. Preventive Veterinary Medicine. 2008;83(3):360–380.

Muskens, J., Barkemaa, H.W., Russchena, E., van Maanena, K., Schukkenb, Y.H., Bakkerc, D., 2000. Prevalence and regional distribution of paratuberculosis in dairy herds in the Netherlands. Veterinary Microbiology 77, 253–261.

National Research Council. 2003. Economic implications of Johne's disease. Pages 9–103 in Diagnosis and Control of Johne's Disease. National Academy Press, Washington, DC.

Nielsen S.S, Weber M., Kudahl A, Marc´e C, Toft N. 2011Stochastic models to simulate paratuberculosis in dairy herds. Revue Scientifique et Technique OIE. 30(2):615.

Nielsen SS, Toft N. 2011.Effect of management practices on paratuberculosis prevalence in Danish dairy herds. Journal of Dairy Science. 94(4):1849–1857.

Nielsen, S.S., Toft, N., Okura, H., 2013. Dynamics of specific anti-Mycobacterium avium subsp. paratuberculosis antibody response through age. PLoS One, 8, p. e63009

Nielsen, S.S., Toft, N., Okura, H., 2013. Dynamics of specific anti-Mycobacterium avium subsp. paratuberculosis antibody response through age. PloS one. 8(4):e63009.

Ott SL, Wells SJ, Wagner BA. Herd-level economic losses associated with Johne's disease on US dairy operations. Preventive Veternary Medicine. 1999;40(3):179-192.

Peeters, A. & Kopec, S., 1996. Production and productivity of cutting grassland in temperate climates of Europe. Grassland Science in Europe 1, 59-73.

Pouillot R, Beaudeau P, Denis JB, Derouin F. 2004. A quantitative risk assessment of waterborne cryptosporidiosis in France using second-order Monte Carlo simulation. Risk Analysis. 24(1):1–17.

Rendac, Tarievenlijst (in Dutch). Accessed sep. 2015. http://www.rendac.nl/nl/footer-quicklinks/rendac-tarieven/

Richardson, E., and S. J. More. 2009. Direct and indirect effects of Johne's disease on farm and animal productivity in an Irish dairy herd. Ir. Vet. J. 62:526–532.

Rideout, B. A., S. T. Brown, W. C. Davis, J. M. Gay, R. A. Giannella, M. E. Hines II, W. D. Hueston, and L. J. Hutchinson. 2003. Diagnosis and Control of Johne's Disease. Committee on Diagnosis and Control of Johne's Disease. National Academy Press, Washington, DC.

Sevenster, M.N., Hueting, D.H., 2007. Energiegebruik in de veevoerketen(in Dutch). Delft CE. 07.6136.01

Tozer, P. R., and A. J. Heinrichs. 2001. What affects the costs of raising replacement dairy heifers: A multiple-component analysis. J. Dairy Sci. 84:1836–1844.

Tamminga, S., Aarts, F., Bannink, A., Oenema, O., Monteny, G.-J., 2004. Actualisation of N and P excretion by cattle (in Dutch). Reeks Milieu en Landelijk Gebied 25, Wageningen, pp. 48 (www.wur.nl).

Thrusfield, M., 1997, Veterinary Epidemiology. Blackwell Publishing P. 280.

van Hulzen, K. J. E. , Nielen, M., Koets, A.P., de Jong, G., van Arendonk, J.A.M., Heuven H.C.M., 2011. Effect of herd prevalence on heritability estimates of antibody response to Mycobacterium avium subspecies paratuberculosis. J. Dairy Sci. 94 :992–997

van Weering, H., Van Schaik, G., Van der Meulen, A., M. Waal, Franken, P., Van Maanen, K., 2007. Diagnostic performance of the Pourquier ELISA for detection of antibodies against Mycobacterium avium subspecies paratuberculosis in individual milk and bulk milk samples of dairy herds Vet. Microbiol., 125. 49–58

Weber, M. F. 2006. Risk management of paratuberculosis in dairy herds. Ir. Vet. J. 59:555–561.

Weber, M.F., van Schaik, G., van Weering, H.J., 2007. Results of a bulk milk quality assurance programme for paratuberculosis. Cattle Practice 15: 3-261

Weber, M.F., 2012, Five years of milk quality assurance for paratuberculosis in the Netherlands Proc. 11th Intern. Colloq. Paratuberculosis, 74

Weber, M.F., van Roermund, H.J.W., Velthuis, A.G.J., de Koeijer, A.A., de Jong, M.C.M., Nielen, M. 2008. Milk quality assurance programmes for paratuberculosis: stochastic simulation of within-herd infection dynamics and economics. Vet. Res. 39:12

Wood P. Algebraic model of the lactation curve in cattle. Nature. 1967;p. 164–165