Monitoring of the intra-dermal tuberculosis skin test performed by Belgian field practitioners

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Abstract

The present study aimed to monitor skin test practices as performed by veterinarian field practitioners in Belgium. For this purpose, an anonymous postal questionnaire was elaborated and dispatched to veterinarians involved in bovine tuberculosis detection. The questionnaire included items focusing on the skin test performance. International experts in the field of bovine tuberculosis were asked to fill the questionnaire and a scoring scale was built as follows: 0 = 'ideal' answer, 1 = acceptable answer, whereas 2 = unacceptable answer. Furthermore, experts were asked to rank the questionnaire's items according to their possible impact on the risk of not detecting reactors. A global score was further calculated for each participant and a comparison of practices was carried out between the two regions of the country, *i.e.* Wallonia and Flanders. Significant differences were observed between both regions, a harmonization at the country level is thus essential. No veterinarian summed a null score, corresponding to the ideal skin test procedure, which suggests that skin-testing is far from being performed correctly. Field practitioners need to be sensitized to the importance of improving compliance with good skin test practice. The authors recommend the questionnaire is suitable for application in other countries or regions.

Keywords: Bovine tuberculosis – *Mycobacterium bovis* – questionnaire – skin test – evaluation strategy

1. Introduction

Despite the implementation of eradication programs, bovine tuberculosis (bTB) still remains of a great zoonotic concern in the European Union (EU) and can even be regarded as a re-

emerging disease in some Member States (MS) (EFSA, 2007a). There are two categories of EU MS, according to their bTB status: officially tuberculosis-free (OTF) or not OTF. The OTF status (notably less than 0.1 % of herds not free of bTB infection) can also be granted to one or more regions within a given MS. The region means a clearly defined part of a country containing an animal subpopulation with a distinct health status with respect to a specific disease for which required surveillance, control and biosecurity measures have been applied for the purpose of intra-community trade (between EU MS) or trade with Third Countries (Scott et al., 2006). Belgium obtained the OTF-status in 2003 and the number of bTB outbreaks has remained low and stable since then thanks to an efficient eradication program: 7 outbreaks were recorded in 2003, 8 outbreaks in 2004, 5 in 2005, 8 in 2006, 5 in 2007 and 12 in 2008 (Federal Agency for the Safety of the Food Chain, FASFC, 2008). Currently, the Belgian bTB surveillance program is based 1) on mandatory skin testing of animals at purchase by the veterinary practitioner in charge of the herd epidemiosurveillance; 2) on systematic *post-mortem* examinations at the abattoir with transmission to the National Reference Laboratory of all suspicious lesions for analysis and; 3) on the testing of herds, according to the European Legislation (European Food Safety Authority, EFSA, 2007b). For the latter, in order to keep the OTF status, former outbreaks, neighbours of outbreaks and herds including animal(s) purchased from outbreaks are regularly tested (EFSA, 2007b). The combination of meat inspection and skin testing is recognized as the best practice for bovine tuberculosis surveillance (EFSA, 2003). The frequency of testing depends on: the introduction of new animals into a herd, the results of tuberculin testing, the detection of suspected and infected animals, the epidemiological investigation related to suspected or infected animals or herds and the follow-up testing of infected and/or eradicated herds during 5 years (EFSA, 2007b).

The skin test is the international standard for the *ante mortem* diagnosis of bTB, and accordingly the cervical Single Intra-dermal Tuberculin test (SIT) is primarily used to screen both individual cattle and herds in continental Europe (Francis et al., 1978; Caffrey, 1994;

European Council, 2004). Over the years, different factors were shown to influence the ability of the skin test to identify infected animals (sensitivity), as well as factors increasing the likelihood of false positive test results (reduced specificity); these factors are listed in Table I. On the other hand, the fact that most outbreaks were identified at the abattoir suggest that most positive animals evade the normal scheme of testing or that skin tests are not performed in the most adequate way. For that reason, the aim of this study was to develop a novel and useful methodology to evaluate the current situation of skin testing practices in regions or in countries (e.g. Belgium), on the basis of an epidemiological questionnaire. The first objective of the study was to collect data on skin test practices by mean of a questionnaire dispatched to bovine field practitioners. The second objective was to compare the obtained answers with predefined scores assigned to each question by comparison to standardized answers given by international experts in the field of bTB. Experts were also asked to rank the items of the questionnaire according to their possible impact on the risk of non-detection of reactors. The questionnaires filled in by the veterinarians were globally scored in order to evaluate their compliance to skin test procedures. A comparison between the practices as performed in both regions of the country was further carried out. To our knowledge, this approach is original and has never been used previously to assess the proficiency of bTB testing strategy.

2. Materials and Methods

2.1. Enrolment of bovine veterinary practitioners

This cross-sectional descriptive study covered the whole country of Belgium, which is divided into three administrative regions. Only two regions are concerned by bovine farming: Wallonia (Southern part of the country) and Flanders (Northern part of the country). In collaboration with the FASFC (Federal Agency for the Safety of the Food Chain), annual meetings are held to present the prophylactic campaigns to veterinary bovine practitioners in charge of the herds' epidemiosurveillance (i.e. screening for the detection of bTB, bovine leukosis and brucellosis). The questionnaire and a reply-paid envelope were distributed to the

veterinarians attending the meetings (N = 859). Several meetings were planned in the whole country (about one per province) in November 2007. The participation to the postal survey was on a voluntary basis. Respondents were assured in the covering letter that results of the survey would remain anonymous and would not be used to identify individuals. They were asked to fill in the questionnaire and send it back directly to the Research Unit at the University of Liège.

2.2. Questionnaires

Demographic information was collected including data on years of experience in rural practice and location (provinces) of activities. The questionnaire was divided into several sections: personal data, tuberculin utilisation and conservation, tuberculin injection protocol (preparation of the injection site, instrument used, delay of reading the response), epidemiological information, decision in case of test-reactor or –suspect, use of avian tuberculin and skin test at purchase of cattle. Items of the questionnaire were presented in two forms: multiple-choice questions and open answer questions. Additional questions concerning veterinarians' personal opinion on the test were included in the survey but not used in the data analysis. Before dispatching, the questionnaire was pre-tested in a group of veterinarians practicing in the cattle sector (N = 10). The questionnaire is available as supplementary material at the UREAR website address: http://www.dmipfmv.ulg.ac.be/epidemiovet/i/Questionnaire_Skin_Testing_Belgium.pdf

2.3. Desing of the scoring scale

A panel of international experts (N = 5) in the domain of bTB were asked to fill in the questionnaire and to give their opinion on what should be the ideal, acceptable and unacceptable answers. The standard questionnaire was then drafted accordingly. Each answer received a score. This scoring scale is depicted in Table II. A score of 0 was allocated to the ideal answer; the acceptable answer was given a score of 1, and a 2-score was retained for the unacceptable answer. As all experts did not agree for each item, the prevailing answer was

selected for each point. The answers provided by the veterinarians were scored according to the experts' opinion.

2.4. Balancing of scores according to the experts' ranking of criteria

A global score was calculated for each participating veterinarian, according to the above scoring scale. This global score was equal to the sum of individual scores obtained for each item of the questionnaire. The items were classified into 5 categories, as shown in Table II: 1) materials (tuberculins and instrument); 2) injection protocol 3) reading; 4) skin-test at purchase and 5) others (epidemiological data). As each parameter or step of the skin-testing process does not have the same impact (weight) on the risk of non-detection of reactors, a panel of international experts in the field of bTB (N = 11) were asked to rank the parameters according to this possible impact on such a risk. Inclusion criteria for experts were a large bTB field experience (> 20 years) and responsibility in the National Reference Laboratory for bTB or membership in the subgroup for bTB of the "EU Task force for monitoring eradication diseases in Member States" or coordinators of bTB eradication programme. Experts allotted 100 points between the criteria on the basis of the potential negative impact they can have on the risk of non-detection of reactors. The parameter with major impact received the maximum of points (the greater the impact on non-detection, the highest the number of points allotted). These points were then summed in order to obtain the total for each item (Table II). A new balanced global score was thus re-evaluated for each veterinarian according to this ranking. Each veterinarian's global score (according to the scoring scale) was balanced according to the classification of items: the score obtained for each parameter was multiplied by the total of points allotted by the experts for the specific item. A new balanced global score was then calculated by summing up the points obtained for each item of the questionnaire. For the elaboration of the standard questionnaire presented in Table II, not all the items of the questionnaire were considered. Some of the less pertinent parameters were left aside in order to minimize the number of items necessary to calculate the global score. The following parameters were considered as less pertinent because they do not have the

same impact on the risk of not detecting a reactor: questions related to the veterinarians' personal opinion, e.g. their personal safety while performing the skin test or the remuneration and valorisation of the act (subjectivity). Other items for which the response rate was very low (questions requiring more precise answers such as the specific number of reactors and/or positive animals reported during the past 5 years) were not considered either. Thirty parameters were eventually selected to establish the global score of compliance. Based on these 30 parameters, the scores obtained by Flemish (FLVT) and Walloon (WAVT) veterinarians were further statistically compared.

2.5. Statistical analyses

A Pearson's correlation coefficient was calculated to evaluate the provincial representativeness (geographical origin), on one hand, between veterinarians who attended the reunions and the total number of rural practitioners, and on the other hand, between veterinarians who attended the meetings and those who participated to the study (test of representativeness). Differences were considered as significantly different for $P \leq 0.05$. The participants' region of origin (Flanders and Wallonia) was the identification information always available, so it was decided to compare the situations between both regions, though there are no differences in terms of farm management. The distribution of scores for FLVT and WAVT was compared considering two scenarios. In the first one, a direct imputation was applied: each missing data point was replaced by a score of 2 (worst case scenario, assuming the absence of answer meant masking an unacceptable answer). In this case, the comparison between the distribution of global scores (FLVT and WAVT) was assessed using a Poisson regression model or, in case of extra-binomial variability, a negative binomial regression. In the second scenario, no imputation of values was applied for missing data and the total score was calculated as the average of the available scores: a comparison between FLVT and WAVT, using this average score, was assessed by means of a boostrapped quantile regression, an iterative method allowing the estimation of the parameters of interest on the

basis of a re-sampling. All statistical analyses were carried out in STATA/SE 10.1 (StataCorp, 2007).

3. Results

3.1. Participation to the study

A hundred and fifty-seven veterinarians returned useable questionnaires between the 30th of November 2007 and the 15th of January 2008. The answer rate was higher in Flanders (N = 111) than Wallonia (N = 46), but there are also proportionally more practitioners in Flanders. Nevertheless, a significant correlation was found between the number of answers and the number of veterinarians per province of activities (Pearson's correlation coefficient of 0.96, with $P < 0.0001$); the participation was thus considered as representative for each province. Missing data were distributed as follows: 41 practitioners had failed to answer one question; 21 vets did not answer two items; 3 vets did not answer three items; 4 vets did not answer 4 items and 4 vets did not answer 5 items. These missing data were homogeneously and proportionally split between WAVT and FLVT.

3.2. Descriptive results

Descriptive results are summarized in Tables III (a, b and c), IV and V. The majority of respondents had an experience in rural practice ranging between 20 and 33 years; few young graduates participated, which could be perceived as a bias as young assistants are generally the ones involved in bTB testing. Tuberculin conservation was an important point of the study, as presented in Table IV. The mean conservation time of tuberculin in the vehicle reached 30 days (range: 1 to 365 days; median: 15 days); the mean minimal conservation time was 1 day (range: < 1 day to 100 days; median: 1 day) and the mean maximal conservation time was not indicated for 74% of the participating veterinarians. The majority of veterinarians use between more than 70% and 100% of the tuberculin doses at their disposal,

as shown in Table V. The veterinarians using avian tuberculin did it within the framework of the single intradermal comparative tuberculin test (SICTT).

A mean delay of 72 hours post-injection is generally respected before the reading of the test result (range 36 hours to 78 hours). Regarding the type of reading of the response, the majority of veterinarians include the qualitative reading, which consists in the observation of inflammatory clinical signs such as oedema, exudation, necrosis, pain or inflammatory reaction of the vessels and local lymph nodes. The simple palpation of the site of injection is also included in this category. Visual observation takes as well part of the reading procedure. The quantitative reading by the measurement of the skin fold thickness with calipers is practiced by a minority of veterinarians, and not always systematically applied. In field conditions, many practitioners first rely on a visual observation and the palpation of the site of injection; only in case of a suspect reaction, they measure the swelling with a caliper.

Only few veterinarians do not perform the skin test at purchase, especially in beef cattle feedlots, where the animals arrive when they are still quite young (less than 6 weeks of age). Veterinarians recommend isolation of animals until reading of the result of skin test performed at purchase but they are not sure this recommendation is systematically followed by the farmers.

3.3. Analytical comparison of practices between both regions

3.3.1. Before balancing the scores

The distribution of global scores with imputation (scenario 1) and the average score without imputation for missing data (scenario 2) are presented in Figure 1. It is important to mention the broad range of individual scores, indicating large variations in how participants are performing the test. According to the first scenario, the average of global scores for FLVT (Mean: 21.66; 95% CI: 20.80 - 22.54) and for WAVT (Mean: 21.02; 95 % CI: 19.72 – 22.39) were not significantly different (Poisson Regression). According to the second scenario, the distribution of mean scores for FLVT (Bootstrapped quantile regression; Mean: 0.72;

Percentile 25: 0.60; Median: 0.70; Percentile 75: 0.83) and WAVT (Bootstrapped quantile regression; Mean: 0.70; Percentile 25: 0.57; Median: 0.70; Percentile 75: 0.80) did not significantly differ either.

3.3.2. After balancing the scores (ranking of criteria)

According to the first scenario (imputation for missing data) no significant difference was observed between FLVT and WAVT for the global balanced score (P = 0.06). When investigating inside each group of criteria (N = 5 categories): 1) materials such as tuberculins and instrument; 2) injection protocol; 3) reading; 4) skin-testing at purchase and; 5) other such as epidemiological data, FLVT obtained a significantly higher score for two categories of criteria: 'material' (Negative Binomial Regression; P = 0.02; FLVT: Mean = 238.45; 95% CI = 222.80-254.10 and WAVT: Mean = 200.96; 95% CI = 175.78-226.13;) and 'others' (Negative Binomial Regression; P = 0.03; FLVT: Mean = 129.93; 95% CI = 121.48-138.38 and WAVT: Mean = 105.87; 95% CI = 91.16-120.58).

The second scenario (without imputation for missing data) revealed that average balanced scores did not differ significantly between FLVT and WAVT. When investigating inside each group of criteria, it appeared FLVT presented a higher average score for the categories 'others' (Bootstrapped quantile regression; $P < 0.0001$; FLVT; Median = 42.44; Percentile 25 = 28.0; Percentile 75 = 52.66, and WAVT: Median = 33.51; Percentile 25 = 24.66; Percentile 75 = 52.66) while WAVT had a higher score for the category 'Reading of the response' (Bootstrapped quantile regression; P = 0.002; FLVT; Median = 37.71; Percentile 25 = 23.5; Percentile 75 = 47.0 and WAVT: Median = 37.96; Percentile 25 = 29; Percentile 75 = 43.25).

4. Discussion and conclusions

Veterinary practitioners participated at a rate of 18.3%, which is a good score for this type of postal questionnaire, for which a good expected rate of answers varies between 5 and 20% (Dufour, 1994). The decision of comparing two regions of the same country was aiming at

implementing such a methodology to compare different regions as well as EU Member States or other countries (multicentre investigations).

Balancing the scores according to experts' ranking was also a crucial point, as each step of the skin-test process does not have the same importance in terms of impact on the risk of not detecting reactors. The non-detection of reactors must be considered from the zoonotic point of view. Both statistical approaches led to the same observations when all the criteria were considered together: no statistical difference was observed between the two regions of the country, which led to the decision of further investigating each criteria category. The first scenario (imputation for missing data) identified two categories of criteria for which the scores differed significantly between practitioners of both regions. For the category 'material', FLVT obtained a significantly higher score than WAVT, which could be explained by two observations. First, FLVT do not claen or disinfect their injection material as often as WAVT; nevertheless, this parameter should be interpreted carefully because some practitioners might as well use disposable products. Moreover, FLVT using syringes do not change the needle as often as WAVT do. According to the experts' opinion, the use of a new needle should be recommended before each herd testing. Using a sole needle for several animals poses a problem in terms of biosecurity as the needle may act as a vector of pathogens. The category of criteria 'others' also showed significant differences according to the first scenario (imputation for missing data). Animals treated with a steroidal anti-inflammatory drug are more likely to be skin-tested by FLVT. A previous study demonstrated that a topical or systemic administration of glucocorticoids can lead to a significant reduction in the size of the bovine tuberculin reaction in infected cattle (Doherty et al., 1995b). Animals suffering from continuous coughing or a chronic pneumonia resistant to a classical treatment are less often skin-tested by FLVT than by WAVT. However, bTB should be included in the differential diagnosis of previous conditions and thus a skin test should be performed to detect a possible bTB infection.

The second scenario, i.e. without imputation for missing data, showed a significant difference between both regions in 'reading the response': FLVT would be more inclined to respect the 72 hours-delay before the lecture. Some veterinarians read the response as early as 24 hours after the injection; however, taking into account that delayed hypersensitivity only starts 8 to 10 hours after the injection to peak after 24 to 72 hours (Coignoul et al., 1989), this is a too 'short delay to read a tuberculin test reaction. The reference reading is thus at 72 hours after injection (Gayot et al., 1977; Desmecht, 1980) as prescribed in the EU legislation and recommended by the OIE.

Other criteria did not present any significant difference between the practitioners of both regions. Nevertheless, the scores did not match the experts' opinion for several parameters. The conservation of tuberculin was ranked as a criterion with major impact. Indeed, improperly stored tuberculin can actually be responsible for tuberculin test false negative results in cattle (De la Rua-Domenech et al., 2006). According to the OIE (World Organization for Animal Health), tuberculin should be kept in best storage conditions away from light and at a temperature between 2 and 8°C (OIE, 2004). Even if the neck remains the main site of injection, some practitioners inject the tuberculin in the caudal fold; however, the skin of the neck is a better injection site for obtaining better skin-test sensitivity results compared to the skin of the caudal fold (OIE, 2004). Furthermore, the cervical SIT is the official screening test in the EU (European Council, 2002 and 2004). Few practitioners spontaneously read the response by measuring the skin-fold thickness with a caliper, which is the recommended type of reading (OIE, 2004). Most of them look for the presence of lesions and palpate the site of injection, only measuring in case of doubt. Before injection, practitioners only check the integrity of the skin. However, the skin-fold thickness should be measured before injection as well (European Council 2002; Anonymous, 2003). Although mandatory, the isolation of purchased animals is not always advised by veterinarians. Nevertheless, when recommending such a quarantine period for purchased animals, there is no certainty that the farmer will follow this recommendation. If a purchased animal gives

evidence of an inconclusive reaction, it may be legally sent back to the herd of origin for future investigations and be followed-up thereafter. In case of intra-community trade or importation from Third Countries, accuracy and validity of the information contained in the veterinary certificate are essential (e.g. pre-movement tuberculosis testing). Indeed the eventual post-movement testing should take into account an eventual pre-movement skin test. Indeed, the risk of having a period between both tests shorter than 6 weeks exists, which will increase the probability of a false negative reaction.

It appears that efforts still need to be done to sensitize the veterinarians to the importance of compliance when using the SIT as a herd screening test for bTB. Indeed, none of the participants reached a score of 0. The significant differences reported between the practices of FLVT and WAVT should be an encouragement towards a harmonization in the execution of SIT at the country level. Whilst the general approach including the analysis of all criteria at the same time showed no statistical difference, it was necessary to further analyse each group of criteria to highlight significant. The establishment of a standardized testing methodology linked with bovine tuberculosis risk factors (veterinary manual) should be recommended to the sanitary authorities. It would then be interesting to repeat the questionnaire-based study within a few years, using the same methodology, in order to check for any improvement and follow-up of recommendations established on the basis of this standard. The ultimate step would be the implementation of multicentre investigations in order to assess the situation at the European level. This could lead to the harmonization of skin test practices around the world to facilitate live animals trade.

The present methodology is original and easy to perform by an independent partner to assess the theoretical knowledge of participating veterinarians in regard to skin-testing. To which degree answers correlate with what veterinarians actually do in practice is still to be assessed. Such a questionnaire can be used in countries as well as in regions, to evaluate and maintain the level of the bTB epidemiosurveillance network. A next step would be to evaluate the

implementation of testing. Moreover, this approach could also be used to develop similar evaluation methods for the surveillance of other diseases.

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Table I. Potential causes of false negative and false positive results to the tuberculin test in cattle

Potential causes of false negative results

A. Factors related to the animal:
   a. Skin-test performed too early after a previous tuberculin test (Doherty et al., 1995a)
   b. Recently infected cattle (Monaghan et al., 1994)
   c. Anergy: lack of immunological response to a specific antigen e.g. terminal stage of a generalized tuberculosis (Pollock and Neill, 2002)
   d. Concurrent infection with immunosuppressive viruses: bovine diarrhoea virus, bovine immunodeficiency virus (Charleston et al., 2001)
   e. Treatment with drugs (e.g. corticosteroids and other immunosuppressive agents) (Doherty et al., 1995a)
   f. Immunosuppression during early post-partum (Kehrli et al., 1989)
   g. Malnutrition: demonstrated in rabbits and guinea pigs (Bell et al., 1986; McMurray et al., 1989) but not in cattle (Doherty et al., 1996)

B. Factors related to tuberculins
   a. Expired product
   b. Product stored under inappropriate conditions (exposed to light and heat for long periods, bacterial or fungal deterioration of the product)
   c. Tuberculin manufacturing errors (e.g. use of inadequate *M. bovis* strain, incorrect calibration of batch potency)*
   d. Potency of tuberculins (Semret et al., 2006)

C. Factors related to the method of administration, reading and recording of the test (tester errors due to inexperience, lack of attention, poor cattle restraining facilities, fractious animals, poorly maintained testing equipment e.g.)
   a. Injection of too much or too little of tuberculin (Lepper et al., 1977; Monaghan et al., 1994)
   b. Subcutaneous (rather than intradermal) injection of tuberculin (Lepper et al., 1977; Monaghan et al., 1994)
   c. Incorrect site of injection
   d. Use of avian tuberculin instead of bovine tuberculin or vice-versa in the single intradermal comparative cervical tuberculin test (SICCT)
   e. Reading the results too early or too late after the time of injection: not within the advised 72h ± 4-6 hours post-tuberculin injection (Lepper et al., 1977; Monaghan et al., 1994)
   f. Human errors in identifying the reactor animal or while registering the skin readings
   g. Tester bias (conscious or unconscious)

Potential causes of false positive test results

A. Co-infection with (or pre-exposure) to an environmental mycobacterium such as (not exhaustive list):
   a. *M. avium-intracellulare* complex resulting in hypersensitivity to bovine tuberculin, prompting the use of the SICCT (Amadori et al., 2002; Hope et al., 2005)
   b. Exposure to infected domestic or wild bird or occasionally with exposure to pigs infected with the M. *avium-intracellulare-scrofulaceum* complex (Brown et al., 1981)

B. Infection with *M. avium subsp. paratuberculosis* (Aranaz et al., 2006; Dunn et al., 2005)

Adapted from De la Rua-Domenech et al., 2006 ; SICCT = Single Intradermal Comparative Cervical Tuberculin test ; * PPD (purified protein derivative) tuberculins used for performing the tests specified should be prepared in accordance with the World Health Organization requirements and should conform to these requirements with respect to source materials, production methods and precautions, added substances, freedom from contamination, identity, safety, potency, specificity and freedom from sensitizing effect (OIE, 2004).

Table II. Single intradermal tuberculin test (SIT) scoring table elaborated on the basis of (inter-) national experts' opinion

(N = 5) and total of points obtained for each criteria (N = 11 experts)

Items of the questionnaire	Scores			Points[¥¥]
	0 (Standard)	1 (acceptable)	2 (unacceptable)	
A. MATERIALS				
1. Tuberculin conservation methods (in general)	Off light, 3-8°C	—	Other answers	70
2. Tuberculin conservation in vehicle	Icebox 4°C	—	Other answers	25
3. Mean tuberculin conservation delay in the vehicle before use	1 day	3-5 days	> 5 days	47
4. Percentage of use of tuberculin doses	90 to 100%	80 to 89%	< 80%	12
5. Tool of injection	Manual syringe	Dermojet, automatic syringe	—	21
6. Use of a syringe previously filled with a tuberculin carpule	No	Yes	—	17
7. Use of a dermojet previously filled with a tuberculin solution	No	Yes	—	17
8. Cleansing/disinfection of SIT material	Cleansing+Disinf.	Disinf. or cleansing	Nor cleansing nor disinf.	28
9. Frequency of cleansing/disinfection of SIT material	After each herd	Once a week	Less often than once a week	25
10. Frequency of needle replacement (syringe)	After each herd; if broken	Once a week	Others	20
11. Frequency of dermojet revision	Yearly	If defective	Others	22
B. INJECTION				
12. Use of avian tuberculin	Never	Occasionally**	Often	36
13. Site of injection	Neck	—	Caudal fold, other	51
14. Shaving of the site of injection	—	Yes	No	22
15. Clipping of the site of injection	Yes	No	—	24
16. Use of scissors to clip the hair of the site of injection	Yes	No	—	44
17. Checking for the absence of swelling or lesion before injection	Yes	—	No	45
18. Evaluation of the skin fold before injection	Spring cutimeter or Slide caliper	Palpation or visual observation	—	48
19. Post-injection verification (formation of a pea-like swollen area)	Yes	—	No	75
C. READING				
20. Type of reading of the response	Quant. + Qual.; Quant.	Qual.; palpation	Visual observation	94
21. Mean delay of reading	72 hours	—	—	58
22. Isolation of a test-reactor and/or –suspect	Yes	—	No	23
23. Delay of warning of the Authority	Immediately	12 to 24 hours	> 24 hours	33
D. SIT AT PURCHASE				
24. Systematic checking of the animal's identification when skin-tested at purchase	Yes	—	No	42
25. Isolation at purchase, until reading of the response	Yes	—	No	19
26. Systematic SIT at purchase	Yes	—	No	53
27. Repetition of SIT if test-suspect at purchase	Yes	No[¥]	—	42
E. OTHERS				
28. Minimal age of calves for carrying out a skin test	6 weeks	< 6 weeks	> 6 weeks	23
29. SIT if steroidal anti-inflammatory treatment	No*	—	Yes	42
30. SIT if chronic pneumonia (resistant to classical TTM)	Yes	—	No	37

Disinf. = Disinfection; Quant. = quantitative; Qual. = qualitative; TTM = treatment; Susp. = suspect; SIT = Single Intradermal Test. * The veterinarian is not always advised by the farmer that an animal has been treated; [¥] If sent to the abattoir. ** A veterinarian might occasionally use avian tuberculin after Authorities' authorization based on the environmental epidemiological context. [¥¥] The total of points summed for all experts is not exactly 1100 (in reality 1115) because some experts used more than 100 points that was allowed.

Table IIIa. Evaluation of the skin test practices to detect bovine tuberculosis according to the veterinarian's area of origin (part I: tuberculin and materials)

	Wallonia (N = 46)			Flanders (N = 111)		
	N answers	*N positive*	*% (95%CI)*	*N answers*	*N positive*	*% (95%CI)*
Tuberculin						
Isothermal box at 4°C in the vehicle	13	5	38.5 (13.9 – 68.4)	61	38	62.3 (49.0 – 74.4)
Use of syringe already containing a tuberculin carpule	17	4	23.5 (6.8 – 49.9)	62	59	95.2 (86.5 – 99.0
Use of a dermojet already containing tuberculin solution	33	12	36.4 (20.4 – 54.9)	50	40	80.0 (66.3 – 90.0)
Use of avian tuberculin for screening purposes (SICCT)	45	17	37.8 (23.8 – 53.5)	110	8	7.3 (3.2 – 13.8)
Materials						
Tool of injection						
• Dermojet	46	33	71.7 (56.5 – 84.0)	111	51	45.9 (36.4 – 55.7)
• Manual syringe	46	10	21.7 (10.9 – 36.4)	111	21	18.9 (12.1 – 27.5)
• Automatic syringe	46	9	19.6 (9.4 – 33.9)	111	42	37.8 (28.8 – 47.5)
Cleansing/disinfection of the material of injection						
• No cleansing nor disinfection	46	6	13.0 (4.9 – 26.3)	109	39	35.8 (26.8 – 45.5)
• Cleaning + disinfection	46	15	32.6 (19.5 – 48)	109	21	19.3 (12.3 – 27.9)
• Cleaning	46	24	52.2 (36.9 – 67.1)	109	45	41.3 (31.9 – 51.1)
• Disinfection	46	1	2.2 (0.06 – 11.5)	109	4	3.7 (1.0 – 9.1)
Frequency of cleansing/disinfection						
• After each herd	40	19	47.5 (31.6 – 63.9)	72	32	44.4 (32.7 – 56.6)
• Once a week	40	2	5.0 (0.6 – 16.9)	72	18	25.0 (15.5 – 36.6)
• Once a month	40	9	22.5 (10.8 – 38.5)	72	10	13.9 (6.9 – 24.1)
• Less often than once a month	40	4	10.0 (2.8 – 23.7)	72	3	4.2 (0.9 – 11.7)
• No particular delay	40	1	2.5 (0.06 – 13.2)	72	5	6.9 (2.3 – 15.5)
Frequency of needle replacement						
• After each herd	18	5	27.8 (9.7 – 53.5)	59	5	8.5 (2.8 – 18.7)
• Once a week	18	2	11.1 (1.4 – 34.7)	59	2	3.4 (0.4 – 11.7)
• When broken	18	8	44.4 (21.5 – 69.2)	59	7	11.9 (4.9 – 22.9)
• Once a month (or less often)	18	3	16.7 (3.6 – 41.4)	59	42	71.2 (57.9 – 82.2)
• No particular delay	18	0	0.0 (0 – 15.3)	59	2	3.4 (0.4 – 11.7)
• After skin-test at purchase	18	0	0.0 (0 – 15.3)	59	1	1.7 (0.04 – 9.1)
Frequency of dermojet revision						
• When defective	33	27	81.8 (64.5 – 93.0)	49	45	91.8 (80.4 – 97.7)
• Once a year	33	4	12.1 (3.4 - 28.2)	49	3	6.1 (1.3 – 16.9)

N = number; CI = confidence interval (Binomial exact); SICCT = single intradermal comparative cervical tuberculin test; *less often than once a month (once or twice a year) or no specific delay.

Table IIIb. Evaluation of the skin test practices to detect bovine tuberculosis according to the veterinarian's area of origin (part II: methods and epidemiological data)

	Wallonia (N = 46)			Flanders (N = 111)		
	N answers	*N positive*	*% (95%CI)*	*N answers*	*N positive*	*% (95%CI)*
Methods						
Site of injection						
• Neck	46	35	76.1 (61.2 – 87.4)	111	104	93.7 (87.4 – 97.4)
• Caudal fold	46	7	15.2 (6.3 – 28.9)	111	2	1.8 (0.2 – 6.4)
• Other (e.g. buttock)	46	5	10.9 (3.6 – 23.6)	111	5	4.5 (1.5 – 10.2)
Preparation of the injection site						
• Hair clipping	45	34	75.6 (60.5 – 87.1)	101	30	29.7 (21.0 – 39.6)
• Hair shaving	39	12	30.7 (17.0 – 47.6)	111	94	84.7 (76.6 – 90.8)
Checking for the absence of swelling/lesion at the site of injection	43	39	90.7 (77.9 – 97.4)	110	98	89.1 (81.7 – 94.2)
Estimation of the skin fold before injection						
• Visual inspection	43	42	97.7 (87.7 – 99.9)	109	107	98.2 (93.5 – 99.8)
• Measurement (calipers)	43	0	0.0 (0.0 – 6.7)	109	1	0.9 (0.02 – 5.0)
• No inspection	43	1	2.3 (0.06 – 12.3)	109	1	0.9 (0.02 – 5.0)
Verification of the site post-injection	46	40	87.0 (73.7 – 95.1)	111	90	81.1 (72.5 – 87.9)
Mean delay of 72 hours before reading the test	45	39	86.7 (73.2 – 94.9)	109	108	99.1 (95.0 – 100.0)
Type of reading						
• Qualitative (and palpation)	46	41	89.1 (76.4 – 96.4)	111	80	72.1 (62.3 – 80.2)
• Visual observation	46	14	30.4 (17.7 – 45.8)	111	52	46.8 (37.3 – 56.6)
• Measurement of the skin fold (calipers)	46	9	19.6 (9.4 – 33.9)	111	12	10.8 (5.7 – 18.1)
Epidemiological data						
Skin-testing of calves from 6 weeks of age	46	36	78.3 (63.6 – 89.1)	111	89	80.2 (71.5 – 87.1)
Skin-testing of animals treated with a SAI	41	29	70.7 (54.5 – 83.9)	108	89	82.4 (73.9 – 89.1)
Skin-testing of animals with chronic respiratory disorders (resistant to treatment)	43	22	51.2 (35.5 – 66.7)	110	27	24.5 (16.8 – 33.7)
Isolation of test reactors and suspects not advised	45	33	73.3 (58.1 – 85.4)	110	50	45.5 (35.9 – 55.2)
To informing the Authority (test-reactor and/or suspect) within 24 hours	45	43	95.6 (84.9 – 99.5)	111	107	96.4 (91.0 – 99.0)
Re-testing of a test-reactor or –suspect						
• After 6 weeks	45	18	40.0 (25.7 – 55.7)	100	41	41.0 (31.3 – 51.3)
• After less than 6 weeks	45	11	24.4 (12.9 – 39.5)	100	27	27.0 (18.6 – 36.8)
• After more than 6 weeks	45	8	17.8 (8.0 – 32.1)	100	15	15.0 (8.6 – 23.5)
	45	6	13.3 (5.1 – 26.8)	100	16	16.0 (9.4 – 24.7)

• Reference to the FASFC • Other	45	2	4.4 (0.5 – 15.1)	100	1	1.0 (0.03 – 5.4)

N = number; CI = confidence interval (Binomial exact); SAI = steroidal anti-inflammatory; FASFC = Federal Agency for the Safety of the Food Chain

Table IIIc. Evaluation of skin test strategies to detect bovine tuberculosis according to the veterinarian's practice main location (part III: purchase)

	Wallonia (N = 46)			Flanders (N = 111)		
	N answers	*N positive*	*% (95% CI)*	*N answers*	*N positive*	*% (95% CI)*
SIT at purchase						
Systematic SIT at purchase	45	43	95.5 (84.9 – 99.5)	111	106	95.5 (89.8 – 98.5)
Isolation of purchased animals until reading	44	5	11.6 (3.8 – 24.6)	110	20	18.2 (11.5 – 26.7)
Repetition of SIT if test-suspect at purchase	44	15	34.1 (20.5 – 50.0)	104	59	56.7 (46.7 – 66.4)
Delay after which the SIT is repeated if test-suspect at purchase						
• After less than 6 weeks	14	6	42.9 (17.7 – 71.3)	49	29	59.2 (44.2 – 73.0)
• After 6 weeks	14	7	50.0 (23.0 – 77.0)	49	14	28.6 (16.6 – 43.3)
• After more than 6 weeks	14	1	7.1 (0.2 – 33.9)	49	6	12.2 (4.6 – 24.7)
Systematic check/control of an animal's ID when skin-tested at purchase	45	42	93.3 (81.7 – 98.6)	110	101	91.8 (85.0 – 96.2)

N = number; CI = confidence interval; SIT = Single Intradermal Tuberculin test; ID = identification; CI = Confidence Interval (Binomial exact)

Table IV. Methods of tuberculin conservation

Tuberculin conservation	**Wallonia** (N answers = 46)		**Flanders** (N answers = 111)	
	N positive	***% (95% CI)***	***N positive***	***% (95% CI)***
Off light, 3-8°C	11	23.9 (12.6 – 38.8)	26	23.4 (15.9 – 32.4)
Off light, 3-8°C + vehicle*	19	41.3 (27.0 – 56.8)	46	41.5 (32.2 – 51.2)
Off light, 3-8°C + injector**	8	17.4 (7.8 – 31.4)	6	5.4 (2.0 – 11.4)
Off light, 3-8°C + vehicle + injector	7	15.2 (6.3 – 28.9)	25	22.5 (15.1 – 31.4)
Off light, >8°C	—	—	2	1.8 (0.2 – 6.4)
Off light, >8°C + injector	—	—	2	1.8 (0.2 – 6.4)
Off light, >8°C + vehicle	1	2.2 (0.06 – 11.5)	3	2.7 (0.6 – 7.7)
Off light, >8°C + vehicle + injector	—	—	1	0.9 (0.02 – 4.9)
TOTAL	46	100.0	111	100.0

CI = Confidence Interval (Binomial exact); * vehicle: some veterinarians keep tuberculin doses in their vehicle most of the time; ** tuberculin is often left in the injector itself

Table V. Utilisation of tuberculin doses

Utilisation of tuberculin (%)	**Wallonia** (N answers = 42)*		**Flanders** (N answers = 109)*	
	N positive	*% (95% CI)*	*N positive*	*% (95% CI)*
≤ 30%	2	4.8 (0.5 – 14.8)	3	2.7 (0.5 – 7.7)
40 – 65%	7	16.7 (6.3 – 28.9)	23	21.1 (13.6 – 29.5)
70 – 80%	13	30.9 (16.0 – 43.5)	33	30.3 (21.4 – 39.1)
85 – 95%	13	30.9 (16.0 – 43.5)	29	26.6 (18.2 – 35.3)
96 – 100%	7	16.7 (6.3 – 28.9)	21	19.3 (12.1 – 27.5)
TOTAL	42	100.0	109	100.0

CI = confidence interval (Binomial exact); * in Wallonia, 42 veterinarians and in Flanders, 109 of them answered that item.