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Nielsen, Søren Saxmose

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Danish Control Programme for Bovine Paratuberculosis

Nielsen, S.S., Associate Professor of Veterinary Epidemiology, Dept. of Large Animal Sciences, Faculty of Life Sciences, University of Copenhagen, Frederiksberg, Denmark

ABSTRACT

Paratuberculosis is widespread in Denmark and as a result, a voluntary control programme was established in 2006, aiming at providing tools for farmers to control infections, and to ultimately reduce the prevalence. Approximately 1140 (23%) of dairy farmers were enrolled in the programme by June 2007. Participating herds test all lactating cows four times/year by using a milk antibody ELISA. The test-results are primarily used for risk-based management of infectious animals. This risk-based approach is aimed to reduce the workload of herd managers, thereby making implementation of changes more feasible than if all cows had to be managed with increased awareness. The test results are also used for communication to farmers, as a central part of the programme. Communication between farmers and advisors also takes place via risk assessments, which helps the farmers identify risk areas of transmission.

Farmers are informed that the control programme is expected to last 6 to 8 years. Therefore, there is a continued need from farmers, their advisors and the central administration to identify tools and methods to ensure ongoing enthusiasm.

A surveillance component may be added to the programme at a later stage, but currently no officially recognised recommendations are available related to trade of live animals. The surveillance component may be a next step to keep farmers in the programme and encourage more farmers to join.

KEYWORDS: Communication, control programme, management, milk ELISA, paratuberculosis

INTRODUCTION

Bovine paratuberculosis is common in the Danish dairy population. Precise estimates of prevalences of infection are not known, partly because of inaccurate diagnostic tests, which render design of prevalence studies difficult. Between-herd prevalences of 47% (Nielsen *et al.* 2000) and 85% (Nielsen, unpublished data) were estimated in studies conducted in 1998 and 1999, respectively. Subsequent to these estimates, the Danish cattle industry launched a major research project to develop new types of herd health recommendations to producers and their advisors (Andersen *et al.* 2000). This project along with later projects focused on definition, diagnosis, epidemiology and economics of paratuberculosis. A major objective of the research projects was continued involvement of farmers and their advisors.

In the late 1990's, most farmers would have said that their farm was free of paratuberculosis, but from 2000 to 2005 increasingly farmers acknowledged a between-herd prevalence in Denmark >50%, and their farm was likely to be infected. A contributing factor may have been that the concept of "paratuberculosis" had changed from only including clinical paratuberculosis to be considered more broadly as an infection with multiple effects, *e.g.* infected animals that could become infectious and cows with production losses without having diarrhoea. These statements cannot be supported by data, except that the interest in control of the infection in late 2005 resulted in a demand from producers that a voluntary control programme be established.

The Danish Cattle Federation decided to launch a control programme, "Operation Paratuberculosis", which began in February 2006. By June 2007, approximately 1,140 (23%) dairy herds of the 4,900 dairy herds in the country joined the programme. The herds in the programme are primarily larger herds, with an average herd size of approximately 137 cows/herd compared to the national average of approximately 110 cows/herd. Prior to establishment of the programme, diagnostic testing was primarily based on bacteriological culture of faecal samples with subsequent culling of animals perceived as "high shedders of *Mycobacterium avium* subsp. *paratuberculosis* (MAP)". Recommendations for reduction of transmission of MAP were based on protecting calves against manure from all adult cattle, *e.g.* removal of the newborn calf immediately after calving, and calvings taking place in special calving pens cleaned after each calving. These management measures were recommended to prevent faecal material from cows getting in direct contact with susceptible calves, irrespective of the infectious status of the cows. This is a non-risk-based approach, where all cows are deemed equal risks as sources of MAP transmission.

This paper provides an overview of the components in the Danish control programme for paratuberculosis among dairy herds, where the central feature is a risk-based management of cows classified as infectious based on a test-strategy including frequent repeated diagnostic testing with a milk antibody ELISA.

AIMS OF "OPERATION PARATUBERCULOSIS"

The specific aims of the control programme "Operation Paratuberculosis" as established by the Danish Cattle Federation are to: 1) provide tools to farmers that wish to control paratuberculosis; and 2) reduce the overall prevalence of paratuberculosis in Denmark. There are currently no state veterinary regulations on control of paratuberculosis, and the programme is voluntary. No surveillance and certification schemes have been established, and it is assumed that most participants so far have joined the programme because their aim is to control the infection rather than certifying their herd as free of MAP infection. It has been clearly communicated to farmers that the certificate "free of paratuberculosis" presently does not exist.

STRUCTURE OF "OPERATION PARATUBERCULOSIS"

Operation Paratuberculosis is administrated by the Danish Cattle Federation in cooperation with the four regional recording and yield control centres (RYK-centres). An advisory board provides input to the paratuberculosis administration regarding requirements for communication and information, use of tools developed, and practical implementation of the available knowledge. The advisory board consists of five external herd health advisors (two practising veterinarians, one veterinary herd health advisor and two herd health advisors with animal science background). Also included in the board are persons from the paratuberculosis administration, communication staff, a researcher, a laboratory representative, a representative from the Danish Cattle Database and a RYK-representative.

Farmers in the programme cover costs for diagnostic testing (23 DKK ~ 2.1 GPB per sample or ~6.3 GPB per cow-year), an annual fee of 250 DKK /herd to RYK, and costs for advisors. There are no subsidies in the programme, except the Danish Cattle Federation covers cost for programme administration other than administrated by RYK.

The RYK-centres receive applications for joining the programme and coordinate sampling according to the test-strategy upon which the programme is based (see next section). The samples tested are the same milk samples used in the Danish milk recording scheme, in which approximately 85% of farmers participate. Farmers outside the milk recording scheme can rent sampling equipment if they wish to participate in the paratuberculosis programme. The sampling is coordinated to ease the flow of samples to the commercial laboratory, which tests the samples for both antibodies to MAP and other milk recording factors such as protein and fat content, and somatic cell count. This system has been established to reduce the costs related to sampling. All samples are bar-coded to improve identification of samples and to reduce handling costs. Samples submitted to the laboratory without barcodes are priced differently than samples with a barcode.

After laboratory analysis, all diagnostic data are transferred to the Danish Cattle Database, and the information is immediately available to farmers. A special software programme is needed to view results directly from the database, but results are also sent from the RYK centres to farmers by mail. Approximately 3360 farmers, 159 veterinary practices and 100 non-veterinary advisors are recorded users of the software programme, but it is unknown how many of these actually access results electronically. The results can be extracted in different formats: a) lists for management of calvings and milk feeding, with cows divided into high- and low-risk cows (including red and yellow marks for "red" and "yellow" cows, respectively (see next section)); b) a report with the last 6 test-results per cow, divided into six "infection groups", to provide advisors and farmers with an overview of all relevant test results; c) a summary including a roughly calculated milk production loss for the herd and estimates of prevalences of potentially infectious cows in the specific herd and for all herds in the control programme; and d) a culling list, in which animals are sorted according to those recommended culled first solely based on the diagnostic data. More reports are being developed as the needs are expressed by advisors.

DIAGNOSIS AND TEST-STRATEGY

Paratuberculosis is considered to cover a variety of conditions, as the infection progresses. The pathogenesis is hypothesised to be as follows: a) Infection with MAP are believed to occur via enteric M-cells, which serve as an entry port into the lymphatic system, where MAP are scavenged by macrophages or dendritic cells, with a subsequent initiation of specific immune responses; b) the immune response initially consists of cell-mediated immune reactions, which controls the infection; c) with progressing MAP infection, the humoral immune response becomes the predominant type (Stabel 2000); d) during the humoral immune reactions, MAP proliferate and the infection is no longer considered to be under control. Therefore, the condition is expected to worsen, resulting in excretion of high numbers of MAP, reduced milk production and eventually diarrhoea, emaciation and death. During the cell-mediated immune reactions, low concentrations of IgG2 may be produced, whereas higher concentrations of IgG1 are expected during the humoral immune responses. Triggers for switching between the two immune responses are unknown, and it is not known whether infections that have progressed to humoral immune responses can regress to cell-mediated immune responses.

MAP are expected to be shed during a major part of the infection course, but it is likely that the probability of detecting shedding is highest a few months after infection and 5 to 10 months prior to becoming clinical (Lepper *et al.* 1989). Although shedding of MAP may occur during all stages of infection, it appears to be greater in the period from approximately 2.5 to 5 years of age (Nielsen & Ersbøll 2006), and the probability of shedding detectable amounts is higher with higher concentrations of antibodies (Toft *et al.* 2005).

The probability of detecting infected animals using an antibody ELISA appears to be affected by the age of the animal, whereas the probability of detecting infectious animals does not appear to be affected of age (Nielsen & Toft 2006). The probability of testing positive by ELISA and the magnitude of the ELISA response is therefore expected to correlate with the infectious status. Most infectious animals are expected to be detected by ELISA if testing is frequent enough. A high test-frequency appears most beneficial between 2 to 4 years of age because majority of infected animals sero-convert in this age-period (Nielsen & Ersbøll 2006).

The timely detection of infectious animals is also important to avoid transmission of MAP from these animals. Therefore, the current test-strategy includes four test-rounds in each herd, which is a requirement in Operation Paratuberculosis. This test-strategy translates to most cows being tested three times per year, and not during the dry period. The presumed probability of detection of infectious animals at various ages with different test-frequencies is considered constant with sensitivities as presented schematically in Figure 1, based on results by Nielsen & Toft (2006).

A high test-frequency is expected to result in an overall low specificity of the combined test result, and the risk of a high number of false-positives should be included in the interpretation and use of the diagnostic results. It is therefore important not to cull all animals, which are test-positive in one test only.

The milk ELISA test, which is primarily used in the Danish control programme, detects both IgG1 and IgG2 (Nielsen 2002). Therefore, it is not possible to distinguish between animals with cell-mediated immune-responses and those with humoral immune responses simply by interpreting the result of one test. However, it is more important to detect as many potentially infectious animals, but this puts more emphasis on cautious use of the diagnostic results.

In practise, the cows are categorised into different risk-categories after each test (Table 1), based on their antibody profiles (Figure 2). The risk categories have different levels of complexity, to assure that the needs of both farmers and advisors requiring low levels of details and those requiring high levels of details are met. The lowest level of complexity is simply dividing cows into high- and low-risk (Table 1, column 1). The more popular and easily communicable is division into three cow-types, "Green", "Yellow" and "Red". "Green cows" are non-infectious and potentially non-infected; "Yellow cows" are controlling the infection, but may become "Red" or be in the initial phase of no control of infection; and "Red cows" no longer control the infection. The cow types to some extend mimic the theoretical progression of the infection, but it should be noted that there are still major differences.

Division into "infection groups" is primarily done to cover not only aspects of transmission but also predictions related to milk production losses. All cows with one positive test-result among the last four tests (infection groups 2, 5 and 9) are considered infectious to some extent. Cows in infection groups 2 and 9 have been estimated to have a 305 day kg. energy corrected milk (305 kg ECM), which is reduced approximately 10 to 12% relative to their herd mates in infection group 0, irrespective of breed and parity (Nielsen *et al.* 2006). Cows of infection groups 3 and 5 did not appear to experience losses, and insufficient information was available regarding cows of infection group 1, because these cows often are in the beginning of first lactation.

Figure 1. Schematic presentation of the probability of testing positive in ELISA given the animal is infectious at different ages, for 3 different test-frequencies: 1, 2 and 4 tests/cow/year. The shaded areas represent the uncertainty related to each line.

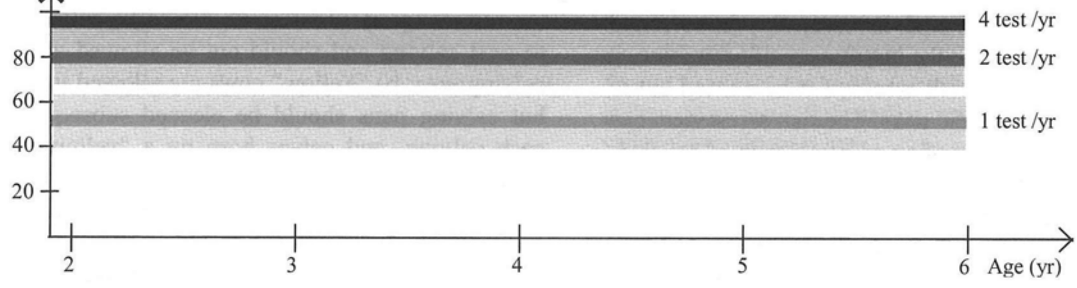
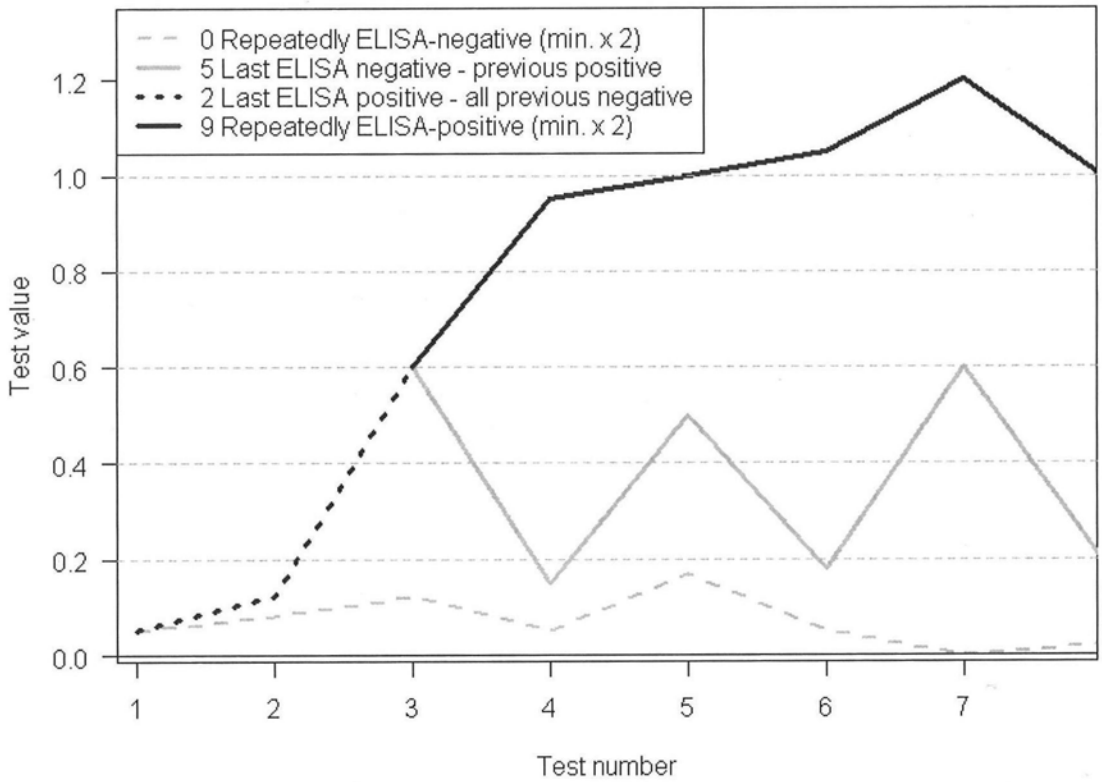


Table 1. Risk levels and categories of cows subsequent to testing by an ELISA§ for detection of antibody in milk samples.

Risk level	"Cow type"	"Infection group"	Definition
Low	Green	0	Repeatedly negative (min. two times)
		1	Negative, but only one sample obtained
		3	Negative, but have been positive more than three samples ago (potentially false-positive?)
High	Yellow	5	Interchangeably positive and negative on repeated tests. Last test was negative
	Yellow	2	Last test was positive, with all previous tests being negative
	Red	9	Repeatedly (minimum last two tests) positive.

§) The individual sample is ELISA-positive at $OD_{Corrected}$ values ≥ 0.3.

Figure 2. Graphical presentation of antibody profiles of cows in infection groups 0, 1, 5 and 9. Test-values > 0.3 are considered ELISA-positive.



MANAGEMENT

Control and eradication of MAP from a herd can be achieved by reducing transmission, as demonstrated by simulation studies (Groenendaal *et al.* 2002, Kudahl *et al.* 2007). Primarily calves, as the most susceptible animals, should be protected from faeces and milk from infectious cows. There are also other transmission routes, and in order for farmers and advisors to recognise the importance of each route, it is suggested that a risk assessment be carried out to illustrate herd-specific risks. The risk assessment can be carried out by use of an advisors' manual (Nielsen & Nielsen 2007). Most frequently, management of the new born calves in the calving area, feeding of calves with milk and purchase of live animals are identified as risks of MAP transmission in a specific herd, although other transmission routes may be of importance.

In Operation Paratuberculosis, risk based management can be initiated when diagnostic results are available. Specific risks should be addressed, depending on the possibilities. Risks related to calvings are primarily recommended to encompass the following: a) "Red" cows should be culled prior to next calving and should not be allowed near the calving area; b) "Yellow" cows are allowed to calve, but calving pens should be cleaned subsequent to each calving, and calves born by a "yellow" dam should be removed immediately; c) housing of calves should be away from the calving area and not in contact with adult cattle; and d) if a calf is born by a high-risk cow, and the calf is to remain in the herd, it should be isolated from other calves that will remain in the herd for more than 1 year, *e.g.* a heifer calf born by a "yellow" dam can be housed with a bull calf, that is sent to slaughter prior to 1 year of age. Regarding milk feeding, any milk (colostrum, surplus milk and milk with a high somatic cell count (SSC)) should not be used from "red" and "yellow" cows, but can be used from "green" cows. A requirement for the separation into "red", "yellow" and "green" cows are that the diagnostic results are updated continuously, *i.e.* are less than 4 months old. Although "red" cows are suggested to be culled, a more specific culling strategy is recommended for a specific farm. This could include: a) level in antibody ELISA of last test(s) (highest first); b) deviations in milk yield relative to expected yield; c) diarrhoea; d) high SCC; e) within-herd prevalence of "red" and "yellow" cows; and f) other factors such as lameness, age, and performance in general. In Table 2 is a brief summary of interpretation of the risk of different cow types and decisions which can be made if a cow is in a specific group.

A simulation study using PTB-Simherd (Kudahl *et al.* 2007) indicated that the strategy suggested in Operation Paratuberculosis was cost-effective after 2 to 3 years with a reduction in prevalence to 1/10 of the initial prevalence after 5 to 8 years, and with a total annual net revenue per cow per year of 500 DKK (~45 GBP) higher after 8 to 10 years, compared to if no control measures were implemented (Kudahl & Nielsen, unpublished data).

MOTIVATION

Control of MAP in a herd is a long process, which usually is considered to take a minimum of a cow generation, *i.e.* 6 to 8 years. This is being communicated to farmers, and they are encouraged not to join the programme if they are not willing to stay in the programme for that period of time. A control programme with a long time frame suggests that continued motivation is necessary in order to make herd managers adhere to the management routines that result in reduced transmission. There is a persistent need for inventing new tools to keep motivation high, and great focus is on this aspect. Initially, estimation of production losses in each herd was used as a motivation factor, along with comparison of prevalences with other herds (see next section). It is clear that further motivation factors are required, but these have not yet been fully developed.

TOOLS

A variety of tools have been developed for advisors. The main tool is a Manual for Advisors (Nielsen & Nielsen 2007), which consists of two main parts: 1) background information about paratuberculosis; and 2) recording forms that should end up in a final action plan. The forms primarily cover: a) description of the aim of the efforts; b) a risk assessment; c) a plan to address the risks identified; and d) a test-strategy. The manual and supplementary material is available electronically from http://www.paratuberkulose.dk, and advisors can fill in action plans electronically. The forms that are filled in electronically can then be uploaded to the Danish Cattle Database for later use. An example of supplementary material is a spreadsheet where the risk assessment ends up in a graphical representation of each of the risks (excerpt shown in Figure 3; English version available at: http://www.ihh.kvl.dk/htm/ssn/risk_ptb.xls).

Diagnostic results for daily management of high risk animals are presented in the "ParaTB Milk feeding list" (Figure 4), where cows are presented in risk groups for management at calving and milk

Table 2. Summary of code systems and the recommended interpretation and resulting decisions of cows tested in the Danish control programme for paratuberculosis.

Code system for cows			Condition			Decision
Risk group	Colour code	Cow type	Infected	Infectious	Affected	
Low	Green	0	?	-	-	No restrictions on use of milk and calvings can occur under regular circumstances. Also, colostrum can be used for colostrum bank. The cow cannot be deemed non-infected
Low	Green	1	?	(-)	-	As inf. grp. 0, except colostrum should not be used for colostrum bank
Low	None	3	?	-	-	Probably a false-positive, but could be considered a potential risk as being infected
High	Yellow	2	++	+++	+++	Should be considered highly infectious, but is not due for culling. More test-information is required. Could be culled if prevalence is low
High	Yellow	5	+	+	(-)	Moderately infectious, probably controlling infection
High	Red	9	++	+++	+++	Should be considered highly infectious. The higher OD-value, the more infectious. Milk production is likely to be reduced, or it can be expected to occur

Figure 3. Excerpt of graphical presentation of risk assessment.

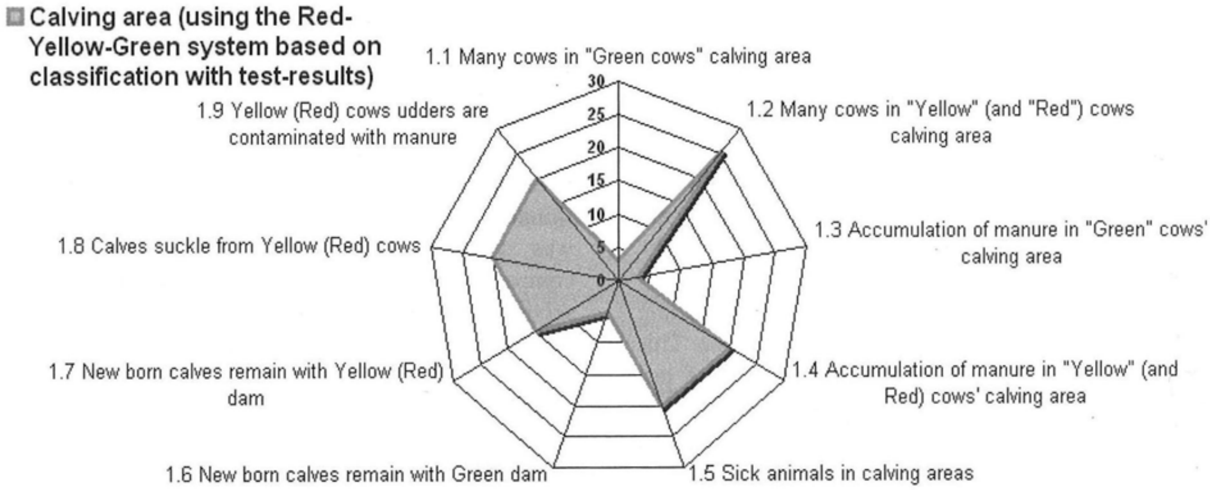


Figure 4. Excerpt from "ParaTB Milk feeding list" for management of high risk animals at calving and milk feeding. The list shown here was out-dated already when printed, because it was printed on Aug. 23, 2006 and test results were > 6 months old at that date. New test results are required for use of the list. Cows in infection groups 2 and 5 are marked yellow, and cows in infection group 9 are marked red in the last column (here shown in gray-scale).

Dansk Kvæg		ParaTB Milk feeding list
Herd with 230 cows	Herd ID xxxxx Test date: 15.02.06	Printed 23.08.06 15.38 Page 1 Den Kgl. Veterinær- og Landbohø 35 28 20 79 135

Based on milk antibody test i Herd xxxxx tested 15.02.06

Risk = High: milk should not be used for feeding of heifer calves; High hygiene around calving

Animal ID	ELISA (15.02.06)	Previous (04.01.06)	Clv.no.	Exp. calving	Milk yield drop	Inf.-group
-00929	0.9	0.1	6		Likely	2 #
-00940	0.3	0.7	4	09.10.06	Very likely	9 *
-00941	1.0	1.2	5	28.09.06	Very likely	9 *
-00958	0.5	1.0	5	10.03.07	Very likely	9 *
-00965	0.7	0.0	5		Likely	2 #
-00982	0.8	0.8	4	18.09.06	Very likely	9 *
-00984	0.6	0.6	3	23.11.06	Very likely	9 *
-00991	0.5 (04.01.06)	-	5	17.03.07	Likely	2 #
-00994	0.1	0.3	3	26.09.06	Possible	5
-01001	0.9	0.9	4		Very likely	9 *
-01001	0.9	0.8	3	16.10.06?	Very likely	9 *
-01013	0.4	0.4	3		Very likely	9 *
-01035	0.5	0.6	3		Very likely	9 *

feeding. A "quick and dirty" calculation of the production losses are available as a part of the ParaTB Milk Feeding list (Figure 5).

INFORMATION AND COMMUNICATION

Information and communication are considered pivotal in the programme. The "Manual for Advisors" and results reports are not only considered to be quick checklists and documents that are posted in the stable. All tools are developed to assist communication between the herd manager and the herd health advisor. Background information about paratuberculosis, both for farmers and advisors, are available in various formats: manual, in farmers' magazines, on http://www.paratuberkulose.dk, and a DVD, which was sent to all Danish dairy farmers in 2006. The production of a DVD was seen as a possibility of providing information to farmers,

Figure 5. A "quick and dirty" calculation of production losses in a herd, where 68 cows have been evaluated. The annual production loss was estimated to 15.000 kg. ECM.

		1st Parity	2nd Parity	>2nd Parity	Total
Avg. kg. ECM		7305	8321	8711	
No. of Cows	**Inf.-grp**				
	0	17	19	16	52
	2	2	1	1	4
	9	0	3	9	12
Annual production loss		1753	3661	9582	14997
	Inf.-grp				
Percent cows	**2+9**	11	17	38	24

which prefer as little reading material as possible. Also, the format provided an opportunity of illustrating points, which cannot easily be illustrated in writing. Continuous development of tools for communication is included in the programme.

CHALLENGES

"Operation Paratuberculosis" has been launched as a programme in which considerable uncertainty is associated with the diagnosis "paratuberculosis", primarily because of the complexity of the infection. Such uncertainty provides room for a variety of interpretations, some of which are not intended. Although the 4900 dairy farmers in Denmark are not many in a global perspective, communication with this amount of people is a challenge considering the complexity of the infection. A major part of the communication is carried out by local herd health advisors. Approximately 1,140 herds currently included in the programme are receiving advisory services from roughly 334 herd health advisors. Approximately 125 advisors have attended courses, where the principles of the programme have been taught. An unknown number of advisors have participated in various meetings, where the principles have been explained, and some advisors are self-taught. However, there still remains an unknown proportion that may be unaware of the principles on which the programme is based. One concern is that of the 334 advisors, 105 have only one herd in the programme, 161 have 2 to 5 herds, 49 have 6 to 10 herds, and 19 have more than 10 herds. Therefore, some advisors will have limited experience in providing advisory services with regard to paratuberculosis, and positive and negative experiences from other advisors therefore need to be communicated. Follow-up on whether MAP transmission is actually reduced in the participating herds is also needed. If not, the farmers should be advised to withdraw from the programme, as testing alone cannot be considered as a way to control MAP infections.

Some farmers may have a low prevalence of paratuberculosis, and surveillance may be preferred over control. Currently, no surveillance option is available. Various test strategies for surveillance are being considered, and it seems most likely that a test strategy with whole-herd antibody ELISA testing will be the preferred strategy. The test-frequency and resulting classifications are yet to be determined.

Implementation of a surveillance component may result in farmers with a low prevalence joining a programme, because so far they have had little incentives for doing so. However, a surveillance component may also result in some farmers, which are in the control programme to switch, even though a control programme is more relevant to them, if they have a high prevalence. The uncertainty in the diagnostic tests will not make such recommendations easier and sufficient information and communication will be even more challenging, the more complexity that is added to the programme.

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