Review of performance and test accuracy of diagnostic tests used during the bovine tuberculosis control program implemented in Germany in 2012

von Valerie-Beau Patricia Pucken

Inaugural-Dissertation zur Erlangung der Doktorwürde der
Tierärztlichen Fakultät der Ludwig-Maximilians-Universität München

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von Valerie-Beau Patricia Pucken

aus Bad Godesberg jetzt Bonn

München 2018

Aus dem Zentrum für Klinische Tiermedizin der Tierärztlichen Fakultät der Ludwig-Maximilians-Universität München

Lehrstuhl für Innere Medizin und Chirurgie der Wiederkäuer

Arbeit angefertigt unter der Leitung von:

Univ.-Prof. Dr. Gabriela Knubben-Schweizer

Gedruckt mit Genehmigung der Tierärztlichen Fakultät der
Ludwig-Maximilians-Universität München

Dekan:	Univ.-Prof. Dr. Reinhard K. Straubinger, Ph.D
Berichterstatter:	Univ.-Prof. Dr. Gabriela Knubben-Schweizer
Korreferenten:	Univ.-Prof. Dr. Rolf Mansfed Univ.-Prof. Dr. Kaspar Matiasek Priv.-Doz. Dr. Sven Reese Univ.-Prof. Dr. Mathias Ritzmann

Tag der Promotion: 10.02.2018

Für dich

"Heute nun
Schreibe ich Dir."
(Hanns-Josef Ortheil)

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ABBREVIATIONS

DNA	Deoxyribonucleic acid
EIA	Sandwich enzyme immunoassay
EU	European Union
FLI	Friedrich-Loeffler-Institute
HSe	Herd level sensitivity
IFN-gamma	Interferon-gamma
NVL	Non-visible lesions
OTF	Officially Bovine Tuberculosis-Free
PCR	Polymerase chain reaction
PPD	Purified protein derivate
SICT test	Single intra-dermal cervical tuberculin test
SICCT test	Single intra-dermal comparative cervical tuberculin test
TB	Tuberculosis

I. INTRODUCTION

Bovine tuberculosis (bovine TB) is a chronic disease which can affect almost all mammals, whereby the infection of cattle, wild animals and humans is of greatest importance. As a compulsorily notifiable disease it is listed in the World Organisation for Animal Health (OIE) *Terrestrial Health Code* (Anonymous, 2017b). Due to negative consequences on trade of animals, animal products and its zoonotic character it concerns public health, international trade and is a significant economic burden (Cousins, D. V., 2001; Thoen, C. O. et al., 2006). As a result the control of bovine TB with stringent test and slaughter regimes is an ambition all over the world. In Europe the success with such control programs is achieved by reaching the "Officially Bovine Tuberculosis-Free (OTF) Status". This status is defined in the European Union (EU) law and is reached if not more than 0.01% of cattle farms have been diagnosed bovine TB positive during the last six years and 99.9% of cattle farms were officially bovine TB free during the last 10 years[1]. Within test and slaughter regimes herds or animals at risk are tested with ante mortem tests like the intradermal tuberculin test or the Bovigam® assay. Animals being tested positive are culled and confirmation of disease is done post mortem with pathological examinations, polymerase chain reaction and bacteriological examination. The proper execution of the diagnostic tests and high test accuracies are essential for the success of such control programs (Humblet, M.-F. et al., 2011; Schiller, I. et al., 2010a).

Since Germany received the status OTF in 1996 the further perpetuation of this status is important for international trade and public health. Bovine TB cases that were found during routine abattoir inspection lead to the implementation of a one-time bovine TB control program in Germany in 2012. During this control program discrepancies in the right execution of the intradermal tuberculin tests led to discussions between stakeholders (Anonymous, 2013; 2014; 2015) and a legal dispute[1]. The objective of the first study was therefore to review farm-animal practitioners' current knowledge on execution of the intradermal tuberculin tests in comparison to current and former legislation and literature. Furthermore, persons involved in the bovine TB control program noticed discrepancies between the

[1] 97/76/EC: Commission Decision of 17 December 1996 laying down the methods of control for maintaining the officially tuberculosis free status of bovine herds in certain Member States and regions of Member States

results of the Bovigam® assay and the pathological findings. Hence the objective of the second study was the estimation of sensitivities and specificities from the tests used within the one-time bovine TB control program in the Allgäu Region. As for bovine TB a perfect reference test is missing the test characteristics were assessed with a latent class analysis within a Bayesian approach. Both studies review on the tests performed within the one-time bovine TB surveillance program and can be therefore valuable for the implementation of future control programs in Germany.[2]

[2] VGH München, Beschluss v. 03.07.2014 – 20 CS 14.1032

II. LITERATURE OVERVIEW

1. Tuberculosis

Since centuries tuberculosis (TB) is known as an infectious disease that occurs in humans and vertebrates all over the world (Cambau, E. et al., 2014; Sattelmair, H., 2005). In humans it is the most common cause of death world-wide among adolescents and adults. In the year 2015 there were approximately 10.4 Million new human TB cases and 1.4 Million human TB-patients died (Anonymous, 2016b). Bovine TB with cattle as its original host, is a major infectious disease which concerns public health, international trade and other areas of public and private interest in many countries of the world (Anonymous; Cousins, D. V., 2001). As a consequence there are strategies and control programs with the aim of reduction in humans and eradication in livestock (Anonymous, 2016b).

1.1. The pathogen

In the year 1882 ROBERT KOCH discovered the tubercle bacillus as the infectious agent of tuberculosis. It was first called *Bacterium tuberculosis* until LEHMANN and NEUMANN suggested to name the agent *Mycobacterium tuberculosis* and to include this species together with the leprosy bacillus into the new genus *Mycobacterium* (Sewpersadh, M., 2012). This genus was placed in its own family of *Mycobacteriaceae* in the order *Actinomycetales* (Rastogi, N. et al., 2001). Today there are four conditions for a bacterium to be included in the genus *Mycobacterium*. These are acid-alcohol fastness, presence of mycolic acids containing 60-90 carbons which can be cleaved by pyrolysis to C22-C26 fatty methyl esters and containing Guanin and Cytosin to 61-71 mol% in the DNA (Shinnick, T. M. et al., 1994).

Mycobacteria can be subdivided into pathogenic, facultative pathogenic and non pathogenic also called saprophytic species. Furthermore, they can be distributed into slow growing (mostly pathogenic) and fast growing (mostly nonpathogenic) mycobacteria. Some slow growing species have the additional distinctive feature

to build carotinoid pigments. Those building the pigment under light are called photochromogens and classified to Runyon group I. The scotochromogens build the pigment in the dark and belong to the Runyon group II. The species that are not able to build pigments and are also slow growing are called nonphotochromogens (Runyon group III). Some authors distribute the fast growers to Runyon group IV (Koch, O. et al., 2012; Rolle, M. et al., 2011; Shinnick, T. M. et al., 1994).

The agents that are pathogenic and causing tuberculosis in humans and animals are summarized into the *Mycobacterium tuberculosis* complex.

1.2. Bovine tuberculosis

Bovine TB, which is primarily caused by *Mycobacterium bovis* (*M. bovis*) and to a lesser extent by *Mycobacterium caprae* (*M. caprae*), has been reported everywhere in the world, except of the Antarctica[3] (Anonymous, 2017a; Skuce, R. A. et al., 2011). Cattle is regarded as the original host. Nevertheless, infections with *M bovis* are reported in many other warm-blooded vertebrates, including humans. Furthermore, *M. caprae*, being first described in 1999, was until now isolated from goats, wild animals, sheep, pigs and humans in Europe (Aranaz, A. et al., 1999; Corner, L. A. L., 2006; Cousins, D. V., 2009; Grange, J. M. et al., 1994; Morris, R. S. et al., 1994; Prodinger, W. M. et al., 2002). Hence, bovine TB is found among several domesticated and wildlife species with the latter known as reservoir for infections in domesticated animals (Corner, L. A. L., 2006; Morris, R. S. et al., 1994; O'Reilly, L. M. et al., 1995).

A horizontal transmission with the inhalation of infectious aerosols is the most frequent cause for spread of bovine TB. The infectious aerosols can be transmitted from nasal mucus of an infected animal or from infected dust particles in the environment. Beside the infection via the respiratory tract also a primary alimentary infection with contaminated feed, drinking water or, especially with calves, milk can occur. A secondary alimentary infection can be due to the swallowing of infected lung exudates. In addition congenital, cutaneous and genital transmissions are also reported (Cousins, D. V., 2001; Hofmann, W. et al.,

[3] Verordnung über anzeigepflichtige Tierseuchen in der Fassung der Bekanntmachung vom 19. Juli 2011 (BGBl. I S. 1404), die zuletzt durch Artikel 3 der Verordnung vom 3. Mai 2016 (BGBl. I S. 1057) geändert worden ist

2005; Menzies, F. D. et al., 2000; Pritchard, D. G., 1988). In an infected herd the spread and occurrence of tuberculosis depends on how many animals are infected, how many young animals are exposed to the infected animals and what kind of interventions are done for isolation (Cousins, D. V., 2001).

By means of a strict test-and- slaughter policy Australia, some Caribbean islands (including Cuba) and some parts of South America have successfully eradicated bovine TB (Skuce, R. A. et al., 2011). The wildlife reservoir is a serious problem in control and eradication of bovine TB in cattle though (Corner, L. A. L., 2006; Michel, A. L. et al., 2010). Eradication can only be successful if the transmission between wildlife and domestic animals is controlled, combined with a strict and effective test-and slaughter program and movement regulations among cattle (Cowie, C. E. et al., 2015; Schiller, I. et al., 2010a).

1.2.1. Eradication of bovine TB in Germany

After the Second World War the control of bovine TB in Germany was one of the most important veterinary tasks. In the year 1952 a voluntary program was started with the Bang's method. This method is based on the following principals: intradermal tuberculin testing of all cattle, separating the reactor cattle from the non-reactor cattle, eradication of the positive animals, the breeding of tuberculosis free young stock and recurring intradermal tuberculin testing in frequent intervals (Bisping, W., 1998). With this program the bovine TB free farms increased to 99,7% until 1961 and on first of July 1996 Germany received the status OTF[1] (Bisping, W., 1998; Hunermund, G. et al., 2006). For this reason the nationwide regularly intradermal tuberculin testing was completed and replaced with the official meat inspection at the abattoir (Köhler, H. et al., 2012; Rolle, M. et al., 2011). The number of farms officially diagnosed as infected with bovine TB was between two and ten farms in the years 1999 to 2006 (0,001 % -0,008 %) based on the epizootic report of the Federal Ministry for Food, Agriculture and Consumer Protection and on the animal health annual report of the Friedrich-Loeffler-Institute (FLI) from 1999 until 2012. Between 1997 and 2007 nearly half (43%) of all bovine TB outbreaks in Germany were confirmed in Bavaria and predominantly caused by *M. caprae*. Out of these 43% bovine TB cases nearly two

thirds (65%) occurred in the Allgäu Region (Gerstmair, E.-M., 2011; Homeier-Bachmann, T. et al., 2016). Due to this regional accumulation and a slight increase with 12 officially confirmed farms in 2007 a one-time tuberculosis surveillance program was implemented from October 2007 until March 2009 with a total of 187.000 tested cattle (Anonymous, 2009b; Gerstmair, E.-M., 2011). In the following years the number of confirmed bovine TB cases went down and hit rock bottom in 2011 with five confirmed bovine TB cases. However, already in the following year there was an increase of 23 bovine TB cases which led to the implementation of another one-time tuberculosis surveillance program starting in November 2012 in the district Oberallgäu and being extended to all regions along the Alps (Anonymous; Homeier-Bachmann, T. et al., 2016; Zellner, G., 2013). Within this surveillance program the number of confirmed bovine TB cases increased to 46 in the year 2013 and decreased to 13 in the year 2014, 12 in the year 2015 and two in 2016 (Anonymous; Homeier-Bachmann, T. et al., 2016). The bovine TB cases that occurred along the Alps were predominantly caused by three different molecular types of *M. caprae* (Domogalla, J. et al., 2013; Moser, I. et al., 2014).

2. Diagnosis of bovine tuberculosis

As an infection of bovine TB is often subclinical, the clinical signs are not characteristically and can include emaciation, weakness, anorexia, dyspnoea, enlargement of lymph nodes and cough. For the diagnosis in the living animal two diagnostic methods are available, the intradermal tuberculin tests and the IFN gamma assay (Rolle, M. et al., 2011). Both base on the delayed type hypersensitivity response (Jungi, T. W., 2000).

Post mortem diagnosis is based on pathological, histopathological, and bacteriological examination. This includes microscopic examination to find acid fast bacilli, polymerase chain reaction (PCR) and bacteriological culture with subsequent cultural and biochemical tests to identify the agent (Cousins, D. V., 2009).

2.1. Ante-mortem tests

2.1.1. Intradermal tuberculin test

The intradermal tuberculin test is worldwide the standard method for detection of tuberculosis and is the required test for international trade (Cousins, D. V., 2009). Two types of tuberculin tests can be distinguished: the single intradermal cervical tuberculin (SICT) test and the single intradermal comparative cervical tuberculin (SICCT) test. The SICT test involves only the intradermal application of bovine purified protein derivate (bovine PPD), whereby with the SICCT test there is an additional intradermal injection of avian purified protein derivate (avian PPD) (Monaghan, M. L. et al., 1994).

2.1.1.1. Single intradermal tuberculin test

When using the SICT test 0,1 ml of bovine PPD is injected intradermally either about 7 cm distal of the base of the tail (caudal fold test) or between the anterior and middle thirds of the neck. The caudal fold test is carried out in North America, New Zealand and Australia. In Europe the SICT test is performed at the neck. The interpretation of the test results is carried out 72 hours after the injection of tuberculin. At the caudal fold site any palpable or visible variation is considered to be positive or inconclusive. The interpretation of the tuberculin test at the skin of the neck is based on clinical observations and recording of increase in skin thickness. A positive result is based on clinical signs such as diffuse or extensive oedema, exudation, necrosis, pain or inflammation of the lymphatic ducts or lymph nodes in that region or if the increase in skin thickness is more than 4 mm. The animal is classified as suspect, if none of the clinical signs, that are mentioned above, are present and if the increase of skin thickness ranges between 2 and 4 mm. A negative result is ascertained if no clinical signs are present and the increase of skin thickness is lower than 2 mm[4] (Cousins, D. V., 2009; Monaghan, M. L. et al., 1994).

As only bovine PPD is used within the SICT test, false positive reactions can occur

[4] Commission Regulation (EC) No 1226/2002 of 8 July 2002 amending Annex B to Council Directive 64/432/EEC

if the animal is infected with other mycobacteria. In particular infections with *Mycobacterium avium* subspecies *avium* and *M. avium* subspecies *paratuberculosis* can lead to such false positive reactions and can increase the rate of false positive detected animals (de la Rua-Domenech, R. et al., 2006; Francis, J. et al., 1978; Rolle, M. et al., 2011).

2.1.1.2. Comparative cervical tuberculin test

For the SICCT test avian PPD and bovine PPD are both injected at different sites of the neck. The injection site of bovine PPD should be 12.5 cm apart from the injection site of the avian PPD or on the other side of the neck[4] (Cousins, D. V., 2009; Monaghan, M. L. et al., 1994). The animal is distinguished as reactor if the increasing of skin thickness at reaction site of the bovine PPD is more than 4 mm greater than that of the avian PPD, or clinical signs, as already mentioned for the interpretation of the SICT test, are present. An inconclusive reaction is evident in cases where the increase of skin thickness on the bovine site was between 2 and 4 mm in comparison to the avian site. A negative reaction is less than 2 mm and without any clinical signs[4]. With the injection of avian PPD false positive reactions resulting from the infection with other mycobacteria are clarified (Rolle, M. et al., 2011).

2.1.2. Bovigam® assay

The Bovigam® assay is the alternative test for international trade and was invented in the year 1985. It is, like the intradermal tuberculin tests, also predicated on the cell mediated immune response (Cousins, D. V., 2009). The investigation of this in vitro test became possible with the availability of bovine cytokines and the development of sensitive biological assays (Wood, P. R. et al., 2001).

For the Bovigam® assay fresh blood is incubated with avian PPD and bovine PPD for 16-24 hours. Within this time frame sensitized lymphocytes will release interferon-gamma (IFN-gamma). Pokeweed Mitogen serves as immunocompetence control and phosphate buffer saline as negative control. The

IFN-gamma production is determined with the sandwich enzyme immunoassay (EIA) method. The sample is declared as positive if the PPD-bovine stimulated blood produces more IFN-gamma than the PPD-avian stimulated blood and the negative control. False positive results are detected if the IFN-gamma level of the PPD-avian stimulated blood is higher compared to the PPD-bovine stimulated blood and the negative control. A negative result is declared as negative if the immunocompetence control gave a positive result (Faye, S. et al., 2011; Gerstmair, E.-M., 2011; Wood, P. R. et al., 2001).

2.2. Post-mortem tests

2.2.1. Necropsy

During necropsy the carcass and its organs are examined for macroscopic lesions. Most of the lesions can be found in the lymph nodes of the head, the mediastinal lymph nodes and the bronchial lymph nodes (Corner, L. A., 1994). However a more detailed examination leads to a significant higher chance to detect infected animals than during a routine abattoir inspection and includes the examination of a wide range of lymph nodes from the head, thorax, abdomen and carcass, the tonsils, lungs, liver, spleen, kidneys, udder or scrotal contents and seminal vessels (Corner, L. A., 1994; Corner, L. A. et al., 1990). The diagnosis of bovine TB with necropsy contains the risk of infected cattle diagnosed as negative due to the problem of non-visible lesions (NVL). The reasons for this NVL are variable, as lesions might be present, but they are too small to be visually discovered or the lesions are just not detected (Corner, L. A., 1994). With a detailed necropsy the possibility of NVL can be reduced.

2.2.2. Polymerase chain reaction

In the early 1990ths the polymerase chain reaction (PCR) was developed to identify members of the *M. tuberculosis* complex. Since then different target sequences were found to detect and specify different mycobacteria. Wards et al. found that the insertion sequence IS *1081* has a high sensitivity for *M. bovis*

(Wards, B. J. et al., 1995). Rodriguez et al. detected a helicase gene to distinguish between *M. bovis* and *M. tuberculosis*, *M. avium* and *M. paratuberculosis* (Rodríguez, J. G. et al., 1999; Rodriguez, J. G. et al., 1995). The *Official Collection of Methods* recommend therefore a real time PCR for the detection of the hypothetical helicase and of the IS *1081* for the evidence of bovine TB (Anonymous, 2017d). The sample is interpreted as positive for bovine TB if both target genes are amplified. Weak PCR signals or the detection of only one target sequence leads to an inconclusive test result (Gerstmair, E.-M., 2011). For the distinction between *M. bovis* and *M. caprae* a multiplex real-time PCR assay can be used, with the *lepA* gene as target sequence (Reddington, K. et al., 2011). The tissues to be examined are the retropharyngeal lymph nodes, parts of the lungs, the intestine, the liver, the spleen, the kidneys and their belonging lymph nodes[5].

2.2.3. Culture

For the bacteriological examination the same tissue samples should be examined as already recommended for the PCR[6] . According to the *Official Collection of Methods* the samples are inoculated on two solid and one liquid media and aerobically incubated for 6 to 8 weeks, at 37°C. Grown colonies are examined for acid fast bacilli with Ziehl-Neelsen stain and further identified with PCR.

3. Estimating test accuracy

For the correct interpretation of test results the estimated test characteristics play an important role. These test characteristics are the sensitivity and specificity of the given tests.

[5] Tuberkulose-Verordnung in der Fassung der Bekanntmachung vom 12. Juli 2013

3.1. Sensitivity and specificity

The accuracy of a test is given as sensitivity and specificity and can be used for validation (Dohoo, I. et al., 2009). One can distinguish between analytical and diagnostic accuracy. The analytic sensitivity and specificity describes the repeatability and resilience of the assay performed under laboratory conditions and in a population with known disease status. With diagnostic sensitivity and specificity the ability of the assay is meant, to diagnose all truly diseased from non diseased in a population where the true disease status is unknown (Rabenau, H. F. et al., 2007). The sensitivity is the proportion of individuals that were tested positive and can be found under all diseased patients. As a question it can formulated as: "How likely is it, that an infected patient has a positive test result?". The formula is written as P(T+|D+). The proportion of test negative individuals which can be found under all healthy patients is the specificity. The formula is: P(T-|D-). With increasing specificity the sensitivity decreases or vice versa (Brenner, H. et al., 1997).

It is often assumed, that sensitivity and specificity are constant values. However they vary with external factors and are not universally applicable (Berkvens, D. et al., 2006; Brenner, H. et al., 1997). This can also be seen in a research of de la Rua-Domenech et al. where several estimated values for test sensitivities and specificities of the SICT test, the SICCT test and the Bovigam® assay are listed from different studies (de la Rua-Domenech, R. et al., 2006). For the SICT test the estimated values for the sensitivity ranges between 63.2% and 100% and for the specificity between 75.5% and 99.0%. For the SICCT test it varies between 52.0% to 100% (sensitivity) and 88.8% to 100% (specificity). The range of the Bovigam® assay was 73.0% to 100% for the sensitivity and 87.7% to 99.6% for the specificity. The studies differ according to the concentration of PPD, the apparent prevalence, test interpretation and the injection site for the intradermal tuberculin tests (de la Rua-Domenech, R. et al., 2006). Another study evaluated the sensitivities and specificities of the SICT test and the Bovigam® assay for standard and severe interpretation for the SICT test and differing cut-off values for the Bovigam® assay. There sensitivities between 53.0% and 83.9% were estimated for the SICT test. For the Bovigam® assay the lowest stated sensitivity was 83.1%, the highest 92%. The estimated specificity for the SICT test was

between 95% and 99.7%. For the Bovigam® assay specificities of 85.7% up to 98% were estimated (Álvarez, J. et al., 2012). For the intradermal tuberculin test it is reported that anti-inflammatory treatment previous to the intradermal tuberculin test or an infection with *Fasciola hepatica* (*F. hepatica*) could alter the test results and influences therefore the test characteristics (Claridge, J. et al., 2012; Doherty, M. L. et al., 1995; Flynn, R., J. et al., 2007).

The test characteristics for necropsy differ with the accuracy of the performed examination (Corner, L. A., 1994). Norby et al. evaluated a sensitivity for necropsy of 86.05% with lesions in only one lymph node. This sensitivity increased to 100% if lesions in two or more lymph nodes were detected. The examination was conducted by a pathologist in the Diagnostic Center for Population and Animal Health of Michigan State University. With the incidence on non visible lesions (NVL) infected cattle could be diagnosed as not infected which has an effect on the sensitivity (Norby, B. et al., 2004).

For the real time PCR with the target genes hypothetical helicase and IS *1081* sensitivities between 59% to 68% and specificities between 75% to 99% are reported, depending if the reference test was the bacterial culture or the pathological examination (Gerstmair, E.-M., 2011). In another study, with the bacteriological culture as reference test, the PCR reached a sensitivity of 72.5% and a specificity of 100% (Köhler, H. et al., 2013). For a real time PCR with the target gene IS *6110* a sensitivity of 90.9% and a specificity of 99.8% was estimated without a gold standard as reference test (Courcoul, A. et al., 2014).

The bacterial culture is still regarded as the gold standard for the diagnosis of bovine TB (Cousins, D. V., 2009). However several factors can influence the sensitivity of the culture as number of examined tissues, number of mycobacteria present in the examined tissue and cross contamination (Strain, S. A. J. et al., 2011a; Strain, S. A. J. et al., 2011b). Therefore, one can only assume a 100% specificity. In a recent study the sensitivity of the bacterial culture was estimated with 79.2% (Courcoul, A. et al., 2014). Hence the culture cannot be assumed as a true gold standard with having 100% specificity and 100% sensitivity. If test characteristics are estimated with bacterial culture as gold standard this can lead to misclassified bias and therefore to over- or underestimating of the prevalence, sensitivity and specificity (de la Rua-Domenech, R. et al., 2006; Hartnack, S. et al.,

2012; Strain, S. A. J. et al., 2011b).

3.2. The latent class analysis theorem

To estimate the sensitivity and specificity of a certain test often a gold standard is used as reference test (Bossuyt, P. M. et al., 2015). If no true gold standard is available a latent class approach can be used (Enøe, C. et al., 2000; Toft, N. et al., 2005). "Latent class" refers to the fact, that the true disease state is always hidden (Walter, S. D. et al., 1988). This latent class approach is based on multiple tests performed on the same animals (Hartnack, S. et al., 2012).

Hui and Walter were the first to describe an estimation of sensitivity, specificity and prevalence by applying two tests simultaneously on each animal of two populations with assuming conditional independence of both tests and different disease prevalence in both populations (Hui, S. L. et al., 1980).

Where binomial test results and arbitrary number of test and populations (R and S) are given, there are always R sensitivities, R specificities and S prevalences to be estimated. If the prevalence and the test characteristics vary for each population, the number of estimable parameters is $S(2R + 1)$ and the degrees of freedom are $S(2^R - 1)$ (Hui, S. et al., 1980; Hui, S. L. et al., 1998). Whenever $S \geq R/(2^{R-1}-1)$ is fulfilled the model can be extended to R tests and S populations (Hui, S. et al., 1980). For one population there are $2R + 1$ estimable parameters and $2^R - 1$ degrees of freedom. As a result for one population there have to be at least three tests, with 7 degrees of freedom (Hui, S. L. et al., 1998; Walter, S. D. et al., 1988). The conditional dependence of tests should be included in the model as the test error rates can be substantially underestimated and the prevalence can be positively or negatively biased (Vacek, P. M., 1985). There are three methods of estimation and computational techniques to use the Hui-Walter model. The Newton-Raphson technique, the Expectation-Maximization (EM) algorithm and the Bayesian approach (Enøe, C. et al., 2000).

3.2.1. Bayesian approach

Thomas Bayes lived from 1702 to 1761 and was a reverend and ordained minister.

As he was educated privately by the French statistician Abrahma de Moivre he stayed interested in statistics and mathematics his whole life (Bolstad, W. M. et al., 2007; Lesaffre, E. et al., 2007). Bayes' Theorem is based on an essay that was found after his death by Richard Price (Bayes, F. R. S. et al., 1763). It is thought, that Bayes was the first one to use probability theory inductively. He developed the mathematical basis for probability conclusion. All parameters involved in conclusions belong to one of the following kinds: 1. known, 2. unknown. The known parameters are accepted values, the unknown parameters are probability distributions, based on prior knowledge which reflects expert opinion (Lesaffre, E. et al., 2007). This expert opinion can be informative or even uninformative (Hartnack, S. et al., 2012).

In the *Standard Operating Procedure for OIE Registration of Diagnostic Kits*, the Bayesian inference and latent class models are described for estimation of diagnostic sensitivities and specificities (Anonymous, 2012).

3.3. Cohen's Kappa

For the evaluation of two tests without a gold standard Cohen's kappa coefficient can be used. This coefficient can assess how well two tests agree with each other. Additional to the truly measured concordances the coincidental concordances are considered as well. A value <1 means that the concordances comply with the coincidental concordances. If the two tests agree in total Cohen's kappa will be 1 (Dohoo, I. et al., 2009).

III. PUBLICATIONS

1. Publication 1

Der Tuberkulin-Hauttest: Literatur, Richtlinie und Umsetzung in der Praxis

The intradermal tuberculin test: literature, directive and implementation in practice

V.-B. Pucken[1], F. Götz[2], R. Mansfeld[1], S. Moder[3], C. Sauter-Louis[1,4], R. K. Straubinger[5], G. Knubben-Schweizer[1]

[1]Klinik für Wiederkäuer mit Ambulanz und Bestandsbetreuung, Tierärztliche Fakultät der Universität München
[2]Landratsamt Ostallgäu
[3]bpt Landesverband Bayern
[4]Friedrich-Loeffler-Institut, Bundesforschungsinstitut für Tiergesundheit
[5]Institut für Infektionsmedizin und Zoonosen, Tierärztliche Fakultät der Universität München

Tierärztliche Praxis 2015; 43 (G): 197-206

Der Tuberkulin-Hauttest: Literatur, Richtlinie und Umsetzung in der Praxis

V.-B. Pucken[1]; F. Götz[2]; R. Mansfeld[1]; S. Moder[3]; C. Sauter-Louis[1,4]; R. K. Straubinger[5]; G. Knubben-Schweizer[1]

[1]Klinik für Wiederkäuer mit Ambulanz und Bestandsbetreuung, Tierärztliche Fakultät der Universität München; [2]Landratsamt Ostallgäu; [3]bpt Landesverband Bayern; [4]Friedrich-Loeffler-Institut, Bundesforschungsinstitut für Tiergesundheit; Greifswald – Insel Riems; [5]Institut für Infektionsmedizin und Zoonosen, Tierärztliche Fakultät der Universität München

Schlüsselwörter
Tuberkulinisierung, Tuberkulose, Rinder, Fragebogen, Richtlinie

Zusammenfassung

Gegenstand und Ziel: Aufgrund vermehrter Tuberkuloseausbrüche in Rinderbeständen in der Region Allgäu in den letzten Jahren wurde die flächendeckende Tuberkulinisierung in dieser Region wieder aufgenommen. Ziel dieser Studie war es, einen Überblick über die aktuellen Kenntnisse der Nutztierpraktiker bezüglich der Technik des Tuberkulinisierens zu erhalten sowie basierend auf der aktuellen Gesetzeslage einen Leitfaden zur Anwendung der Methode zu erstellen. **Material und Methoden:** Das Wissen und die Erfahrungen der Nutztierpraktiker zur aktuellen Tuberkulinisierung wurden mithilfe eines Fragebogens erfragt, gesammelt und ausgewertet. Die Antworten der Tierärzte wurden mit der aktuellen und den vorherigen Fassungen des Anhangs B der Verordnung (EG) Nr. 1226/2002 der Kommission verglichen. **Ergebnisse:** An der Befragung nahmen insgesamt 137 Tierärzte teil. Sieben Fragebögen waren nicht auswertbar, sodass die Antworten von 130 Tierärzten in die Auswertung eingingen. Vierundvierzig dieser 130 Teilnehmer tuberkulinisierten zum Zeitpunkt der Befragung. Von 44 ausgefüllten Fragebögen der tuberkulinisierenden Teilnehmer konnten 42 in die abschließende Auswertung einfließen. Ein Großteil der Nutztierpraktiker nimmt die Tuberkulinisierung so vor, wie sie in der Verordnung (EG) Nr. 1226/2002 der Kommission vom 8. Juli 2002 zur Änderung von Anhang B der Richtlinie 64/432/EWG des Rates gefordert ist. Bei der Kontrolle der Ergebnisse weichen jedoch viele Praktiker von den Vorgaben in der Verordnung (EG) Nr. 1226/2002 ab. Die Tierärzte, die am stärksten davon abweichen, tuberkulinisieren entweder nur einzelne Tiere oder nicht im stark betroffenen Regierungsbezirk Schwaben. **Schlussfolgerungen:** In den besonders von der Tuberkulose betroffenen Gebieten wird die Methode des Tuberkulinisierens von den im Rahmen dieser Studie befragten Tierärzten nahezu so ausgeführt, wie es die Verordnung (EG) Nr. 1226/2002 fordert. Beim Ablesen der Ergebnisse sollte allerdings ein einheitlicheres und sorgsameres Vorgehen angestrebt werden. Der im Rahmen dieser Studie verfasste Leitfaden kann dabei helfen. Die Informationen aus der Literatursichtung zeigen zudem, dass es bisher kein standardisiertes Verfahren zur Tuberkulinisierung gibt.

Keywords
Skin test, tuberculosis, cattle, questionnaire, council directive

Summary

Objective: Because of an increase in the number of cases of bovine tuberculosis in southern Germany (Allgäu region, mainly in the administrative district Swabia) during recent years, blanket tuberculosis testing was resumed in this region. The aim of this study was to review the veterinarians' current knowledge regarding the technique of the intradermal tuberculin test. As a consequence, a guide with precise instructions for the execution and interpretation of intradermal tuberculin testing in cattle based on the current legislation should be created. **Material and methods:** Using a questionnaire, farm-animal practitioners' knowledge and experiences of intradermal tuberculin testing were surveyed, collected and evaluated. Legislative texts on tuberculosis (particularly testing of tuberculosis) were evaluated in their current and previous versions, and compared with the experiences reported by the veterinarians. **Results:** A total of 137 veterinarians participated and 130 returned questionnaires could be evaluated. Forty-four of the 130 participants were involved in tuberculosis testing when the survey was performed. Of these 44 questionnaires, 42 were incorporated in the final evaluation. The majority of the veterinarians perform the intradermal tuberculosis test as laid down in the Commission Regulation (EC) no. 1226/2002 of 8 July 2002 amending Annex B to Council Directive 64/432/EEC. However, many practitioners do not comply with the requirements of the Commission Regulation (EC) no. 1226/2002 when evaluating the results of the intradermal tuberculosis test. Veterinarians showing the least accordance with required standards only test single animals or work in areas other than Swabia. **Conclusions:** In areas severely affected by tuberculosis, the technique of intradermal tuberculosis testing is performed almost as demanded by the Commission Regulation (EC) no. 1226/2002. However, a more uniform and careful approach should be sought when monitoring the results. The guide designed in the context of this study can help to improve the performance of the intradermal tuberculosis test. The information from the literature review also shows that there is currently no standardized method of intradermal tuberculosis testing.

Korrespondenzadresse
Prof. Dr. Gabriela Knubben-Schweizer
Klinik für Wiederkäuer mit Ambulanz und Bestandsbetreuung
der Ludwig-Maximilians-Universität München
Sonnenstraße 16
85764 Oberschleißheim
E-Mail: G.Knubben@lmu.de

The intradermal tuberculin test: literature, directive and implementation in practice
Tierärztl Prax 2015; 43 (G): ■■■
http://dx.doi.org/10.15653/TPG-141145
Eingegangen: 24. Dezember 2014
Akzeptiert nach Revision: 7. Mai 2015
Epub ahead of print: 2. Juli 2015

Einleitung

In Deutschland wurde die Rindertuberkulose (bTB) von 1952 bis 1996 mittels regelmäßiger Tuberkulinisierung überwacht und durch das Merzen positiver Tiere bekämpft (2, 21). Dabei wurde das Bang'sche Tuberkulosebekämpfungsverfahren angewandt, das nach folgendem Prinzip funktioniert: Tuberkulinisierung aller Rinder, Trennung der Reagenten von den Nichtreagenten, Merzung der positiven Tiere, Aufzucht von tuberkulosefreien Kälbern und in regelmäßigen Abständen wiederkehrende Kontrolle mittels Tuberkulinisierung der Bestände (2). Dadurch gelang es, den Anteil tuberkulosefreier Bestände von 10% im Jahr 1952 auf 99,7% im Jahr 1961 zu erhöhen (2, 16). Die Tuberkulosebekämpfung war so erfolgreich, dass Deutschland mit der Entscheidung der Kommission vom 17. Dezember 1996 (97/76/EG) den Status "amtlich frei von Tuberkulose" erhielt. Laut Definition der EU bedeutet dies, dass „in sechs aufeinanderfolgenden Jahren höchstens 0,01% der Rinderbestände mit Tuberkulose infiziert waren, und mindestens 99,9% der Rinderbestände sind seit 10 Jahren amtlich anerkannt tuberkulosefrei" (Richtlinie 97/12/EG des Rates vom 17. März 1997 Anhang A, Teil I, Nummern 4, 5 und 6). Bis heute konnte in Deutschland dieser Status aufrechterhalten werden.

Aufgrund der Entscheidung des Rates vom 17. Dezember 1996 wurde die flächendeckende Tuberkulinisierung seit 1997 ausgesetzt. Zur Tuberkuloseprävention dient seitdem die amtliche Fleischuntersuchung am Schlachthof (17, 21, 26). Bei dieser werden jedoch, laut einer in Australien durchgeführten Studie (6), rund 47% der Tiere mit makroskopisch erkennbaren Veränderungen übersehen. Des Weiteren gibt es Untersuchungen, in denen Tiere mit Tuberkulose, aber ohne makroskopisch sichtbare Veränderungen (non visible lesions) beschrieben sind (12, 29), die bei der Fleischuntersuchung am Schlachthof nicht erkannt werden können.

In den Jahren 1999 bis 2006 schwankte die Zahl der Betriebe in Deutschland, in denen die bTB amtlich festgestellt wurde, zwischen zwei und zehn pro Jahr (0,001–0,006%). Im Jahr 2007 waren es 12 Betriebe und im darauffolgenden Jahr 23 (Grundlagen: monatliche Tierseuchenberichte des Bundesministeriums für Ernährung, Landwirtschaft und Verbraucherschutz, Tiergesundheitsjahresberichte des FLI von 1999 bis 2012). Zwischen 1997 und 2007 wurden 43% aller bTB-Ausbrüche in Deutschland im Bundesland Bayern registriert, 65% davon in der Region Allgäu (7). Aufgrund der Zunahme an Tuberkuloseausbrüchen sowie der regionalen Häufung erfolgte von Oktober 2007 bis März 2009 eine flächendeckende Tuberkulinisierung in der Region Allgäu (7). Untersucht wurden dabei ca. 187 000 Rinder (13). In den darauffolgenden Jahren sank die Zahl der Tuberkuloseausbrüche in Deutschland auf ein Minimum von fünf Ausbrüchen im Jahr 2011. Im Jahr 2012 wurde jedoch wieder ein Anstieg verzeichnet, wobei am Ende des Jahres in insgesamt 23 Betrieben die bTB amtlich festgestellt wurde (Grundlage: Tierseuchenbericht des Bundesministeriums für Ernährung und Landwirtschaft, BMEL). Infolge der erneuten Häufung von Rindertuberkulose begann man im November 2012 wiederum mit der flächendeckenden Tuberkulinisierung im Oberallgäu, die 2013 auf die gesamte Region entlang der Alpenkette ausgeweitet wurde (Amtsblatt Nr. 45 für den Landkreis Oberallgäu; Bayerisches Staatsministerium für Umwelt und Gesundheit).

Bei der flächendeckenden Tuberkulinisierung von 2007 bis 2009 wurde der Intrakutan-Monotest (Monotest) eingesetzt. Die aktuelle Tuberkulinisierung (2013–2014) findet mit dem Simultantest statt. Alternativ kann am lebenden Tier nach § 1 der Verordnung zum Schutz gegen die Tuberkulose (Tuberkulose-Verordnung) ein Interferon-Gamma-Freisetzungstest (z. B. Bovigam® oder zugelassene Produkte anderer Anbieter) zur Diagnostik angewendet werden (31).

Die Diagnose der bTB mithilfe des Tuberkulins wird seit über 100 Jahren als diagnostisches Mittel im Rahmen der Tuberkulosebekämpfung genutzt (20). Dabei hat sich im Lauf der Zeit die intrakutane Tuberkulinprobe gegenüber den anderen Testmethoden (subkutane Tuberkulinisierung mit anschließender Temperaturkontrolle, konjunktivale Tuberkulinisierung, Tuberkulinisierung durch Injektion am Lid) vom Zeit- und Arbeitsaufwand am praktikabelsten erwiesen (14, 20). Sie gilt als wichtigste und wirksamste Methode, um bTB am lebenden Tier zu diagnostizieren (29). In Europa haben sich dabei vor allem der Monotest am Hals sowie der Simultantest durchgesetzt (11).

Beim zervikalen Monotest wird das Rindertuberkulin am Hals *intrakutan* injiziert. Im positiven Fall tritt nach 72 Stunden eine allergische Reaktion Typ IV (verzögerter Typ) mit Schwellung, Verhärtung, Schmerz und vermehrter Wärme an der Applikationsstelle auf (20, 26). Bei einer Zunahme der Hautfaltendicke um mindestens 4 mm oder dem Vorliegen von klinischen Veränderungen (diffuser oder extensiver Ödembildung, Ulzeration, Nekrose, Entzündung der Lymphknoten oder der Lymphgänge im Injektionsbereich) ist der Monotest als positiv zu beurteilen (Verordnung [EG] Nr. 1226/2002 Anhang B 2.2.5.3.1) (30). Durch das gemeinsame Vorkommen einzelner Antigene bei verschiedenen Mykobakterien können jedoch falsch positive Ergebnissen auftreten (26). Besonders Tiere, die mit *Mycobacterium avium* subsp. *avium* sowie *M. avium* subsp. *paratuberculosis* infiziert sind, können auf die Injektion des Tuberkulins positiv reagieren (11). Die Sensitivität des Monotests wird mit 80,0–91,0%, die Spezifität mit 75,5–96,8% angegeben (27).

Falsch positive Reaktionen im Monotest lassen sich durch den Simultantest abklären (26). Bei diesem wird sowohl Rinder- als auch Geflügeltuberkulin an zwei parallel zur Schulterlinie liegenden Injektionsstellen intrakutan injiziert. Ist beim Ablesen die Zunahme der Hautfaltendicke an der Injektionsstelle des Rindertuberkulins mindestens 4 mm größer als an der Injektionsstelle des aviären Tuberkulins oder liegen klinische Veränderungen wie oben beschrieben vor, ist der Hinweis auf bTB gegeben (Verordnung [EG] Nr. 1226/2002 Anhang B 2.2.5.3.2) (30). Die Sensitivität des Simultantests wird mit 55,1–93,5%, die Spezifität mit 88,8–100% angegeben (27).

Durch das vermehrte Auftreten der Rindertuberkulose in den letzten Jahren ist es nicht auszuschließen, dass die Tuberkulinisie-

rung in Zukunft auch in derzeit nicht betroffenen Gebieten wieder regelmäßig durchgeführt wird.

Mit dieser Studie wurde ein Leitfaden erstellt, an dem sich Tierärzte, die in die Tuberkulinisierung involviert sind, orientieren können.[1] Dieser beruht auf den Erfahrungen von praktizierenden Tierärzten, der Literatur und den aktuellen Gesetzestexten. Der Leitfaden ist vor allem für diejenigen gedacht, die vor 1997 keine Erfahrungen sammeln konnten und sich hinsichtlich der Technik der Tuberkulinisierung unsicher sind. Ferner geben die gesammelten Informationen einen Überblick über die aktuellen Kenntnisse der Großtierpraktiker zur Technik des Tuberkulinisierens.

Material und Methoden

Fragebogen

Ein Fragebogen wurde entwickelt, um die Erfahrungen der Nutztierpraktiker zur aktuellen Tuberkulinisierung ausführlich erfassen zu können.[2] Er beinhaltete 36 Fragen und basierte auf der Studie von Prof. Dr. Claude Saegerman und Dr. Marie-France Humblet in Belgien (15). Ferner wurde der Fragebogen mit mehreren Tierärzten aus verschiedenen Tätigkeitsgebieten (Nutztierpraxis, Universität, Veterinäramt), die alle praktisch oder theoretisch in die Tuberkulinisierung involviert sind, besprochen. Nachdem der Fragebogen auf eine Online-Plattform (https://www.soscisurvey.de/) gestellt wurde, durchlief er eine kurze Pretest-Phase, in der ihn vier Tierärzten nochmals auf Unstimmigkeiten und Verständnisprobleme überprüften.

Die Nutztierpraktiker wurden via bpt-Mitteilungsblatt, Bekanntmachung am bpt-Kongress 2013 in Mannheim und an der Mitgliederversammlung des Tierärztlichen Bezirksverbandes Schwaben sowie über den E-Mail-Verteiler der Fachgruppe Bestandsbetreuung Rind des bpt auf den Fragebogen aufmerksam gemacht.

Die Frage „Testen Sie in der Regel alle Tiere einer Herde?“ musste während der laufenden Umfrage um eine Antwortoption („Anderes“) erweitert werden.

Die Antworten wurden anonym gesammelt und mithilfe des Tabellenkalkulationsprogramms Microsoft Excel 2010 (Microsoft Corporation) ausgewertet. Der Einfachheit halber werden im Folgenden alle Teilnehmer/Teilnehmerinnen des Fragebogens als Teilnehmer bezeichnet.

Literatur

Die Literatur zur Tuberkulose aus den Jahren 1936 bis 2014 wurde gesichtet und relevante Publikationen zu den Themen Bekämpfungsmaßnahmen, Diagnostik im Allgemeinen und Tuberkulinisierung im Speziellen wurden erfasst. Berücksichtigung fanden außerdem die Anlage B der Richtlinie 64/432/EWG des Rates vom 26. Juni 1964 (23) sowie die geänderten Versionen aus den Jahren 1980 (24), 1997 (25), 2002 (30) und 2014 in der englischen Fassung sowie in der übersetzten deutschen Fassung. Des Weiteren wurde die Verordnung zum Schutz gegen die Tuberkulose des Rindes in der aktuellen und der vorhergehenden Fassung (31, 32) in die Studie einbezogen.

Ergebnisse und Diskussion

Rücklaufquote des Fragebogens

Von den insgesamt 137 ausgefüllten Fragebögen mussten sieben aus der Auswertung ausgeschlossen werden, weil sich die Teilnehmer in den Angaben widersprachen oder sie nicht in Deutschland tuberkulinisiert hatten. Somit standen für die Auswertung die Antworten von 130 Teilnehmern zur Verfügung.

Im Jahr 2012 waren in Deutschland 1218 Nutztierärzte gemeldet (Grundlage: Statistik der Bundestierärztekammer, 2012). Hätten alle Nutztierärzte den Fragebogen erhalten, läge die Rücklaufquote bei 10,7% deutschlandweit und bei 12,6% in dem am stärksten von der Tuberkulose betroffenen Bundesland Bayern. Vor dem Hintergrund, dass der Fragebogen nicht allen Nutztierpraktikern in Deutschland zur Verfügung stand sowie in Anbetracht der geringen Rücklaufquote müssen die folgenden Ergebnisse vorsichtig betrachtet werden.

Daten zu den Teilnehmern

Nach den Landkreisangaben in 118 Fragebögen waren die Teilnehmer dieser Studie sowohl in Nord- als auch in Süddeutschland beheimatet (▶Abb. 1a). Die restlichen Fragebögen enthielten entweder nur eine sehr vage oder gar keine Angabe bezüglich des Standorts der Befragten. Die Mehrzahl der aktuell tuberkulinisierenden Tierärzte (n = 44) arbeitet im Regierungsbezirk Schwaben (▶Abb. 1b), der derzeit am stärksten von der Tuberkulose betroffen ist (Grundlagen: Tierseuchenbericht des BMELV, Deutsches Tierärzteblatt 8/2012–4/2014).

Von den 130 Teilnehmern waren 63,9% männlich und 30,8% weiblich. Keine Angabe zu ihrem Geschlecht machten 5,3% der Teilnehmer. Das Durchschnittsalter betrug 48 Jahre (27–85 Jahre).

Erfahrungen mit der Tuberkulinisierung

Vor 1997 tuberkulinisierten 74 (knapp 57%) der 130 Teilnehmer. In die aktuell durchgeführte Tuberkulinisierung sind 44 (knapp 34%) dieser 130 Teilnehmer involviert, wobei von diesen 44 Tierärzten 15 (34%) nicht vor 1997 tuberkulinisiert haben. Dies zeigt, wie wichtig es ist, jüngere Kollegen in die Tuberkulinisierung einzuweisen.

Zwei Fragebögen konnten nicht in die Auswertung zur Tuberkulinisierung in der Praxis miteinbezogen werden, da die Befrag-

[1] Der Leitfaden ist als Supplementary Material zum Artikel auf der Internetseite der Zeitschrift (www.tieraerztliche-praxis.de) kostenlos abrufbar.

[2] Der Fragebogen ist als Supplementary Material zum Artikel online verfügbar.

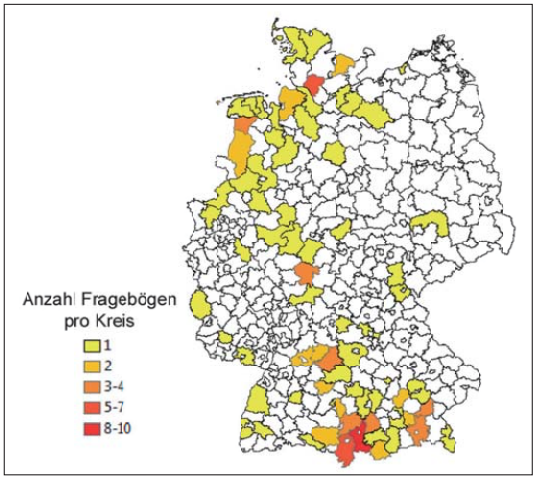


Abb. 1a Herkunft von 118 Fragebögen zum Thema Tuberkulinisierung von Tierärzten mit und ohne Erfahrung mit der Tuberkulinisierung in Deutschland
Fig. 1a Origin of 118 questionnaires about the intradermal tuberculosis test by veterinarians with and without experience with tuberculosis testing in Germany.

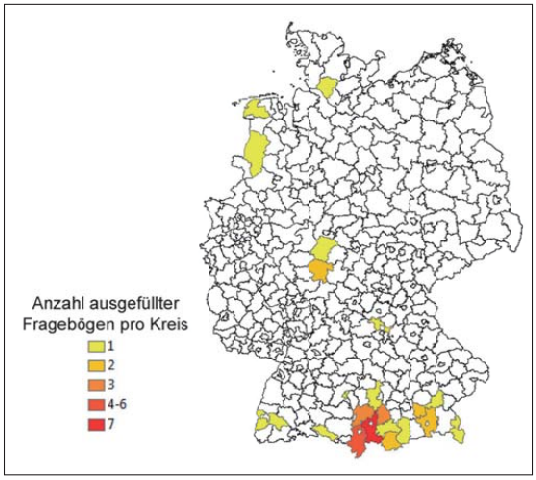


Abb. 1b Herkunft von 44 Fragebögen zum Thema Tuberkulinisierung von Tierärzten, die Erfahrung mit der Tuberkulinisierung in Deutschland haben
Fig. 1b Origin of 44 questionnaires about the intradermal test by veterinarians with experience with tuberculosis testing in Germany.

ten nur ihr Alter, ihren Landkreis und die Anzahl der Herden, die sie bis dato tuberkulinisiert hatten, angaben. Somit verblieben 42 Fragebögen für die weitere Auswertung. Aufgrund der Tatsache, dass manche Angaben fehlten oder widersprüchlich waren (was zum Ausschluss der entsprechenden Frage von der Auswertung führte), entspricht die maximale Anzahl der Antworten nicht bei jeder Frage der Gesamtteilnehmerzahl von 42.

28 Teilnehmer hatten schon vor der Einstellung der Tuberkulinisierung im Jahr 1997 tuberkulinisiert. Diese konnten aufgrund dessen auch auf ihre Erfahrungen von früher zurückgreifen und somit einen subjektiven Vergleich zwischen damals und heute in Bezug auf Kommunikation mit dem Landwirt, Handhabung der Geräte, Rechtslage und Ergebnisinterpretation ziehen (▶Abb. 2).

Einzelne Aspekte des Fragebogens

Wenige Teilnehmer empfinden, verglichen mit früher, die **Kommunikation mit dem Landwirt** aktuell als schwieriger. Aufgrund der Tatsache, dass sich die Rechtslage im aktuell durchzuführenden Tuberkulinisierungsverfahren geändert hatte (10, 34), kam es während der laufenden Untersuchungen zu Verunsicherungen in Bezug auf die Durchführung und Aussagekraft der eingesetzten diagnostischen Tests auf Seiten der Tierärzte wie auch bei den Landwirten, die sich zum Teil erschwerend auf die Kommunikation zwischen Tierarzt und Landwirt ausgewirkt haben könnten. Die Änderung der Untersuchungsverfahren in manchen Landkreisen (vom zervikalen Monotest zum Simultantest, zeitweise

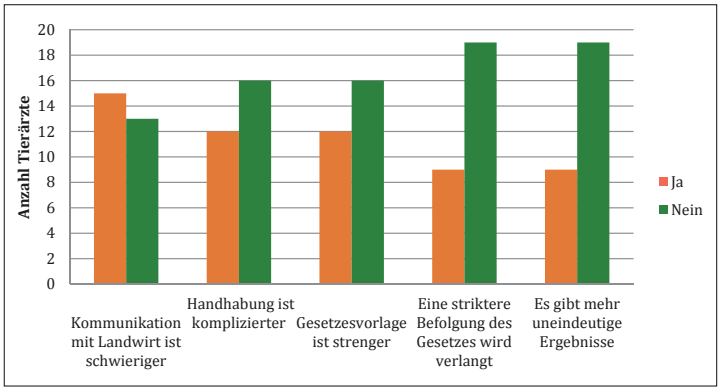


Abb. 2
Tuberkulinisierung vor 1997 und heute – Vergleich der Aussagen aus 28 Fragebögen
Fig. 2
Intradermal tuberculosis testing before the year 1997 and the current testing – comparison of the answers from 28 questionnaires.

auch Bovigam®) förderte diese Verunsicherung zusätzlich. Grundsätzlich steigern gute Information und das Verständnis von Zusammenhängen die Akzeptanz. Eine offene Kommunikation der angewandten Testmethoden mit einer genauen Erläuterung der Erkrankung und deren Diagnostik führt möglicherweise zu einem besseren Verständnis der Landwirte für diese Bekämpfungsmaßnahme.

Die **Methodik der Tuberkulinisierung** ist seit 1980 nahezu unverändert geregelt (24, 30). Demzufolge hat sich die Durchführung nicht geändert. Einige Teilnehmer der Studie empfanden die heutige Handhabung der Methodik dennoch als komplizierter als früher. Dies könnte an dem geänderten Pistolensystem liegen. Früher wurde das Tuberkulin in Ampullen vertrieben, die man in die Ampullenspritze einlegen konnte. Aktuell ist in Deutschland nur Tuberkulin zugelassen, das in Durchstechflaschen geliefert wird (22). Daher sind die alten Tuberkulinspritzen nicht mehr einsatzfähig und es müssen die neuen Spritzen mit Aufziehsystem verwendet werden.

Auch das **Empfinden einer strengeren Rechtslage**, das 12 Tierärzte angaben, kann sich insofern nicht auf die Methodik der Tuberkulinisierung beziehen. Die einschlägige Verordnung hat sich jedoch mehrfach geändert und aufgrund der Änderung der Falldefinition auch die Handhabung fraglicher und positiver Tiere (10, 32, 34).

Jeweils neun Tierärzte waren der Ansicht, dass das Gesetz eine striktere Befolgung verlangt oder mehr fragliche Ergebnisse zu verzeichnen sind als früher.

Das **Alter**, ab dem Rinder tuberkulinisiert werden, wird vom zuständigen Landratsamt festgelegt und kann sich so von Landkreis zu Landkreis unterscheiden. Diese Tatsache sowie eventuelle Besonderheiten (z. B. Untersuchung einzelner Tiere) berücksichtigte der Fragebogen vor der Ergänzung der Antwortoptionen auf die Frage „Testen Sie in der Regel alle Tiere einer Herde?“ nicht. Daher kann keine generelle Aussage darüber getroffen werden, welche Tiere untersucht werden und ab welchem Alter die Untersuchung erfolgt. Aus den Antworten geht jedoch hervor, dass sich jeder Tierarzt an die Anweisungen des zuständigen Landratsamtes hält.

Lokalisation der Tuberkulinisierung: Neun Teilnehmer tuberkulinisieren explizit nur am Hals des Tieres, die restlichen Befragten tuberkulinisieren entweder sowohl an Hals und Schulter oder nur an der Schulter.

In der deutschsprachigen Literatur wird bei der Darstellung der Tuberkulinisierung eine Region kurz vor der Schulterblattgräte beschrieben (8, 25). So wird es an deutschsprachigen Universitäten gelehrt und auch in der ersten Fassung der Anlage B der Richtlinie 64/432/EWG des Rates vom 26. Juni 1964 wird auf eine Region an der Schulter oder am Hals des Tieres verwiesen (23). Dies konkretisierte sich ab der Richtlinie 80/219/EWG, in der ein Punkt zwischen vorderem und mittlerem Halsdrittel beschrieben wird (24). Die aktuelle Fassung der Richtlinie 64/432/EWG von 2002 beinhaltet allerdings widersprüchliche Angaben, wenn die englische mit der deutschen Version verglichen wird: Laut der Commission Regulation (EC) No. 1226/2002 heißt es: „...the site for injection of avian tuberculin shall be about 10 cm from the crest of the neck and the site for the injection of bovine tuberculin about 12.5 cm lower…” (5). Diese Angabe wurde in der Verordnung (EG) Nr. 1226/2002 folgendermaßen übersetzt: „…so liegt die Injektionsstelle für Geflügeltuberkulin ungefähr 10 cm vor oder hinter der Schulterblattgräte und für Rindertuberkulin ungefähr 12,5 cm tiefer…“ (30). Das Wort „crest“ (= der Scheitel bzw. Nacken) wurde fälschlicherweise mit Schulterblattgräte übersetzt.

Um diese widersprüchlichen Vorgaben in eine einheitliche Fassung zu bringen, gab das Friedrich-Loeffler-Institut gemeinsam mit dem Paul-Ehrlich-Institut eine Empfehlung heraus, in der als Tuberkulin-Injektionsstelle der Hals am „Übergangsbereich zwischen dem ersten und dem mittleren Nackendrittel“ beschrieben wird (19). Diese Stelle ist jedoch bei einem im Fressgitter oder in einer Anbindevorrichtung fixierten Tier für den Tierarzt schwer zugänglich und zudem mehr mechanischen Einflüssen ausgesetzt als die Stelle an der Schulter. Die Empfehlung wird deshalb auf der Homepage www.wir-sind-tierarzt.de als „praxisuntauglich“ diskutiert (33). Möglicherweise ist die deutschsprachige Version die für Deutschland maßgebende Gesetzesgrundlage (s. auch Beschluss Az. 20 CS 14.1031 vom 3. Juli 2014 des Bayerischen Verwaltungsgerichtshofs). Insofern lässt sich nicht abschätzen, wie die Rechtsprechung in einem konkreten Fall ausfallen würde. In anderen Ländern wird die Tuberkulinisierung jedoch auch an der Schwanzfalte praktiziert (1, 4) und es ist fraglich, inwiefern die Reaktion an einer anderen Körperstelle abweicht.

Haarentfernung an der Injektionsstelle: Laut der Verordnung (EG) Nr. 1226/2002 soll die Injektionsstelle[3] geschoren und gesäubert werden. Vierzig von 41 Teilnehmern entfernen das Haar vor der Injektion. Dabei nutzen 24 die Schermaschine und 14 einen Rasierer oder den Scherenschlag. Nur ein Teilnehmer aus Niedersachsen gab an, das Haar nicht zu entfernen. Zwei Teilnehmer machten zur Art der Haarentfernung keine Angaben. Die Empfehlung des Friedrich-Loeffler- und des Paul-Ehrlich-Instituts sieht die Entfernung der Haare „mit einer Schere oder Schermaschine“ vor und zwar „ohne vorherige Desinfektion der betreffenden Hautstelle“ (19). Das Entfernen der Haare dient dem exakteren Ablesen der Hautfaltendicke, dem einfacheren intrakutanen Injizieren des Tuberkulins und erleichtert beim Ablesen das Wiederauffinden der Injektionsstelle. Das Scheren stellt dabei eine leichte, akkurate und rasch durchführbare Methode dar. Bei der Rasur esteht das Problem, dass Hautläsionen gesetzt werden und somit eine zusätzliche Reizung der Haut auftreten kann.

Reinigung der Injektionsstelle: Bei den Fragen, ob die Teilnehmer vor der Tuberkulinisierung die Haut reinigen oder desinfizie-

[3] Im Gesetzestext befindet sich im Originalwortlaut ein Tippfehler: „Die Infektionsstellen scheren und säubern“ (englisch: „Injection sites shall be clipped and cleaned“).

ren, gaben sechs von 39 an, die Haut vor der Tuberkulinisierung zu reinigen. Ein Teilnehmer, der einzelne Tiere untersucht, „desinfiziert" die Haut, jedoch ohne vorherige Reinigung. Die restlichen Teilnehmer reinigen oder desinfizieren die Haut nicht. Ob mit der in der Verordnung (EG) Nr. 1226/2002 geforderten Säuberung der Injektionsstelle nur die Beseitigung von grobem Schmutz, wie beispielsweise das Wegwischen von Haaren und Schmutz mit der Hand, oder eine gründlichere Reinigung (z. B. mit Wasser oder Alkohol) gemeint ist, geht aus dem Gesetzestext nicht hervor.

Laut der Verordnung (EG) Nr. 1226/2002 muss die **Hautfaltendicke vor der Injektion** des Tuberkulins innerhalb des geschorenen Bereichs mit einem „Greifzirkel" gemessen und anschließend dokumentiert werden. Mit „Greifzirkel" ist das Kutimeter oder die Schieblehre gemeint. Es handelt sich wahrscheinlich um einen Fehler bei der Übersetzung des Wortes „callipers" aus dem englischen Gesetzestext.

Bei der Durchführung des Simultantests messen 31 von 41 Teilnehmern an der Stelle der Injektion des bovinen sowie des aviären Tuberkulins zuvor die Hautfaltendicke. Zwei Teilnehmer gaben an, im mittleren Bereich zwischen den Injektionsstellen zu messen. Vier Teilnehmer messen nur an der bovinen Injektionsstelle. Jeder dieser 37 Teilnehmer nutzt zur Messung der Hautfaltendicke ein Kutimeter oder eine Schieblehre, wobei das Kutimeter deutlich bevorzugt wird. Vier weitere Teilnehmer gaben an, nicht zu messen.

Fünfunddreißig dieser 37 Teilnehmer dokumentieren bei allen Tieren die Hautfaltendicke. Zwei Teilnehmer machen dies nur bei Tieren, bei denen sie im Vergleich zu den restlichen Tieren der Herde eine deutlich abweichende Hautfaltendicke feststellen. Die Hautdicken von Rindern können stark variieren (28). Daher ist es unerlässlich, bei jedem Tier und an beiden Injektionsstellen die Hautfaltendicke zu messen und sorgfältig zu dokumentieren. Nur so kann eine eventuelle spätere Zunahme der Hautfaltendicke korrekt ermittelt werden. Die Messung der Hautfaltendicke vor Injektion des Tuberkulins sowie bei der Ablesung des Ergebnisses nach 72 ± 4 Stunden sollte immer dieselbe Person mit demselben Gerät an beiden Injektionsstellen vornehmen. So lassen sich systematische Fehler aufgrund von instrumentellen Ungenauigkeiten und persönlichen Fehlern gering halten.

Injektion des Tuberkulins: Nach dem Scheren und Säubern der Injektionsstelle sowie dem Messen und Dokumentieren der Hautfaltendicke wird das Tuberkulin injiziert und die korrekte Injektion durch die Palpation der entstandenen intrakutanen Quaddel überprüft. Dieses Vorgehen wird durch die Verordnung (EG) Nr. 1226/2002 geregelt. Sechsunddreißig von 41 Tierärzten kontrollieren grundsätzlich, ob eine intrakutane Quaddel spürbar ist. Zwei Teilnehmer kontrollieren dies nicht immer oder nur optisch, drei Teilnehmer führen nie eine entsprechende Kontrolle durch. Die Injektion des Tuberkulins muss streng intrakutan erfolgen, da nur so gewährleistet ist, dass genügend Tuberkulin in der Haut verbleibt, um eine Typ-IV-Reaktion auszulösen. Aufgrund der oft schlechten Lichtverhältnisse im Stall ist die Palpation eine schnelle und sichere Methode, um zu überprüfen, ob das Tuberkulin korrekt deponiert wurde. Die optische Kontrolle reicht in diesem Fall nicht aus.

Kanülenwechsel/-reinigung: Die Verordnung (EG) Nr. 1226/2002 sah bis Anfang November 2014 für die Injektion eine sterile Kanüle vor. Dazu äußerte sich das Friedrich-Loeffler-Institut 2013 wie folgt: „Grundsätzlich hält das Friedrich-Loeffler-Institut aus veterinärhygienischen Gründen einen Wechsel der Kanüle nach jedem Tier für erforderlich, um eine parenterale Verschleppung von Infektionserregern zu unterbinden. Im Rahmen der Tuberkulinisierung werden allerdings Systeme eingesetzt, bei denen ein Wechsel der Kanüle nach jedem Tier in der Praxis kaum durchführbar ist. Viele Injektionssysteme sind dafür auch nicht ausgelegt. Eine neue Kanüle sollte aber auf jeden Fall dann verwendet werden, wenn Rinder in einer neuen epidemiologischen Einheit untersucht werden sollen oder wenn die Kanülenspitze Schaden genommen hat (fühlbare Widerhaken, Rauigkeiten, Schwierigkeiten beim Einstechen in die Haut). Es wird auch dringend empfohlen, die Kanüle grundsätzlich zu wechseln, wenn ein Blutstropfen an der Kanülenspitze anzeigt, dass ein Gefäß getroffen wurde" (18).

Auf die Frage nach dem Kanülenwechsel gaben 36 von 40 Teilnehmern an, die Kanüle mindestens nach jeder epidemiologischen Einheit (was maximal der Größe einer Herde entspricht) zu wechseln, womit sich 90% der befragten Tierärzte grundsätzlich an die Empfehlung des Friedrich-Loeffler-Instituts halten. Die Hälfte davon wechselt die Kanüle, wenn sie stumpf ist. Nur zwei Teilnehmer wechseln die Kanüle seltener. Drei Befragte gaben zusätzlich an, die Kanüle auch beim Auftreten eines Blutstropfens zu wechseln oder beim Aufziehen von neuem Tuberkulin (nach ca. 20 Tieren). Einen Kanülenwechsel nach jedem Tier gaben zwei Teilnehmer an, die nur einzelne Tiere untersuchen.

Auch in der Standardliteratur wird bei „Massenbehandlungen" nicht für jedes Tier, sondern für „mindestens jeden zu therapierenden Bestand, besser aber für jedes 5. und 10. Tier" eine neue, sterile Kanüle verlangt (9). Gerade die Frage, wie oft die Kanüle gereinigt oder gewechselt werden sollte, sorgt allerdings während der aktuellen Tuberkulinisierung in Deutschland für Unsicherheit. Die Tatsache, dass die Hälfte der Teilnehmer angab, die Kanüle nicht nach jeder Herde zu reinigen, lässt sich damit erklären, dass diese Tierärzte die Kanüle vermutlich nach jeder Herde entsorgen. Wie aus Gesprächen mit Praktikern hervorgeht, wird in manchen Praxen die Kanüle wiederverwendet, jedoch nach Reinigung und Sterilisation.

Zwanzig von 40 Teilnehmern reinigen oder desinfizieren die Kanüle nach jeder Herde (das genaue Prozedere wurde im Fragebogen jedoch nicht ermittelt). Genau so viele gaben an, dies nicht zu tun. Ein Befragter, der lediglich einzelne Rinder tuberkulinisiert, reinigt und desinfiziert die Kanüle nach jedem Tier.

Neben der Praktikabilität des Kanülenwechsels ist dieser auch im Sinne der Reproduzierbarkeit der Methode zu hinterfragen, denn jeder Kanülenwechsel wird infolge des geringen Injektionsvolumens (100 µl) und des Totraums der Kanülen Abweichungen bedingen, die sich auf das Volumen des zu injizierenden Tuberkulins negativ auswirken können. In der Praxis ist dies vermutlich

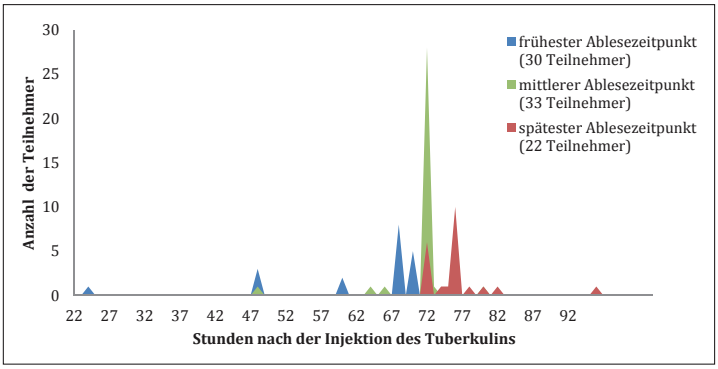


Abb. 3
Ablesezeitpunkte der Hautfaltendicke nach der Injektion des Tuberkulins

Fig. 3
Point in time when the reading of the thickness of the skin after injection of tuberculin takes place.

wenig relevant, da die Kanüle vor der Injektion mittels Durchdrücken der Tuberkulinspritze „befüllt" wird, um mögliche Luftbläschen zu entfernen. Der Nachteil dieses Vorgehens ist ein erhöhter Tuberkulinverbrauch.

Ein aktuelles Urteil vom 3.7.2014 des Bayerischen Verwaltungsgerichtshofes (Az. 20 CS 14.1032) bestätigt, dass die Untersuchung eines Rinderbestandes auf Tuberkulose nur unter der Maßgabe erfolgen darf, dass für jedes zu untersuchende Tier eine sterile Kanüle verwendet wird. Daraufhin wurden Untersuchungen ausgesetzt. Am 14.11.2014 wurde eine angepasste Übersetzung der Verordnung EG 1226/2002 im Amtsblatt Nr. L 329 der Europäischen Union veröffentlicht, in der es unter 2.2.5.1. (Anhang B) „Vorgehensweise" nun heißt: „Dazu kann die kurze, sterile Kanüle (abgeschrägte Seite nach außen) einer graduierten, mit Tuberkulin aufgezogenen Spritze schräg in die tieferen Hautschichten eingeführt werden." (Englische Version: „A short sterile needle, bevel edge outwards, with graduated syringe charged with tuberculin, inserted obliquely into the deeper layers of the skin may be used.").

Das Bayerische Staatsministerium für Umwelt und Verbraucherschutz schlussfolgert daraus: „Daher ist es nunmehr wieder möglich, Untersuchungen unter Anwendung der sogenannten „Stallkanüle" vorzunehmen" (Schreiben vom 19.11.2014 an die Regierungen und das Bayerische Landesamt für Gesundheit und Lebensmittelsicherheit [LGL]). Auch in anderen Ländern, wie beispielsweise den USA, Kanada oder der Schweiz, ist die Injektion mit einer sterilen Kanüle gesetzlich nicht vorgegeben (1, 3, 4).

Hautfaltendicke nach der Injektion: Laut Verordnung (EG) Nr. 1226/2002 muss die Hautfaltendicke an jeder Injektionsstelle 72 (± 4) Stunden nach der Injektion gemessen und aufgezeichnet

Tab. 1
Antworten der Nutztierpraktiker zu den nicht im Text berücksichtigten Fragen. Im Feld „keine Angabe" wurden alle Teilnehmer aufgelistet, die entweder „keine Angabe" ankreuzten oder diese Frage nicht beantworten. Bei der Frage nach dem Injektionsgerät konnten mehrere Antworten gegeben werden, die alle bei der Auswertung berücksichtigt wurden.

Table 1
Answers of the veterinarians to questions not considered in the text. In the field „no answer" all participants were listed that are either checked „not specified" or did not answer the question at all. Several answers were possible on the question on the syringe. All answers were included in the analysis.

Frage	Antwortoptionen		Zahl der Antworten
Halten Sie es für wichtig, die Empfehlungen des Herstellers hinsichtlich Lagerung, Anwendung, Verfallsdatum (des Tuberkulins) zu beachten?	Wichtig		31
	Eher wichtig		9
	Eher unwichtig		0
	Unwichtig		1
	Keine Angabe		1
Tuberkulinisieren Sie hochtragende Kühe (ab der 6. Woche vor der Abkalbung) zeitgleich mit den anderen Kühen der Herde?	Ja		33
	Nein. Ich tuberkulinisiere die hochtragenden Kühe	< 6 Wochen nach der Abkalbung	2
		> 6 Wochen nach der Abkalbung	2
		Keine Angabe	1
	Keine Angabe		4
Dokumentieren Sie Auffälligkeiten an den Tieren (Abmagerung, Lahmheit, Hochträchtigkeit etc.)?	Ja		11
	Nein		29
	Keine Angabe		2

Tab. 1 Fortsetzung
Table 1 Continued.

Frage	Antwortoptionen		Zahl der Antworten
Hat eine vermutete Leberegelerkrankung (Fasziolose) einen Einfluss auf Ihr Ableseverhalten?	Ja		1
	Nein		36
	Keine Angabe		5
Was benutzen Sie für die Injektion des Tuberkulins?	Revolverspritze		13
	Mc Lintock		1
	Syntena II		24
	HSW		3
	Anderes:	Syntena I	2
		Einmalspritze	1
	Keine Angabe		3
Haben Sie schon einmal einen Blutstropfen an der Kanüle bzw. auf der Haut nach der Injektion des Tuberkulins gesehen?	Ja, öfters		4
	Ja, ab und zu		8
	Ja, sehr selten		19
	Nein, nie		9
	Keine Angabe		2
Was verstehen Sie als epidemiologische Einheit?	Eine Gruppe		6
	Eine Herde		28
	Eine Bucht		4
	Anderes:	Alles in einem Stallraum	2
		Einen Betrieb	1
	Keine Angabe		1
Wie oft haben Sie Tiere, die ein fragliches Ergebnis aufweisen?	Ca. 1 Tier alle 10 Tiere		1
	Öfter als 1 Tier alle 10 Tiere		0
	Seltener als 1 Tier alle 10 Tiere		27
	Nie		11
	Keine Angabe		3
Raten Sie dem Landwirt, positive bzw. nicht eindeutig positive Tiere zu isolieren?	Ja, immer		19
	Ja, aber nur wenn er Platz/Kapazität dafür hat		9
	Nein		9
	Keine Angabe		5
Glauben Sie, die Tuberkulinisierung richtig und sicher ausführen zu können?	Ja		27
	Eher ja		13
	Eher nein		0
	Nein		0
	Keine Angabe		2
Falls Sie vor 1997 nicht tuberkulinisiert haben, hatten Sie das Gefühl bei Ihren ersten Tuberkulinisierungen genügend vorbereitet gewesen zu sein?	Ja		9
	Nein		5
	Vor 1997 schon tuberkulinisiert		24
	Keine Angabe		4

werden. Die Mehrzahl der Teilnehmer liest die Ergebnisse in diesem vorgeschriebenen Zeitfenster ab. Nur wenige Befragte lesen deutlich zu früh (24 Stunden) oder zu spät (> 92 Stunden) ab (▶Abb. 3). Die betreffenden Tierärzte stammen nicht aus Bayern oder tuberkulinisieren nur einzelne Tiere. Zwei von insgesamt 40 Teilnehmern gaben keine Antwort auf diese Frage. Alle anderen beantworten zumindest eine der drei Möglichkeiten (▶Abb. 3).

Niemand gab an, nicht alle Tiere zu kontrollieren. Nur ein Teilnehmer ließ diese Frage unbeantwortet. Die Zunahme der Hautfaltendicke messen 18 von 39 Teilnehmern bei jedem Tier, 21 tun dies nur bei deutlicher, adspektorisch erfassbarer Veränderung, wovon sieben angaben, zusätzlich auch palpatorisch die Hautfaltendicke zu überprüfen. Ein Kutimeter oder eine Schieblehre benutzen 35 von 40 Teilnehmern. Zwei Teilnehmer gaben an, nur zu palpieren. Eine Dokumentation der Hautfaltendicke nehmen 32 von 40 Teilnehmern vor, acht gaben an, dies nicht oder nur bei deutlicher, adspektorisch und palpatorisch erfassbarer Hautdickenzunahme zu tun.

Da auch eine diffuse ödematöse Schwellung auftreten kann, reicht eine alleinige Adspektion nicht aus. Die palpatorische Kontrolle auf Schmerzhaftigkeit und Wärme und eine anschließende erneute Messung der Hautfaltendicke sind unabdingbar, denn gerade eine diffuse Ödembildung lässt sich nur mit einer wiederholten Messung der Hautfaltendicke nachweisen. Zudem ist nur eine Messung der Hautfaltendickenzunahme genau genug, um bei einer Schwellung an beiden Injektionsstellen ein positives von einem fraglichen oder negativen Ergebnis zu unterscheiden. Wie oben erwähnt sorgt das Messen durch dieselbe Person mit demselben Kutimeter für den geringsten systematischen Fehler.

Befundkontrolle/-übermittlung: Siebenunddreißig von 39 Tierärzten informieren bei positiven Befunden das Veterinäramt sofort oder innerhalb der nächsten 24 Stunden. Dreißig Teilnehmer überlassen die fraglichen Fälle dem Veterinäramt, sechs kontrollieren die Tiere erneut nach 6 Wochen. Ein Teilnehmer gab an, nur eine erneute Kontrolle durchzuführen, wenn weitere Tiere keine eindeutig negativen Ergebnisse aufweisen oder das Tier durch Schwäche und Abmagerung auffällt. Zwei Teilnehmer machten dazu keine Angabe. Die weitere Handhabung der Tiere mit fraglichen Befunden wird, zumindest im Veterinäramt Ostallgäu, von den Veterinärämtern selbst geregelt (F. Götz, persönliche Mitteilung). Es ist jedoch nicht auszuschließen, dass Veterinärämter diese Aufgabe dem Tierarzt überlassen. Daher kann auf der Basis der Angaben in dieser Studie nicht darüber geurteilt werden, inwiefern sich die Tierärzte an dieser Stelle richtig verhalten.

Sonstige Aspekte: Bemerkenswert ist, dass 19 von 42 Teilnehmern angaben, auch Tiere zu testen, die kurz zuvor mit einem Entzündungshemmer behandelt wurden. Zwanzig verneinten dies und drei machten dazu keine Angabe. Da ein Entzündungshemmer die Reaktion auf das Tuberkulin potenziell negativ beeinflusst, könnte damit ein falsch negatives Ergebnis ermittelt werden.

Fazit für die Praxis

Die meisten befragten Tierärzte führen die Tuberkulinisierung korrekt und entsprechend der Verordnung (EG) Nr. 1226/2002 der Kommission vom 8. Juli 2002 zur Änderung von Anhang B der Richtlinie 64/432/EWG des Rates (30) durch. Lediglich einige wenige Teilnehmer halten sich nicht an die gesetzlichen Vorschriften. Diese Tierärzte kommen aber größtenteils nicht aus dem am stärksten betroffenen Regierungsbezirk Schwaben oder tuberkulinisieren nur einzelne Tiere. Die Befragung zeigt jedoch auch, dass beim Ablesen der Ergebnisse ein einheitlicheres und sorgsameres Vorgehen angestrebt werden muss. Die Literaturrecherche lässt erkennen, dass bei der Wahl der Injektionsstelle sowie hinsichtlich Sterilität der Injektionskanüle weltweit kein standardisiertes Verfahren existiert.

Eine Orientierung für ein einheitliches Tuberkulinisieren in Deutschland bietet der mithilfe dieser Studie verfasste Leitfaden. Dieser ist als Supplementary Material zu diesem Artikel online frei verfügbar.

Die Antworten auf die restlichen Fragen sind in ▶Tab. 1 dargestellt.

Danksagung

Wir danken Herrn Dr. Mages, Herrn Dr. Schuh, Herrn Dr. Stadler, Frau Prof. Dr. Müller, Herrn Prof. Dr. F. J. Conraths sowie Herrn Prof. Dr. W. Klee für die Unterstützung dieses Projekts bzw. die kritische Durchsicht des Manuskripts. Teile dieser Studie wurden von den Firmen Hauptner und WDT finanziert.

Interessenkonflikt

Die Autoren bestätigen, dass kein Interessenkonflikt besteht.

Literatur

1. Anonym. Bovine Tuberculosis Eradication: Uniform Methods and Rules, effective January 1, 2005. Washington: United States Department of Agriculture, Animal and Health Inspection Service; 2005.
2. Bisping W. Zur Geschichte der Bekämpfung der Rindertuberkulose – Beiträge aus dem Institut für Mikrobiologie und Tierseuchen der Tierärztlichen Hochschule Hannover. Berl Münch Tierärztl Wochenschr 1998; 111: 362–367.
3. Bundesamt für Lebensmittelsicherheit und Veterinärwesen BLV. Technische Weisungen über die Untersuchung auf bovine Tuberkulose. Schweiz: Schweizerische Eidgenossenschaft; 2010.
4. Canadian Food Inspection Ageny. Accredited Veterinarians Manual, 3.1 Tuberculosis Testing: Government of Canada 2014; http://www.inspection.gc.ca/animals/terrestrial-animals/diseases/accredited-veterinarian-s-manual/chapter-3/eng/1345233051622/1345233162747?chap=1.
5. Commission Regulation (EC) No 1226/2002 of 8 July 2002 amending Annex B to Council Directive 64/432/EEC 2002 (09.07.2002).
6. Corner LA, Melville L, McCubbin K, Small KJ, McCormick BS, Wood PR, Rotzel JS. Efficiency of inspection procedures for the detection of tuberculous lesions in cattle. Aust Vet J 1990; 67: 389–392.

7. Das Bayerische Landesamt für Gesundheit und Lebensmittelsicherheit (LGL). TB-Situation in Bayern – TBC-Projekt Allgäu. Symposium: Rindertuberkulose und Blauzungenkrankheit in Mitteleuropa. Bozen, 20.05.2009.
8. Dirksen G, Gründer H-D, Stöber M. Innere Medizin und Chirurgie des Rindes. Stuttgart: Parey 2006.
9. Dirksen G, Gründer H-D, Stöber M. Die klinische Untersuchung des Rindes. Stuttgart: Enke 2012.
10. Erste Verordnung zur Änderung der Tuberkulose-Verordnung 2013 (15.03.2013).
11. Francis J, Seiler RJ, Wilkie IW, Oboyle D, Lumsden MJ, Frost AJ. Sensitivity and specificity of various tuberculin tests using bovine PPD and other tuberculins. Vet Rec 1978; 103: 420–425.
12. Gavier-Widen D, Cooke MM, Gallagher J, Chambers MA, Gortazar C. A review of infection of wildlife hosts with Mycobacterium bovis and the diagnostic difficulties of the 'no visible lesion' presentation. N Z Vet J 2009; 57: 122–131.
13. Gerstmair E-M. Validierung molekularbiologischer und immunologischer Nachweisverfahren für die Tuberkulose bei Rindern und Tuberkulosemonitoring beim Rotwild. Dissertation, Ludwig-Maximilians-Universität München 2011.
14. Götze R, Müller J. Zur Methodik und Technik der Tuberkulinisierung beim Rinde. Berl Tierärztl Wochenschr 1936: 151–155.
15. Humblet M-F, Walravens K, Salandre O, Boschiroli M, Gilbert M, Berkvens D, et al. Monitoring of the intra-dermal tuberculosis skin test performed by Belgian field practitioners. Res Vet Sci 2011; 91: 199–207.
16. Hünermund G, Kropp R. Die Bekämpfung und Ausrottung der Rindertuberkulose in Deutschland. Pneumologie 2006; 60: 772–776.
17. Köhler H, Moser I, Menge C. Tuberkulose des Rindes: wieder ein Thema?! Tagungsband „Infektionskrankheiten des Rindes – Neues und Wichtiges für die tierärztliche Praxis"; Stiftung Tierärztliche Hochschule Hannover und Akademie für Tiergesundheit e. V. 2012; 143–149.
18. Mantel T. Newsletter der Bayerischen Landestierärztekammer 2013; 05/2013.
19. Menge C, Köhler H, Moser I, Werner E, Gyra H. Tuberkulose des Rindes: Empfehlung zur Tuberkulin-Injektionsstelle 2015; http://www.fli.bund.de/fileadmin/dam_uploads/Publikationen/Stellungnahmen/Tuberkulin_Stellungnahme_PEI-FLI_20150205.pdf.
20. Monaghan ML, Doherty ML, Collins JD, Kazda JF, Quinn PJ. The tuberculin test. Vet Microbiol 1994; 40: 111–124.
21. Moser I, Köhler H, Menge C. Die Tuberkulose des Rindes – überraschend wieder oder immer noch präsent? Tierärztl Prax 2014; 42 (G): 240–249.
22. Paul-Ehrlich-Institut. Tuberkuline für Tiere 2014; http://www.pei.de/DE/arzneimittel/weitere-tierarzneimittel/tuberkuline-tiere/tuberkuline-tiere-inhalt.html.
23. Richtlinie 64/432/EWG des Rates vom 26. Juni 1964 zur Regelung viehseuchenrechtlicher Fragen beim innergemeinschaftlichen Handelsverkehr mit Rindern und Schweinen 1964 (29.07.1964).
24. Richtlinie 80/219/EWG des Rates vom 22. Januar 1980 zur Änderung der Richtlinie 64/432/EWG in Bezug auf Tuberkulose und Brucellose 1980 (21.02.1980).
25. Richtlinie 97/12/EG des Rates vom 17. März 1997 zur Änderung und Aktualisierung der Richtlinie 64/432/EWG zur Regelung viehseuchenrechtlicher Fragen beim innergemeinschaftlichen Handelsverkehr mit Rindern und Schweinen 1997 (25.04.1997).
26. Rolle M, Mayr A, Selbitz H-J, Truyen U, Valentin-Weigand P. Tiermedizinische Mikrobiologie, Infektions und Seuchenlehre. Stuttgart: Enke 2011.
27. Schiller I, Oesch B, Vordermeier HM, Palmer MV, Harris BN, Orloski KA, Buddle BM, Thacker TC, Lyashchenko KP, Waters WR. Bovine tuberculosis: a review of current and emerging diagnostic techniques in view of their relevance for disease control and eradication. Transbound Emerg Dis 2010; 57: 205–220.
28. Schotterer A. Vergleichende Hautuntersuchungen bei Rindern. Z Tierzücht Züchtungsbiol Tierern 1933; 26: 203–218.
29. Trylich C. Diagnosis and no visible lesions in tuberculosis of cattle. Can Vet J 1960; 1: 545–550.
30. Verordnung (EG) Nr. 1226/2002 der Kommission vom 8. Juli 2002 zur Änderung von Anhang B der Richtlinie 64/432/EWG des Rates (29.06.2013).
31. Verordnung zum Schutz gegen die Tuberkulose des Rindes (Tuberkulose-Verordnung) (17.02.2014).
32. Verordnung zur Änderung der Tuberkulose-Verordnung und sonstiger tierseuchenrechtlicher Verordnungen 2009 (17.06.2009).
33. Wagner A. Tierarztfrust über praxisuntaugliche TBC-Test-Empfehlung 2015; http://www.wir-sind-tierarzt.de/2015/02/praxisuntaugliche-tbc-test-empfehlung/.
34. Zweite Verordnung zur Änderung der Tuberkulose-Verordnung 2013 (12.07.2013).

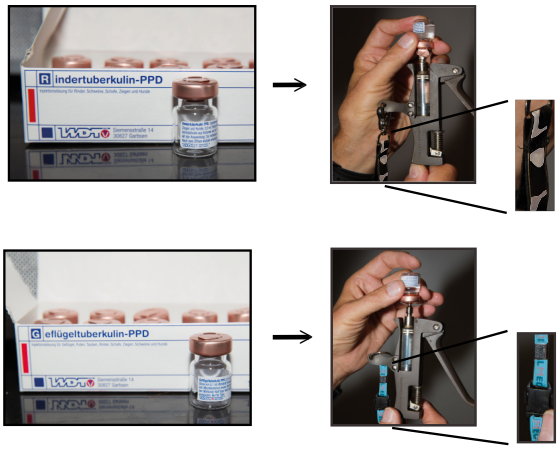
Durchführung der Tuberkulinprobe beim Rind

WDT
Das Tierarztunternehmen.

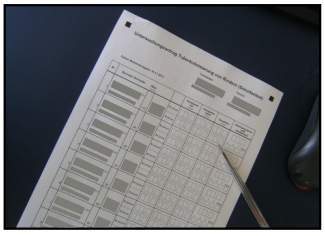
HAUPTNER
Herberholz

Folgende Dinge sollten vorbereitet sein, um diese bei der Tuberkulinisierung griffbereit zu haben:

1. Kutimeter
2. Tuberkulinspritze für bovines Tuberkulin
3. Tuberkulinspritze für aviäres Tuberkulin
4. Neue Kanülen
5. Schermaschine mit einem Ersatzakku und einem weiteren Scherkopf
6. Das sonst im Kühlschrank gelagerte Tuberkulin, entweder schon aufgezogen in den Spritzen, oder noch in den Ampullen, zum Aufziehen direkt vor Ort (siehe untere Bilder)
7. Stirnlampe für Ställe mit schlechten Lichtverhältnissen
8. Zange für das leichtere Auswechseln der Kanülen
9. Stift zur Dokumentation der Hautdicke

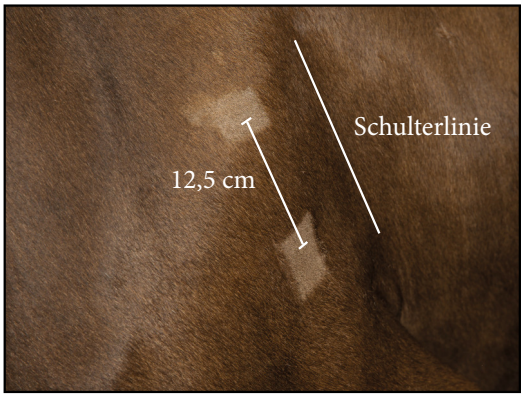


Das deutliche Markieren der jeweiligen Tuberkulinspritzen (bovin und aviär) erleichtert die Unterscheidung bei schlechten Lichtverhältnissen im Stall und schützt vor Verwechslungen.
Die Markierungen sollten entweder gut zu reinigen sein oder nur einmal verwendet werden.



Die Betriebsliste vorher ausdrucken und kontrollieren.

Die folgende Anleitung lehnt sich an die Richtlinie 97/12/EG des Rates vom 17. März 1997 an.



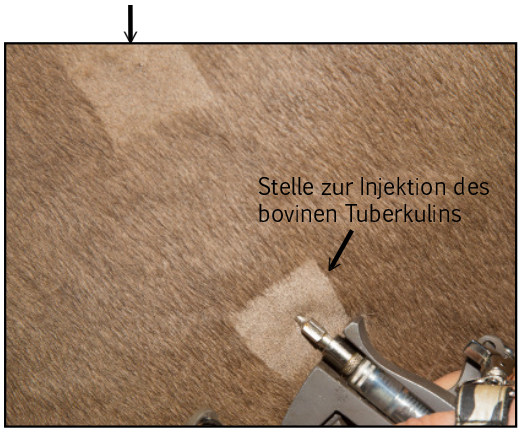
Die Injektionsstellen für das Geflügel- sowie das Rindertuberkulin sollten in einer paralell zur Schulterlinie velaufenden Linie liegen. Die Injektionstelle des Rindertuberkulins liegt etwa 12,5 cm tiefer als die Injektionsstelle des Geflügeltuberkulins.[1]

„Injektionsstellen scheren und reinigen.“[1]

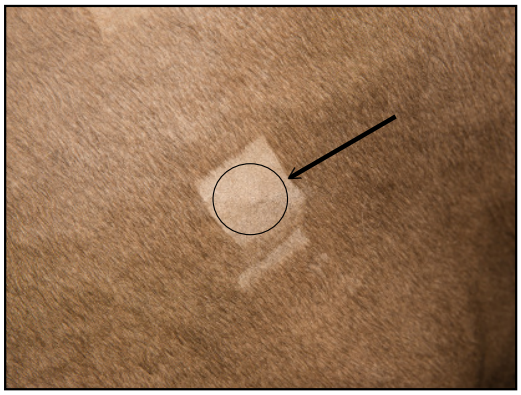
„Innerhalb der geschorenen Bereiche zwischen Zeigefinger und Daumen eine senkrechte Hautfalte bilden und deren Dicke mit Schublehre messen und aufzeichnen.“[1]

Die Dokumentation sollte direkt im Stall von einer zweiten Person durchgeführt werden.

Stelle zur Injektion des aviären Tuberkulins („der Vogel fliegt oben“)



„Eine kurze sterile Nadel, an der eine tuberkulingefuellte Meßspritze befestigt ist, Schrägkante nach außen, schiefwinklig in die tieferen Hautschichten einführen. Die Tuberkulindosis injizieren.“[1]



„Die Injektion wurde korrekt durchgeführt, wenn sich beim Abtasten eine kleine, linsengroße Quaddel an jeder Injektionsstelle feststellen läßt.“[1]

„Die Hautfaltendicke an jeder Injektionsstelle 72 Stunden nach der Injektion wieder messen und aufzeichnen.“[1]

[1] Verordnung (EG) Nr. 1226/2002 der Kommission vom 8. Juli 2002 zur Änderung von Anhang B der Richtlinie 64/432/EWG des Rates

2. Publication 2

Evaluating Diagnostic Tests for Bovine Tuberculosis in the Southern Part of Germany: A Latent Class Analysis

V.-B. Pucken[1*], G. Knubben-Schweizer[1], D. Döpfer[2], A. Groll[3], A. Hafner-Marx[4], S. Hörmansdorfer[4], C. Sauter-Louis[5], R. K. Straubinger[6], P. Zimmermann[4], S. Hartnack[7]

[1]Clinic for Ruminants with Ambulatory and Herd Health Services, Centre for Clinical Veterinary Medicine, LMU Munich, Oberschleißheim, Germany;

[2]Food Animal Production Medicine, School of Veterinary Medicine, University of Wisconsin-Madison, USA;

[3]Department of Mathematics, Workgroup Financial Mathematics, LMU, Munich, Germany;

[4]Bavarian Health and Food Safety Authority, Oberschleißheim, Germany;

[5]Institute for Epidemiology, Friedrich-Loeffler-Institute, Greifswald, Germany;

[6]Institute for Infectious Diseases and Zoonoses, Department of Veterinary Science, LMU Munich, Germany;

[7]Section of Epidemiology, Vetsuisse Faculty, University of Zurich, Zurich, Switzerland

PLoS ONE 12(6):e0179847.https://doi.org/10.1371/journal.pone.0179847

PLOS ONE

RESEARCH ARTICLE

Evaluating diagnostic tests for bovine tuberculosis in the southern part of Germany: A latent class analysis

Valerie-Beau Pucken[1]*, Gabriela Knubben-Schweizer[1], Dörte Döpfer[2], Andreas Groll[3], Angela Hafner-Marx[4], Stefan Hörmansdorfer[4], Carola Sauter-Louis[5], Reinhard K. Straubinger[6], Pia Zimmermann[4], Sonja Hartnack[7]

1 Clinic for Ruminants with Ambulatory and Herd Health Services, Centre for Clinical Veterinary Medicine, LMU Munich, Oberschleißheim, Germany, **2** Food Animal Production Medicine, School of Veterinary Medicine, University of Wisconsin, Madison, Wisconsin, United States of America, **3** Workgroup Financial Mathematics, Department of Mathematics, LMU Munich, Munich, Germany, **4** Bavarian Health and Food Safety Authority, Oberschleißheim, Germany, **5** Institute for Epidemiology, Friedrich-Loeffler-Institute, Greifswald, Germany, **6** Institute for Infectious Diseases and Zoonoses, Department of Veterinary Science, LMU Munich, Munich, Germany, **7** Section of Epidemiology, Vetsuisse Faculty, University of Zurich, Zurich, Switzerland

* valerie.pucken@vetsuisse.unibe.ch

OPEN ACCESS

Citation: Pucken V-B, Knubben-Schweizer G, Döpfer D, Groll A, Hafner-Marx A, Hörmansdorfer S, et al. (2017) Evaluating diagnostic tests for bovine tuberculosis in the southern part of Germany: A latent class analysis. PLoS ONE 12(6): e0179847. https://doi.org/10.1371/journal.pone.0179847

Editor: Odir Antônio Dellagostin, Universidade Federal de Pelotas, BRAZIL

Received: January 31, 2017

Accepted: June 5, 2017

Published: June 22, 2017

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Funding: This research was funded by Bayerisches Staatsministerium für Ernährung, Landwirtschaft und Forsten (www.stmelf.bayern.de/) (grant number: A/13/37, awarded to VBP). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Abstract

Germany has been officially free of bovine tuberculosis since 1996. However, in the last years there has been an increase of bovine tuberculosis cases, particularly in the southern part of Germany, in the Allgäu region. As a consequence a one-time tuberculosis surveillance program was revisited with different premortal and postmortal tests. The aim of this paper was to estimate diagnostic sensitivities and specificities of the different tests used within this surveillance program. In the absence of a perfect test with 100% sensitivity and 100% specificity, thus in the absence of a gold standard, a Bayesian latent class approach with two different datasets was performed. The first dataset included 389 animals, tested with single intra-dermal comparative cervical tuberculin (SICCT) test, PCR and pathology; the second dataset contained 175 animals, tested with single intra-dermal cervical tuberculin (SICT) test, Bovigam® assay, pathology and culture. Two-way conditional dependencies were considered within the models. Additionally, inter-laboratory agreement (five officially approved laboratories) of the Bovigam® assay was assessed with Cohen's kappa test (21 blood samples). The results are given in posterior means and 95% credibility intervals. The specificities of the SICT test, SICCT test, PCR and pathology ranged between 75.8% [68.8–82.2%] and 99.0% [96.8–100%]. The Bovigam® assay stood out with a very low specificity (6.9% [3.6–11.1%]), though it had the highest sensitivity (95.7% [91.3–99.2%]). The sensitivities of the SICCT test, PCR, SICT test, pathology and culture varied from 57.8% [48.0–67.6%] to 88.9% [65.5–99.7%]. The prevalences were 19.8% [14.6–26.5%] (three-test dataset) and 7.7% [4.2–12.3%] (four-test dataset). Among all pairwise comparisons the highest agreement was 0.62 [0.15–1]). In conclusion, the specificity of the Bovigam® assay and the inter-laboratory agreement were lower than expected.

Competing interests: The authors have declared that no competing interests exist.

Introduction

Bovine tuberculosis (bTB) which is caused by *Mycobacterium caprae* and *Mycobacterium bovis* is an important public and animal health problem and an international trade issue in Europe and worldwide [1–6]. Therefore, using reliable, fast and cost-effective diagnostic methods is essential for the control of bTB.

National control programs rely on testing of cattle and removal of animals which are diagnosed as positive [7]. Infected animals are detected with tuberculin skin tests or the Bovigam® gamma-interferon (IFN-γ) assay. The tuberculin skin test, the prescribed test for international trade, is used as a single intradermal cervical tuberculin (SICT) test or single intra-dermal comparative cervical tuberculin (SICCT) test in Europe and as caudal fold tuberculin (CFT) test in North America, Australia and New Zealand [7, 8]. Accuracy of the skin tests varies widely due to different factors, affecting the host and the test itself. The exact estimation of the test characteristics in the field is therefore difficult [8–10]. However, the intradermal skin tests normally have a high specificity although sensitivity can be somewhat lower [9]. The low test sensitivity and the logistics of holding cattle for 3 days to read the test has led to the development of the Bovigam® assay, in 1985 [11]. Australia was the first country to officially accept this test for the diagnosis of bovine tuberculosis in 1991 [11]. In comparison to the skin test the Bovigam® assay almost always showed a better sensitivity but an equal or inferior specificity. The Bovigam® assay is supposed to have the ability to detect bTB earlier in the course of infection [11, 12]. In many countries it is used for serial or parallel testing together with the intradermal skin tests [7, 13]. For post-mortem diagnosis of previously positive-tested animals, bacteriological culture and PCR can be used following necropsy [1, 14, 15].

In many countries the test-and-cull regime led to the status Officially Bovine Tuberculosis free (OTF) [9, 16, 17]. Germany reached this status in 1997 [18]. Owing to the OTF status nationwide periodic surveillance using intradermal skin testing was replaced with surveillance by official meat inspection at the abattoir [19, 20]. Remarkably, there is an apparent increase of bTB cases since 2007, many of them detected during routine meat inspections and with a particular high prevalence in the southern part of Germany. These unexpected bTB cases led to a revision of the tuberculosis regulations in 2009, 2012 and 2013 with commencement of the act in 2009, 2013 and 2014. Within this revision the Bovigam® assay and the PCR were added as new diagnostic methods. Furthermore, the increase of bTB cases lead to the implementation of a one-time tuberculosis surveillance program in Germany in 2012 to verify the nation's OTF status [21]. At the beginning of this surveillance program the SICCT test or a serial testing with the SICT test and the Bovigam® assay were used for ante-mortem diagnosis [22]. This was the first time that the Bovigam® assay was used as a field test in Germany. However, as a consequence of irregular test results, the testing regime was changed in March 2013 with the SICCT test as the only ante-mortem test. Moreover, the PCR analysis as described in the national *Official Collection of Methods* was used since then as additional post-mortem method [1, 22–24].

As described in the literature the sensitivities and specificities of bTB diagnostic tests vary widely [12, 13, 25, 26]. This leads to difficulties in identifying truly infected animals as well as in identifying risk factors for bTB [27]. Diagnostic accuracies of bTB diagnostic tests are often estimated using bacteriological culture as the so-called gold standard for confirmation of bTB [26, 28–30]. A gold standard is considered as a test that has known properties with a high sensitivity and specificity. Because bacteriological culture has limitations in sensitivity this may lead to a misclassification of data [13, 31]. By using a latent class approach the test characteristics can be assessed in the absence of a gold standard [32–34]. This latent class approach can be used within a Bayesian model and is based on multiple tests performed on the same animals.

"Latent class" refers to the fact that the true disease state is always hidden [35]. In the *Standard Operating Procedure for OIE Registration of Diagnostic Kits* the Bayesian inference and latent class models are described to use for estimation of diagnostic sensitivities and specificities [36].

The aim of this study was to assess the diagnostic accuracies of the tests used within the bTB surveillance program in Germany between 2010 and 2014. To the best of our knowledge a latent class analysis for the diagnostic tests of bTB has never been applied in Germany.

Material and methods

With the aim to obtain robust diagnostic test accuracy estimates for different pre- and post-mortem tests diagnosing bovine tuberculosis, a Bayesian latent class approach was performed. Regarding the Bovigam® assay agreement between blood samples tested by five different laboratories and between blood samples taken from two different anatomical locations was assessed with Cohen's kappa coefficient.

Ethics statement

The test results which were used for the Bayesian latent class approach were collected within the context of the officially ordered tuberculosis-surveillance program ("Untersuchungsprogramm: Rindertuberkulose in den Landkreisen der Alpenkette; AZ: 42a-G8755-2013/2-450) prior to this study and were not specifically taken for the purpose of this study. The program was conducted according to Directive 64/432/EEC on animal health problems affecting intra-EU trade in bovine animals and swine, Council Directive 80/219/EEC of 22 January 1980 amending Directive 64/432/EEC as regards tuberculosis and brucellosis and Council Directive 97/12/EC of 17 March 1997 amending and updating Directive 64/432/EEC on health problems affecting intra-Community trade in bovine animals and swine [37–39] to verify the OTF status.

The data used for the Bovigam® assay agreement existed prior to our research. The blood samples were taken in the context of the study "Optimierung der Methode Bovigam®—Test beim Rind—vergleichende Untersuchungen an 21 Tieren des Betriebs Spitalhof, Kempten" which was carried out by the Institute for Infectious Diseases and Zoonoses, Department of Veterinary Science, LMU Munich under the direction of Prof. Dr. med. vet. Reinhard K. Straubinger, Ph.D and were approved by the government of Upper Bavaria (approving authority for animal research). According to the approval no. 5.2-1-54-2532.3-26-13 there is no conflict with animal protection law.

Bayesian latent class models

Animal samples. Out of 5736 animals tested between 2012 and 2014 in the districts Ober- and Ostallgäu (Bavaria), two data subsets with test results from multiple tests run in parallel were chosen. The first dataset comprised test results from 175 animals which had been tested from December 2012 to March 2013 by the SICT test, the Bovigam® assay, culture and pathological examination. The second dataset comprised test results from 389 animals which had been tested from April 2013 to February 2014 by the SICCT test, the PCR and which have been examined pathologically. The data was collected as binary data and to some extent, for the SICT test, the SICCT test and the Bovigam® assay, also as metric data.

Diagnostic tests. The SICT test and the SICCT test were performed by field or official veterinarians. Examination of the test results were carried out in accordance to Commission Regulation (EC) No 1226/2002 of 8 July 2002 amending Annex B to Council Directive 64/432/EEC [15]. For the skin tests 0.1 ml of bovine respectively bovine and avian Purified Protein Derivate (PPD) (Wirtschaftsgenossenschaft Deutscher Tierärzte eG) was injected

intradermally in the neck or shoulder of the cattle. For the latent class analysis the inconclusive reactors were assigned twice, once as negative reactors (standard interpretation) and once as positive reactors (severe interpretation). An inconclusive reactor means an increase of skin thickness between 2 and 4 mm (SICT test) respectively 1 and 4 mm (SICCT test) and no occurrence of clinical signs.

For the Bovigam® assay heparinized blood was taken two to 28 days (mean of 8.45 days) after the SICT test by field practitioners. The blood was sent within 6 to 7 hours at room temperature to the laboratory of the Bavarian Health and Food Safety Authority. The Bovigam® assay was carried out according to the manufacturer's instructions. In brief, the blood samples were stimulated overnight with avian and bovine PPD. IFN-γ production of the lymphocytes was then determined by using a sandwich ELISA. Identification of infected animals based on the prescription in the manufacturer's user manual for Germany. This means that the mean optical density (OD) of a sample being stimulated with bovine PPD minus the OD of the same sample stimulated with avian PPD, was greater or equal 0.1.

The pathological examinations were performed at different places (pathology of the Bavarian Health and Food Safety Authority, carcass disposal plants) by veterinarians. Attention was given to the retropharyngeal lymph nodes, lung, gut, spleen, kidneys, liver and the associated lymph nodes as well as organs or lymph nodes with pathological-anatomical changes.

For polymerase chain reaction (PCR) samples were collected during necropsy from the retropharyngeal lymph nodes, lung, gut, spleen, kidneys, liver and the associated lymph nodes. Furthermore, pieces of organs or lymph nodes with pathological-anatomical changes were taken [23]. All samples were investigated in the laboratory of the Bavarian Health and Food Safety Authority. To increase the detection of mycobacteria samples with pathological findings were homogenized. From inconspicuous samples approximately 25 mg were used for DNA extraction. PCR aiming at detecting *Mycobacterium tuberculosis* complex-pathogens was performed for each sample separately according to the *Official Collection of Methods* [1]. The targeted sequences for PCR amplification are a hypothetical helicase and the insertion element (IS) *1081* [40, 41]. According to the official guidelines, results were interpreted as positive if both target sequences were found, as inconclusive if only one target sequence or only weak PCR signals were detected and as negative if no signals were observed. In agreement with the Friedrich-Loeffler-Institute (FLI) single runs were performed for each organ / lymph node.

Bacteriological culture was performed according to the *Official Collection of Methods* [1]. Organs were cultured as aggregate samples, except for organs with macroscopic lesions, which were cultured separately. As liquid media BD BACTEC™ MGIT™ was used. Löwenstein-Jensen and Stonebrink agar slants were used as solid culture media.

Statistical analysis. A Bayesian latent class approach assuming no gold standard, i.e. a perfect diagnostic test without any misclassification, was performed for the three-test dataset (SICCT test, PCR, necropsy) and the four-test dataset (SICT test, Bovigam® assay, necropsy, culture). The skin tests were considered with both their standard and severe interpretation, separately. In total, for the four-test dataset, there were four sensitivities, four specificities, one prevalence and twelve two-way covariances to be estimated, leading in total to 21 unknown parameters [42]. For the three-test dataset there were three sensitivities, three specificities, one prevalence and six two-way covariances to be estimated, leading in total to 13 unknown parameters. Due to the principle of parsimony, higher order terms of covariances were not considered. The specificity of culture was fixed to "1", assuming that no false positive test result exists. This reduces the number of parameters to be estimated for the four-test dataset. For all other estimable parameters, first uninformative beta priors (1,1) were utilized. Second, informative priors basing on expert opinion and published test accuracies [9, 10] were utilized for the sensitivities and specificities of the SICT and the SICCT test. This was done for sensitivity

analysis, respectively, for setting constraints to have still an identifiable model for the three-test dataset with taking the covariances into account [42]. To incorporate the prior information beta distributions (a,b), modeled by beta buster (http://252s-weblive.vet.unimelb.edu.au:3838/users/epi/beta.buster/), were used. For the SICT test we assumed—being 95% sure—that the sensitivity is greater than 50% with a mode at 70% (a = 13,3221; b = 6,2809) and that the specificity is greater than 70% with a mode at 85% (a = 23.903, b = 5.042). Similarly, for the SICCT test we assumed that the sensitivity is greater than 45% with a mode at 65% (a = 12.1979, b = 7.0296) and the specificity is greater than 80% with a mode at 90% (a = 42.5732, b = 5.6192). The presence of conditional dependencies between tests was checked by assessing separately the impact of each covariance term compared to a covariance term set to 0 on the other estimates. Presence of conditional dependencies was assessed graphically (histograms). Additionally, to assess if higher-level conditional dependencies potentially affect the results, random effect models based on the model from Qu et al. 1996 were also explored using the R package randomLCA [43, 44].

Model selection was based on DIC (Deviance Information Criterion) with lower values indicating a better model fit. For a sensitivity analysis of the three-test dataset considering the covariances uninformative priors were used. The best fitting model of the four-test dataset was additionally run with a higher cut off of the Bovigam® assay (OD difference 0.2 instead of 0.1). Due to missing values for continuous Bovigam® assay results, only 171 animals could be included. The models were implemented in JAGS (Just Another Gibbs Sampler) version 3.4.0 for Markov Chain Monte Carlo Simulation (http://mcmc-jags.sourceforge.net/), the software R version 3.0.3 (https://www.r-project.org/) and the package coda [45]. The model code is given in the supplementary online material (S1 Text). For all models the first 20,000 iterations were discarded as burn-in and based on the next 200,000 iterations the posterior distributions of the unknown parameters were derived. Three chains were run from different starting points. Convergence was checked visually by inspecting the density plots of the three chains.

The positive and negative predictive values of the skin tests (standard and severe interpretation), Bovigam® assay (OD difference 0.1 and 0.2), PCR, necropsy and culture were derived based on the estimated prevalence and posteriors obtained from the different models.

Kappa test of agreement

Animal samples and testing. Blood was taken from 21 cows (Braunvieh breed) at six different time points from the *V. jugularis*. All animals belonged to one farm and were tested previously as positive or inconclusive with the SICT test. On two time points the blood was additionally taken from the *V. caudalis mediana* resp. *V. subcutanea abdominis*. Immediately after collection, the blood was sent to five laboratories, all over Germany. After arriving at the laboratories the blood was directly examined with the Bovigam® assay. Due to the fact that the laboratories were distributed all over Germany the time between blood collection and further examination was between 4 to 29 h with a median of 8.0 h. The samples were not blinded.

Statistical analysis. To determine if the laboratories were classifying approximately the same proportion of individuals as positive, first McNemar's test was applied for each given time point between all possible pairwise laboratory comparisons [46]. McNemar's test was performed with the software R version 3.0.3 (https://www.r-project.org/) with the package exact2x2 [47]. For the inter-laboratory agreement the time point with the best accordance of the proportion of positive test results was chosen to determine Cohen's kappa. Also the agreement between the test results of the Bovigam® assay from the differing localizations was estimated using McNemar's test and Cohen's kappa. Cohen's kappa was calculated online with Graphpad software (http://graphpad.com/quickcalcs/kappa2/).

Table 1. Prevalence and diagnostic test accuracies of different models considered from the dataset with 175 animals tested with SICT test, Bovigam® assay, culture [sp = 100%] and necropsy.

Model	Prevalence (95% CI)	SICT test (95% CI)		Bovigam® assay (95% CI)		Culture (95% CI)		Necropsy (95% CI)	
		se	sp	se	sp	se	sp	Se	sp
1	**7.7 (4.2–12.3)**	**70.3 (44.9–90.5)**	**75.8 (68.8–82.2)**	**95.7 (91.3–99.2)**	**6.9 (3.6–11.1)**	**88.9 (65.5–99.7)**	**fixed at 100**	**76.8 (51.6–94.4)**	**99.0 (96.8–100)**
2	7.7 (4.2–12.3)	70.1 (53.5–84.7)	77.1 (70.9–82.8)	95.8 (91.3–99.2)	6.9 (3.6–11.1)	88.9 (65.0–99.7)	fixed at 100	76.9 (51.7–94.4)	98.9 (96.8–99.9)
3	7.9 (4.3–12.6)	70.2 (45.1–90.4)	76.4 (69.7–82.6)	83.3 (74.2–93.5)	23.5 (17.4–30.3)	88.9 (64.8–99.7)	fixed at 100	77.0 (51.2–94.6)	98.9 (96.7–99.9)
4	7.9 (4.3–12.5)	98.2 (94.9–99.9)	4.1 (1.7–7.5)	95.7 (91.2–99.2)	6.9 (3.7–11.2)	87.5(62.8–99.5)	fixed at 100	77.1 (51.4–94.6)	99.1 (97.0–100)

Model 1: SICT test [standard interpretation, uninformative priors], Bovigam® assay [cut-off = 0.1], culture [sp = 100%], no covariances
Model 2: SICT test [standard interpretation, informative priors], Bovigam® assay [cut-off = 0.1], culture [sp = 100%], no covariances
Model 3: SICT test [standard interpretation, uninformative priors], Bovigam® assay [cut-off = 0.2], culture [sp = 100%], no covariances
Model 4: SICT test [severe interpretation, uninformative priors], Bovigam® assay [cut-off = 0.1], culture [sp = 100%] no covariances
se, sensitivity
sp, specificity

https://doi.org/10.1371/journal.pone.0179847.t001

Results

Bayesian latent class models

The raw data, comprising the dichotomized test results of the four-test and the three-test dataset, are presented in S1 and S2 Tables.

Four-test dataset. Posterior means and corresponding 95% credibility intervals resulting from Bayesian latent class models are shown in Table 1.

There was no evidence, based on DIC and visual inspection of covariance histograms that including any covariance term led to a better model fit (S3 Table and S1 Fig). Including covariance terms did also not alter the posterior means. Adding random effects to model higher level conditional dependencies did not improve model fit.

If informative instead of flat priors were used for SICT test the DIC decreased slightly (392.6 instead of 393.7) and the posterior means were only marginally affected. If a higher cut-off of 0.2 instead of 0.1 for the Bovigam® assay was applied, then the sensitivity of the Bovigam® assay decreased from 95.7% (91.3–99.2%) to 83.3% (74.2–93.5%) and the specificity increased from 6.9% (3.6–11.1%) to 23.5% (17.4–30.3%). The estimated posteriors of the other tests and the prevalence differed maximally around 0.4%. The dichotomized test results are presented in S4 Table. When interpreting the inconclusive test results of the SICT test as positive the specificity of the SICT test was extremely low with 4.1% (1.7–7.5%).

The positive and negative predictive values for the SICT test (standard and severe interpretation), Bovigam® assay (cut-off 0.1 and 0.2), necropsy and culture are presented in S5 Table.

Three-test dataset. The posterior sensitivities and specificities for the three-test dataset resulting from the Bayesian latent class models are presented in Table 2. With the incorporated informative priors the sensitivity of the SICCT test increased by 2%. The estimated test characteristics of the other tests were only marginally affected. With regard to the histograms (S2 Fig), the posteriors and the DIC (S6 Table) dependence between the sensitivity of the PCR and necropsy seemed to be the most likely. Within this model the sensitivities of the PCR and the necropsy decreased and the prevalence increased. The remaining posteriors range around the same values.

By running this model with flat priors the DIC increased to 754.8 instead of 750.7 and the sensitivities of PCR and necropsy decreased around 5.4% and 6.0%. The other estimated

parameters differed maximal around 2%. The density plots of the estimated probability distributions showed better convergence for the model with the SICCT test as standard interpretation. The specificity for the SICCT test was extremely low with 12.0% (8.7–15.8%) for the severe interpretation.

The positive and negative predictive values for the SICCT test (standard and severe interpretation), PCR and necropsy based on the estimated posteriors and prevalence of the three test data set can be found in S7 Table.

Inter- and intra-laboratory agreement

The raw data utilized for assessing agreement between the different laboratories and the different localizations are presented in S8 and S9 Tables. Based on McNemar's test to assess if the proportions of samples classified as positive differed significantly between the laboratories or the anatomical location, the time point with most non-significant tests was chosen (Table 3) [46]. Estimated *p*-values for McNemar's test ranged from 0.03 to 1.00. The inter-laboratory agreement between Laboratory 2 and 3 reached a Cohen's kappa value of 0.62 (95% confidence interval from 0.15 to 1.00). The other agreements constituted between -0.16 (95% confidence interval from -0.32 to -0.01) and 0.38 (95% confidence interval from 0.01 to 0.76).

For the agreement between the varying localizations nearly all McNemar's tests are non-significant (Table 4), thus indicating the proportion of positive test results did not significantly differ between the laboratories. One estimated *p*-value based on McNemar's test was 0.03, giving evidence that there is a disagreement between the two proportions of Bovigam® test results [46]. The best agreement was seen for Laboratory 3 by comparing the Bovigam® assay results between the blood of the *V. jugularis* and *V. subcutanea abdominis* (1.00). Also the agreement between the results of the *V. jugularis* and the *V. caudalis mediana* of this laboratory reached at least a Cohen's kappa value of 0.62 (95% confidence interval from 0.00 to 1.00). Laboratory 5 had a substantial agreement by the comparison between *V. jugularis* and *V. caudalis mediana*. All other agreements were below 0.54 indicating a poor to moderate agreement.

Discussion

Due to the detection of bovine tuberculosis at several occasions during regular abattoir meat inspections in the Allgäu region, a new tuberculosis control program was implemented in

Table 2. Prevalence and diagnostic test accuracies of different models considered from the dataset with 389 animals tested with SICCT test [standard interpretation], PCR and necropsy.

Model	Prevalence (95% CI)	SICCT test (95% CI)		PCR (95% CI)		Necropsy (95% CI)	
		se	sp	se	sp	se	sp
1	17.3 (13.5–21.5)	55.5 (43.3–67.7)	91.7 (88.3–94.6)	80.6 (69.1–90.6)	99.2 (97.6–100)	90.6 (80.6–98.0)	99.1 (97.2–100)
2	17.2 (13.4–21.4)	57.5 (46.5–68.1)	91.5 (88.4–94.2)	80.6 (69.1–90.6)	99.1 (97.5–100)	90.7 (80.7–98.0)	99.1 (97.2–100)
3	**19.8 (14.6–26.5)**	**57.8 (48.0–67.6)**	**92.8 (89.2–96.3)**	**70.6 (52.0–86.0)**	**99.0 (97.4–99.9)**	**78.4 (58.6–93.7)**	**98.9 (96.8–100)**
4	21.8 (15.1–30.9)	56.1 (46.3–66.2)	94.5 (89.7–99.5)	65.2 (44.6–84.5)	99.0 (97.4–99.9)	72.4 (49.8–92.5)	99.0 (96.9–100)
5	15.6 (11.4–20.2)	94.9 (89.5–98.8)	12.0 (8.7–15.8)	87.9 (73.0–99.4)	98.8 (96.9–100.0)	92.2 (81.3–99.6)	97.6 (94.6–99.9)

Model 1: SICCT test [standard interpretation, uninformative priors], no covariances

Model 2: SICCT test [standard interpretation, prior information of se and sp], no covariances

Model 3: SICCT test [standard interpretation, prior information of se and sp], covariance between sensitivity PCR and necropsy

Model 4: SICCT test [standard interpretation, uninformative priors], covariance between sensitivity PCR and necropsy

Model 5: SICCT test [severe interpretation, uninformative priors], no covariances

se, sensitivity

sp, specificity

https://doi.org/10.1371/journal.pone.0179847.t002

Table 3. Comparison of the Bovigam® assay test results from five different laboratories by the calculated *p*-value based on McNemar's test, Cohen's kappa values with the 95% confidence interval and the proportions of the test results of one given time point.

Comparison between:	*p*-value	Kappa	CI	Proportions of test results[a]		
				pos/pos	neg/neg	disconcordant
Lab 1 / Lab 2	**0.69**	**0.31**	-0.12 to 0.74	57%	14%	29%
Lab 1 / Lab 3	**0.38**	**0.30**	-0.15 to 0.74	63%	11%	26%
Lab 1 / Lab 4	**1.00**	**0.26**	-0.23 to 0.75	61%	11%	28%
Lab 1 / Lab 5	**0.75**	**0.00**	-0.42 to 0.42	38%	14%	48%
Lab 2 / Lab 3	**1.00**	**0.83**	0.50 to 1.00	79%	16%	5%
Lab 2 / Lab 4	**1.00**	**0.26**	-0.23 to 0.75	61%	11%	28%
Lab 2 / Lab 5	**0.22**	**0.38**	0.01 to 0.76	52%	19%	29%
Lab 3 / Lab 4	**1.00**	**0.09**	-0.42 to 0.61	63%	6%	31%
Lab 3 / Lab 5	**0.06**	**0.41**	0.05 to 0.77	58%	16%	26%
Lab 4 / Lab 5	**0.45**	**0.11**	-0.33 to 0.55	50%	11%	39%

Lab, Laboratory; neg, negative; pos, positive; CI, 95% confidence interval

[a]The number of analyzable test results ranged from 16–21 animals

https://doi.org/10.1371/journal.pone.0179847.t003

November 2012 in Germany. Within this testing regime the Bovigam® assay was performed for the first time as a field test in Germany. The test results gained from this control program were utilized to estimate the sensitivities and specificities of the different tests with a latent class analysis. This was especially of interest as the persons involved in this testing program recognized contradictory test outcomes for the Bovigam® assay which led to distrust and termination of the testing with the Bovigam® assay. These contradictory test outcomes seem to be corroborated by the raw data presented in S1 Table, where out of 175 tested animals 115 were diagnosed positive only with the Bovigam® assay.

The estimated test characteristics of the SICT and the SICCT test, the PCR, necropsy and culture are in line with already published data [9, 10, 13, 28, 31, 48, 49]. For the Bovigam® assay an extremely low specificity was estimated. In this population with an estimated true prevalence of 7.7 the positive predictive values of the Bovigam® assay would be 7.9% (OD difference of 0.1) respectively 8.54% (OD difference of 0.2).

This finding could be corroborated with additional intra- and inter-laboratory testing of agreement.

In this study no-gold-standard-models, relying on Bayesian latent class approaches, which are increasingly used in medical and veterinary sciences, were used [50, 51]. The specificity of the culture was set at 100%, as a positive result is assumed to be truly positive [7]. The best

Table 4. Comparison of the Bovigam® assay test results between differing localizations by calculating *p*-values based on McNemar's test and Cohen's kappa values.

		Lab 1	Lab 2	Lab 3	Lab 4	Lab 5
V.j. / V.s.a.	***p*-value**	**1.00**	**0.13**	**1.00**	**0.38**	**0.03**
	kappa	**0.29**	**0.41**	**1.00**	**0.46**	**0.37**
	CI	-0.12–0.72	0.00–0.86	1.00–1.00	0.03–0.83	0.08–0.74
V.j. / V.c.m.	***p*-value**	**0.45**	**0.63**	**0.50**	**1.00**	**0.25**
	kappa	**0.16**	**0.54**	**0.62**	**-0.03**	**0.70**
	CI	-0.22–0.59	0.03–0.90	0.00–1.00	-0.37–0.43	0.36–1.00

V.j.: *V. jugularis; V.s.a.*: *V. subcutanea abdominis; V.c.m.*: *V. caudalis mediana*; CI: 95% confidence interval

https://doi.org/10.1371/journal.pone.0179847.t004

fitting model was chosen by DIC. Additionally, as the DIC has its limitations, histograms and posteriors were evaluated [52–54]. In order to comply with good statistical practice all possible two way covariances were taken into account [55]. A conditional dependency could only be seen between the sensitivity of PCR and necropsy. These two examination methods do not rely on similar biological basics, but were related as sample selection and sample size for the PCR were associated with pathological examination. Due to the fact that both datasets were quite small, conditional dependence between other tests could not be excluded. Inclusion of prior information of the SICT test, for a sensitivity analysis of the four-test dataset, did not influence the posteriors. For the three-test dataset the DIC increases by running a sensitivity analysis with flat priors, indicating a worse model fit. As already shown by Álvarez et al. [10] the test characteristics of the skin tests alter with a severe interpretation insofar that the sensitivity increases and the specificity decreases. Within our data a strong shift to lower specificities could be seen for the severe interpretation of the skin tests. This outcome seems to be data driven, as in both datasets most of the animals had an inconclusive skin test result. Therefore and because of the poorer convergence for the models with severe interpretation, which could be due to the small amount of true positive test results, the focus was set on the skin tests standard interpretation.

For the skin tests, PCR, necropsy and culture, the estimated sensitivities and specificities are in accordance of test characteristics from other publications [9, 10, 13, 28, 31, 48, 49]. The wide credibility intervals for the sensitivities (19.6 to 45.6) could be explained by the small data pool of true positive animals. With regard to the estimated test characteristics of the skin tests it has to be considered that these could have been affected by the performance of the skin tests [56]. The test characteristics of the pathological examination were within both datasets 76.8% (51.6–94.4%) and 78.4% (58.6–93.7%), respectively, for the sensitivity and around 99.0% (96.8–100%) for the specificity. The fact that the pathological examination was done in different localizations from different persons as well as the small data pool of true positive animals explains the wide credibility interval for the sensitivity. This spectrum bias appears to be present in both subpopulations. Within our estimated test characteristics the SICCT test is less sensitive although more specific than the SICT test. PCR and necropsy are less sensitive than culture. Therefore, culture is still an essential diagnostic tool.

In the literature the test characteristics of the Bovigam® assay are stated as between 66.9–100% for sensitivity and 70–99.6% for specificity [9, 12, 49]. We estimated a quite high sensitivity of 95.7% (91.3–99.2%), but an extremely low specificity of 6.9% (3.6–11.1%). This stands in line with the experience of the persons involved in the bovine TB testing. With setting the cut-off higher an increase in the specificity was expected, as already reported by others [57, 58]. Indeed, the specificity raised to 23.5% (17.4–30.3%) thereby the sensitivity decreased to 83.3% (74.2–93.5%), This shows again that the model itself is robust. To our knowledge such low specificities were never recognized before for the Bovigam® assay. Although it was already stated that the Bovigam® assay is more sensitive, but less specific than the SICCT test [59]. And it was shown that fewer than 20% of the animals tested positive in the Bovigam® assay were also positive in culture or pathology [60]. Van Dijk [61] showed that the Bovigam® assay is likely to have false positive results and this in a higher amount than the SICCT test. With a decrease of the prevalence the amount of false positive test results even increases [61].

Among the influential factors discussed in literature a previously performed skin test is discussed to have an effect on the specificity of the Bovigam® assay. Within our study the SICT test was performed two to 28 (mean of 8.45 days) days prior to the Bovigam® assay. Several studies discuss the effect of a previous skin test (either CFT test or SICCT test) towards the IFN-γ production in natural or experimental infected cattle. Whereas the CFT test leads to a clear increase of IFN-γ production, this influence is not obvious after the SICCT test [62]. The

previously performed skin test in this study may have had an impact on the estimated specificity within the examined subpopulation. A genetic influence and an association between the breed and the outcome of the SICCT test were reported by Amos et al. [63]. This was not seen for the Bovigam® assay [64]. An influence of breed and genetics might be present in the Allgäu region, but further investigations have to be made to confirm this. The Bovigam® assay was only carried out within the regions Ober- and Ostallgäu during November 2012 until March 2013. This regional and seasonal limitation could have had an impact on the high amount of false positive test results [64, 65]. The correlation between season and occurrence of saprophytic mycobacteria might be associated with this [66, 67]. Moreover, infections with *Mycobacterium avium* subspecies *paratuberculosis* (MAP) may lead to false positive results for the Bovigam® assay [68]. Since the tested animals have not been examined for a concurrent MAP infection this impact could not be excluded. Furthermore, an infection with *Fasciola hepatica* is also reported to influence the IFN-γ response. Although this is until now only stated for the skin test and in context of false negative test results [69]. The specificity of the Bovigam® assay tests varies also with the concentration and potency of PPDs [70], which can differ remarkably [71, 72]. These influences might explain to some extend the estimated low specificities of the Bovigam® assay. Besides, the fact that bovine tuberculosis, in the regions Ober- and Ostallgäu was caused by *Mycobacterium caprae* may have influenced the Bovigam® assay results, too, as bovine tuberculosis in other regions worldwide is predominantly caused by *Mycobacterium bovis*. However, the low inter- and intralaboratory agreements between the Bovigam® assay outcomes could not be fully explained by this. The transportation time as well as the experience seems to influence the Bovigam® assay test outcomes, as between the laboratories with the shortest transportation time (Laboratory 3, data not shown) and the most experience (Laboratory 2 and 3, data not shown) the best, but still only substantial agreement was estimated. By comparing the intralaboratory agreement between the different laboratories again the laboratory with the most experience and the shortest transportation time (Laboratory 3) had the best agreement between the results of the blood taken from the *V*. *jugularis* and the *V*. *subcutanea abdominis*. A longer storage or transportation of the blood samples might lead to a decrease in the mean OD or the IFN-γ production [73–75]. With regard to the sensitivity and specificity of the Bovigam® assay several studies state that blood could also be processed 24 h later without statistical significant changes [76, 77]. However, Laboratory 3 reached also only a substantial agreement of 0.62 between the Bovigam® assay test results of the *V*. *jugularis* and the *V*. *caudalis mediana*. As for the intralaboratory agreement only the localization of the blood collection altered, much better accordance between the Bovigam® assay test results were expected, as blood taken from differing localizations should not differ [78, 79]. But the smaller diameter of the tail vain could lead to more damage and therefore micro-clotting, resulting in captured lymphocytes and therefore lower IFN-γ release [80]. Despite, there are conflicting views if an equal distribution of all lymphocyte subpopulations all over the body can be assumed in general. Regarding this, it must be taken into account that a blood sample can only give a snapshot. Although all five laboratories were officially approved none of them reported good concordance for the Bovigam® assay test results. It seems that the Bovigam® assay is a diagnostic tool with some disadvantages. Many influences including external factors (MAP, saprophytic mycobacteria, previous skin test and genetic components) and factors directly connected with the test performance, as the concentration of the PPDs, transportation time of the blood, localization of blood collection and also the experience of the laboratories might lead to differing test results. A higher specificity of the Bovigam® assay, especially in low prevalence herds and animals having a co-infection with MAP, can be achieved by using the proteins ESAT6 and CFP10 instead of PPDa and PPDb [68, 81]. Also working out and evaluating an individual test performance (proteins, protein concentration, cut-off etc.) for

each Bovigam® assay application as a field test might lead to better test characteristics [64, 70]. This could be demonstrated in this study in so far as the specificity increased by setting the cut-off to 0.2.

To our knowledge the use of a latent class analysis for the estimation of test characteristics for bTB diagnostic tests was never done before in Germany. An important strength of this study is that the data were gained from surveillance and therefore originates from a special epidemiologic situation. But this means also that only a subpopulation was tested and the animals were not chosen randomly. According to this background information our findings cannot be generalized. Additionally a new version of the Bovigam® assay has been developed since 2013 to which our findings cannot be transferred [82].

Conclusion

With this latent class analysis the test characteristics of different diagnostic tests used in the current bovine TB outbreak in Southern Germany could be estimated. Within this study an extremely low specificity and a low inter- and intralaboratory agreement were estimated for the Bovigam® assay. These findings might be due to influences affecting the environment or the immune system of the cow. Also factors that are associated with the testing procedure and the laboratories chosen might have had an effect. Therefore, the change during the testing regime towards SICCT test as only ante-mortem test was correct and founded. Despite the fact that the Bovigam® assay has been further advanced, a previous test evaluation prior to future surveillance programs is highly recommended. The estimated test characteristics for the other tests were in an acceptable range.

Supporting information

S1 Text. Bayesian latent-class model code for four diagnostic tests.
(DOCX)

S1 Table. Number of test result combinations in the four-test dataset (n = 175), the inconclusive test results of the SICT test once considered as negative (standard interpretation) and once as positive (severe interpretation). [b] cut-off: 0.1.
(DOCX)

S2 Table. Number of test result combinations in the three-test dataset (n = 389), the inconclusive test results of the SICCT test once considered as negative (standard interpretation) and once as positive (severe interpretation).
(DOCX)

S3 Table. DIC, prevalence and diagnostic test accuracies of different models, without and with covariances of the sensitivities between the different tests, considered from the dataset (n = 175) tested with SICT test [standard interpretation, uninformative priors], Bovigam® assay [cut-off = 0.1], culture [sp = 100%] and necropsy. Model 1: no covariances; Model 2: covariance sensitivity SICT test, Bovigam® assay; Model 3: covariance sensitivity SICT test, culture; Model 4: covariance sensitivity SICT test, pathology; Model 5: covariance sensitivity Bovigam® assay, culture; Model 6: covariance sensitivity Bovigam® assay, pathology; Model 7: covariance sensitivity culture, pathology; CI, credibility interval; se, sensitivity; sp, specificity.
(DOCX)

S4 Table. Dichotomized test results of the Bovigam® assay for two different cut-offs.
(DOCX)

S5 Table. Positive and negative predictive values of the SICT test, Bovigam® assay, culture [sp = 100%] and necropsy calculated from the prevalence and diagnostic test accuracies obtained from the models of Table 1. PPV, positive predictive value; NPV, negative predictive value.
(DOCX)

S6 Table. DIC, prevalence and diagnostic test accuracies of different models, without and with covariances of the sensitivities and specificities between the different tests, considered from the dataset (n = 389) tested with SICCT test [standard interpretation; prior information], PCR and necropsy. Model 1: no covariances; Model 2: covariance sensitivity SICCT test, PCR; Model 3: covariance sensitivity SICCT test, pathology; Model 4: covariance sensitivity PCR, pathology; Model 5: covariance specificity SICCT test, PCR; Model 6: covariance specificity SICCT test, pathology; Model 7: covariance specificity PCR, pathology; CI, credibility interval; se, sensitivity; sp, specificity.
(DOCX)

S7 Table. Positive and negative predictive values of the SICCT test, PCR and necropsy calculated from the prevalence and diagnostic test accuracies obtained from the models of Table 2. PPV, positive predictive value; NPV, negative predictive value.
(DOCX)

S8 Table. Test results of the Bovigam® assay from five officially approved laboratories; the results of the time point with the best accordance of the proportion of positive test results is shown. Pos, positive; neg, negative; n.a., not analyzable.
(DOCX)

S9 Table. Test results of the Bovigam® assay from five officially approved laboratories. The assayed blood was taken at two time points respectively from two differing localizations. *V.j.*, *V. jugularis; V.s.a.*, *V. subcutanea abdominis; V.c.m.*, *V. caudalis mediana*; pos, positive; neg, negative;?, inconclusive; n.a., not analyzable.
(DOCX)

S1 Fig. Histograms from the covariances of the sensitivities between the different tests, considered from the four-test dataset (n = 175) tested with SICT test [standard interpretation; no prior information], Bovigam® assay [cut–off = 0.1], culture [sp = 100%] and necropsy.
(PDF)

S2 Fig. Histograms from the covariances of the sensitivities and specificities between the different tests, considered from the three-test dataset (n = 389) tested with SICCT test [standard interpretation; prior information], PCR and necropsy.
(PDF)

Acknowledgments

We gratefully acknowledge Dr. Johann Mages as well as Dr. Franz Götz and all official and field veterinarians involved for their contribution in gaining and collecting samples and data. Furthermore, we want to thank Prof. Dr. Dr. Mathias Büttner for his support.

Author Contributions

Conceptualization: GKS RKS SH.

Data curation: AG DD VBP SH.

Formal analysis: VBP SH AG.

Funding acquisition: GKS.

Investigation: PZ SH AHM RKS VBP.

Methodology: SH VBP.

Project administration: GKS SH VBP.

Resources: GKS CSL.

Software: SH.

Supervision: GKS SH.

Validation: SH RKS VBP.

Visualization: VBP SH.

Writing – original draft: VBP SH.

Writing – review & editing: SH GKS PZ SH AG RKS.

References

1. Bovine tuberculosis (*Mycobacterium bovis* and *Mycobacterium caprae*). 2015 28.05.2015. In: Official Collection of Methods [Internet]. Jena: Friedrich-Loeffler-Institute [1–22]. Available from: https://www.fli.de/de/publikationen/amtliche-methodensammlung/.
2. Sattelmair H. Die Tuberkulose des Rindes-ein Beitrag zur Geschichte der Haustierkrankheiten. Berlin: Freie Universität Berlin, Germany; 2005.
3. Livingstone PG, Hancox N, Nugent G, de Lisle GW. Toward eradication: the effect of Mycobacterium bovis infection in wildlife on the evolution and future direction of bovine tuberculosis management in New Zealand. New Zealand veterinary journal. 2014; 63(sup1):4–18. https://doi.org/10.1080/00480169.2014.971082 PMID: 25273888
4. Miller RS, Farnsworth ML, Malmberg JL. Diseases at the livestock-wildlife interface: status, challenges, and opportunities in the United States. Preventive veterinary medicine. 2013; 110(2):119–32. https://doi.org/10.1016/j.prevetmed.2012.11.021 PMID: 23254245
5. Robinson PA. A history of bovine tuberculosis eradication policy in Northern Ireland. Epidemiology and Infection. 2015:1–14. https://doi.org/10.1017/S0950268815000291 PMID: 25778830
6. Zinsstag J, Schelling E, Roth F, Kazwala R, Thoen CO, Steele JH, et al. Economics of Bovine Tuberculosis. Mycobacterium Bovis Infection in Animals and Humans: Blackwell Publishing Ltd; 2008. p. 68–83.
7. Cousins DV. Bovine tuberculosis. 2009. In: Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2014 [Internet]. Paris: World Organisation for Animal Health; [1–16]. Available from: http://www.oie.int/international-standard-setting/terrestrial-manual/access-online/.
8. Monaghan ML, Doherty ML, Collins JD, Kazda JF, Quinn PJ. The tuberculin test. Veterinary Microbiology. 1994; 40(1–2):111–24. https://doi.org/10.1016/0378-1135(94)90050-7 PMID: 8073619
9. de la Rua-Domenech R, Goodchild AT, Vordermeier HM, Hewinson RG, Christiansen KH, Clifton-Hadley RS. Ante mortem diagnosis of tuberculosis in cattle: a review of the tuberculin tests, gamma-interferon assay and other ancillary diagnostic techniques. Res Vet Sci. 2006; 81(2):190–210. Epub 2006/03/04. https://doi.org/10.1016/j.rvsc.2005.11.005 PMID: 16513150.
10. Álvarez J, Perez A, Bezos J, Marqués S, Grau A, Saez JL, et al. Evaluation of the sensitivity and specificity of bovine tuberculosis diagnostic tests in naturally infected cattle herds using a Bayesian approach. Veterinary Microbiology. 2012; 155(1):38–43. https://doi.org/10.1016/j.vetmic.2011.07.034 PMID: 21890284
11. Wood PR, Jones SL. BOVIGAM: an in vitro cellular diagnostic test for bovine tuberculosis. Tuberculosis (Edinburgh, Scotland). 2001; 81(1):147–55. https://doi.org/10.1054/tube.2000.0272 PubMed Central PMCID: PMC11463236. PMID: 11463236

12. Bezos J, Casal C, Romero B, Schroeder B, Hardegger R, Raeber AJ, et al. Current ante-mortem techniques for diagnosis of bovine tuberculosis. Research in Veterinary Science. 2014; 97, Supplement: S44–S52. https://doi.org/10.1016/j.rvsc.2014.04.002
13. Courcoul A, Moyen JL, Brugere L, Faye S, Henault S, Gares H, et al. Estimation of sensitivity and specificity of bacteriology, histopathology and PCR for the confirmatory diagnosis of bovine tuberculosis using latent class analysis. PLoS One. 2014; 9(3):e90334. Epub 2014/03/15. https://doi.org/10.1371/journal.pone.0090334 PMID: 24625670; PubMed Central PMCID: PMCPmc3953111.
14. Corner LA. Post mortem diagnosis of Mycobacterium bovis infection in cattle. Veterinary Microbiology. 1994; 40(1–2):53–63. https://doi.org/10.1016/0378-1135(94)90046-9 PMID: 8073629
15. Commission Regulation (EC) No 1226/2002 of 8 July 2002 amending Annex B to Council Directive 64/432/EEC, (09/07/2002, 2002).
16. Hunermund G, Kropp R. The battle against cattle tuberculosis in Germany. Pneumologie (Stuttgart, Germany). 2006; 60(12). https://doi.org/10.1055/s-2006-944284 PMID: 17163317
17. Official Journal of the European Union, Commission implementing decision of 12 October 2011 amending Decision 2003/467/EC as regards the declaration of Latvia as officially tuberculosis-free Member State and the declaration of certain administrative regions in Portugal as officially enzootic-bovine-leukosis-free regions (notified under document C(2011) 7186), Pub. L. No. OJ L 268(13.10.2011, 2011).
18. Richtlinie 97/12/EG des Rates vom 17. März 1997 zur Änderung und Aktualisierung der Richtlinie 64/432/EWG zur Regelung viehseuchenrechtlicher Fragen beim innergemeinschaftlichen Handelsverkehr mit Rindern und Schweinen, (25.04.1997, 1997).
19. Köhler H, Moser I, Menge C, editors. Tuberkulose des Rindes: wieder ein Thema?! Infektionskrankheiten des Rindes–Neues und Wichtiges für die tierärztliche Praxis; 2012; Stiftung Tierärztliche Hochschule Hannover Akademie für Tiergesundheit e. V.
20. Rolle M, Mayr A, Selbitz H-J, Truyen U, Valentin-Weigand P. Tiermedizinische Mikrobiologie, Infektions und Seuchenlehre. Stuttgart: Enke Verlag; 2011.
21. Moser I, Köhler H, Menge C. Die Tuberkulose des Rindes– überraschend wieder oder immer noch präsent? Tierärztliche Praxis Großtiere. 2014; 42(4):240–9.
22. Heigl F. Rinder-und Rotwild-Tuberkulose im Oberallgäu, Eine aktuelle Übersicht. 2013.
23. Verordnung zum Schutz gegen die Tuberkulose des Rindes, (12.07.2013, 2013).
24. Zellner G. Untersuchungsprogramm "Rindertuberkulose in den Landkreisen der Alpenkette". In: Gesundheit BSfUu, editor. München2013.
25. Corner LA, Melville L, McCubbin K, Small KJ, McCormick BS, Wood PR, et al. Efficiency of inspection procedures for the detection of tuberculous lesions in cattle. Australian veterinary journal. 1990; 67(11):389–92. https://doi.org/10.1111/j.1751-0813.1990.tb03020.x PMID: 2085291
26. Norby B, Bartlett PC, Fitzgerald SD, Granger LM, Bruning-Fann CS, Whipple DL, et al. The sensitivity of gross necropsy, caudal fold and comparative cervical tests for the diagnosis of bovine tuberculosis. Journal of Veterinary Diagnostic Investigation. 2004; 16(2):126–31. https://doi.org/10.1177/104063870401600206 PMID: 15053363
27. Szmaragd C, Green LE, Medley GF, Browne WJ. Impact of Imperfect Test Sensitivity on Determining Risk Factors: The Case of Bovine Tuberculosis. PLoS ONE. 2012; 7(8):e43116. https://doi.org/10.1371/journal.pone.0043116 PMID: 22912804
28. Gerstmair E-M. Validierung molekularbiologischer und immunologischer Nachweisverfahren für die Tuberkulose bei Rindern und Tuberkulosemonitoring beim Rotwild. München: Ludwig-Maximians Universität München; 2011.
29. Buddle BM, McCarthy AR, Ryan TJ, Pollock JM, Vordermeier HM, Hewinson RG, et al. Use of mycobacterial peptides and recombinant proteins for the diagnosis of bovine tuberculosis in skin test-positive cattle. The Veterinary record. 2003; 153(20):615–20. Epub 2003/12/05. PMID: 14653340.
30. Quirin R, Rasolofo V, Andriambololona R, Ramboasolo A. Validity of intradermal tuberculin testing for the screening of bovine tuberculosis in Madagascar. The Onderstepoort journal of veterinary research. 2001; 68(3):231. PMID: 11769356
31. Hartnack S, Torgerson P. The accuracy of the Single Intradermal Comparative Skin Test for the diagnosis of Bovine Tuberculosis estimated from a systematic literature search. J Mycobac Dis. 2012; 2(120):2161–1068.1000120.
32. Hui SL, Walter SD. Estimating the Error Rates of Diagnostic Tests. Biometrics. 1980; 36(1):167–71. https://doi.org/10.2307/2530508 PMID: 7370371
33. Hui SL, Zhou XH. Evaluation of diagnostic tests without gold standards. Statistical Methods in Medical Research. 1998; 7(4):354–70. https://doi.org/10.1177/096228029800700404 PMID: 9871952

34. Bermingham ML, Handel IG, Glass EJ, Woolliams JA, Bronsvoort BMDC, McBride SH, et al. Hui and Walter's latent-class model extended to estimate diagnostic test properties from surveillance data: A latent model for latent data. Scientific Reports. 2015; 5. https://doi.org/10.1038/srep11861 PMID: 26148538

35. Walter SD, Irwig LM. Estimation of test error rates, disease prevalence and relative risk from misclassified data: a review. Journal of Clinical Epidemiology. 1988; 41(9):923–37. https://doi.org/10.1016/0895-4356(88)90110-2 PMID: 3054000

36. Standard Operating Procedure for OIE Registration of Diagnostic Kits, Guide and Administrative Forms Paris: World Organisation for Animal Health (OIE); 2012. Available from: http://www.oie.int/doc/ged/D12069.PDF.

37. Directive 64/432/EEC on animal health problems affecting intra-EU trade in bovine animals and swine, (1965).

38. Council Directive 80/219/EEC of 22 January 1980 amending Directive 64/432/EEC as regards tuberulosis and brucellosis (1980).

39. Council Directive 97/12/EC of 17 March 1997 amending and updating Directive 64/432/EEC on health problems affecting intra-Community trade in bovine animals and swine, (1997).

40. Rodriguez JG, Mejia GA, Del Portillo P, Patarroyo ME, Murillo LA. Species-specific identification of Mycobacterium bovis by PCR. Microbiology. 1995; 141(9):2131–8. https://doi.org/10.1099/13500872-141-9-2131 PMID: 7496524

41. Taylor GM, Worth DR, Palmer S, Jahans K, Hewinson RG. Rapid detection of Mycobacterium bovis DNA in cattle lymph nodes with visible lesions using PCR. BMC Veterinary Research. 2007; 3:12–. https://doi.org/10.1186/1746-6148-3-12 PMID: 17567891

42. Berkvens D, Speybroeck N, Praet N, Adel A, Lesaffre E. Estimating Disease Prevalence in a Bayesian Framework Using Probabilistic Constraints. Epidemiology. 2006; 17(2):145–53 https://doi.org/10.1097/01.ede.0000198422.64801.8d PMID: 16477254

43. Qu Y, Tan M, Kutner MH. Random Effects Models in Latent Class Analysis for Evaluating Accuracy of Diagnostic Tests. Biometrics. 1996; 52(3):797–810. https://doi.org/10.2307/2533043 PMID: 8805757

44. Beath K. randomLCA: Random Effects Latent Class Analysis. R package version 1.0–11 ed2017.

45. Plummer M, Best N, Cowles K, Vines K. CODA: Convergence Diagnosis and Output Analysis for MCMC. R News. 2006; 6(1):7–11.

46. Dohoo I, Martin W, Stryhn H. Veterinary epidemiologic research. Charlottetown, Canada2009.

47. Fay MP. Two-sided Exact Tests and Matching Confidence Intervals. R Journal. 2010; 2(1):53–8.

48. Koöhler H, Hoffmann B, Moser I, Moöbius P. Novel probe-based real-time PCR for rapid diagnosis of bovine Tuberculosis in tissue samples with suspect lesions. 16th International Symposium of the World Association of Veterinary Laboratory Diagnosticians; Berlin2013.

49. Clegg TA, Duignan A, Whelan C, Gormley E, Good M, Clarke J, et al. Using latent class analysis to estimate the test characteristics of the γ-interferon test, the single intradermal comparative tuberculin test and a multiplex immunoassay under Irish conditions. Veterinary Microbiology. 2011; 151(1–2):68–76. https://doi.org/10.1016/j.vetmic.2011.02.027 PMID: 21470800

50. Lewis F, Torgerson P. A tutorial in estimating the prevalence of disease in humans and animals in the absence of a gold standard diagnostic. Emerging Themes in Epidemiology. 2012; 9(1):9. https://doi.org/10.1186/1742-7622-9-9 PMID: 23270542

51. van Smeden M, Naaktgeboren CA, Reitsma JB, Moons KGM, de Groot JAH. Latent Class Models in Diagnostic Studies When There is No Reference Standard—A Systematic Review. American Journal of Epidemiology. 2013. https://doi.org/10.1093/aje/kwt286 PMID: 24272278

52. Ward EJ. A review and comparison of four commonly used Bayesian and maximum likelihood model selection tools. Ecological Modelling. 2008; 211(1–2):1–10. https://doi.org/10.1016/j.ecolmodel.2007.10.030

53. Spiegelhalter DJ, Best NG, Carlin BP, van der Linde A. The deviance information criterion: 12 years on. Journal of the Royal Statistical Society: Series B (Statistical Methodology). 2014; 76(3):485–93. https://doi.org/10.1111/rssb.12062

54. Spiegelhalter DJ, Best NG, Carlin BP, Van Der Linde A. Bayesian measures of model complexity and fit. Journal of the Royal Statistical Society: Series B (Statistical Methodology). 2002; 64(4):583–639. https://doi.org/10.1111/1467-9868.00353

55. Vacek PM. The Effect of Conditional Dependence on the Evaluation of Diagnostic Tests. Biometrics. 1985; 41(4):959–68. https://doi.org/10.2307/2530967 PMID: 3830260

56. Pucken VB, Götz F, Mansfeld R, Moder S, Sauter-Louis C, Straubinger RK, et al. Der Tuberkulin-Hauttest: Literatur, Richtlinie und Umsetzung in der Praxis. Tierärztliche Praxis Großtiere. 2015;(2015-07-02 00:00:00). https://doi.org/10.15653/TPG-141145

57. Faye S, Moyen J-L, Gares H, Benet J-J, Garin-Bastuji B, Boschiroli M-L. Determination of decisional cut-off values for the optimal diagnosis of bovine tuberculosis with a modified IFNγ assay (Bovigam®) in a low prevalence area in France. Veterinary Microbiology. 2011; 151(1–2):60–7. https://doi.org/10.1016/j.vetmic.2011.02.026 PMID: 21420258

58. Antognoli MC, Remmenga MD, Bengtson SD, Clark HJ, Orloski KA, Gustafson LL, et al. Analysis of the diagnostic accuracy of the gamma interferon assay for detection of bovine tuberculosis in US herds. Preventive veterinary medicine. 2011; 101(1):35–41.

59. Vordermeier M, Ewer K. Specificity Trial of the BOVIGAM® IFN-Gamma Test in GB Cattle In: TB Research Group VLA, editor. New Haw, Addlestone,: DEFRA; 2006.

60. anonym. VLA Surveillance Project SB4008: Laboratory Testing and Epidemiology Support for the National Gamma Interferon Field Trial, Part 1—Report on the 195 herds recruited into the trial DEFRA, 2006.

61. van Dijk J. Towards Risk-Based Test Protocols: Estimating the Contribution of Intensive Testing to the UK Bovine Tuberculosis Problem. PLoS ONE. 2013; 8(5):e63961. https://doi.org/10.1371/journal.pone.0063961 PMID: 23717517

62. Schiller I, Vordermeier HM, Waters WR, Whelan AO, Coad M, Gormley E, et al. Bovine tuberculosis: effect of the tuberculin skin test on in vitro interferon gamma responses. Veterinary immunology and immunopathology. 2010; 136(1–2):1–11. Epub 2010/03/12. https://doi.org/10.1016/j.vetimm.2010.02.007 PMID: 20219253.

63. Amos W, Brooks-Pollock E, Blackwell R, Driscoll E, Nelson-Flower M, Conlan AJK. Genetic Predisposition to Pass the Standard SICCT Test for Bovine Tuberculosis in British Cattle. PLoS ONE. 2013; 8(3):e58245. https://doi.org/10.1371/journal.pone.0058245 PMID: 23554880

64. Gormley E, Doyle M, Duignan A, Good M, More SJ, Clegg TA. Identification of risk factors associated with disclosure of false positive bovine tuberculosis reactors using the gamma-interferon (IFNγ) assay. Veterinary research. 2013; 44(1):117. https://doi.org/10.1186/1297-9716-44-117 PMID: 24308747

65. Lauzi S, Pasotto D, Amadori M, Archetti IL, Poli G, Bonizzi L. Evaluation of the specificity of the gamma-interferon test in Italian bovine tuberculosis-free herds. Veterinary journal (London, England: 1997). 2000; 160(1):17–24. Epub 2000/08/19. https://doi.org/10.1053/tvjl.1999.0444 PMID: 10950131.

66. Donoghue H, Overend E, Stanford J. A longitudinal study of environmental mycobacteria on a farm in south-west England. Journal of applied microbiology. 1997; 82(1):57–67. PMID: 9113878

67. Cooney R, Kazda J, Quinn J, Cook B, Muller K, Monaghan M. Environmental mycobacteria in Ireland as a source of non-specific sensitisation to tuberculins. Ir Vet J. 1997; 50:370–3.

68. Barry C, Corbett D, Bakker D, Andersen P, McNair J, Strain S. The effect of Mycobacterium avium complex infections on routine Mycobacterium bovis diagnostic tests. Veterinary medicine international. 2011; 2011:7 pages. https://doi.org/10.4061/2011/145092 PMID: 21772961

69. Claridge J, Diggle P, McCann CM, Mulcahy G, Flynn R, McNair J, et al. Fasciola hepatica is associated with the failure to detect bovine tuberculosis in dairy cattle. Nature Communications. 2012; 3:853. https://doi.org/10.1038/ncomms1840 PMID: 22617293

70. Cagiola M, Feliziani F, Severi G, Pasquali P, Rutili D. Analysis of possible factors affecting the specificity of the gamma interferon test in tuberculosis-free cattle herds. Clinical and diagnostic laboratory immunology. 2004; 11(5):952–6. Epub 2004/09/11. https://doi.org/10.1128/CDLI.11.5.952-956.2004 PMID: 15358658; PubMed Central PMCID: PMCPMC515264.

71. Good M, Clegg TA, Murphy F, More SJ. The comparative performance of the single intradermal comparative tuberculin test in Irish cattle, using tuberculin PPD combinations from different manufacturers. Veterinary microbiology. 2011; 151(1):77–84.

72. Bakker D. Practicalities of the immune-based diagnostic assay for the control of bovine tuberculosis. VI International M Bovis conference; Cardiff2014.

73. Gormley E, Doyle MB, McGill K, Costello E, Good M, Collins JD. The effect of the tuberculin test and the consequences of a delay in blood culture on the sensitivity of a gamma-interferon assay for the detection of Mycobacterium bovis infection in cattle. Veterinary immunology and immunopathology. 2004; 102(4):413–20. Epub 2004/11/16. https://doi.org/10.1016/j.vetimm.2004.08.002 PMID: 15541794.

74. Schiller I, Waters WR, Vordermeier HM, Nonnecke B, Welsh M, Keck N, et al. Optimization of a whole-blood gamma interferon assay for detection of Mycobacterium bovis-infected cattle. Clinical and Vaccine Immunology. 2009; 16(8):1196–202. https://doi.org/10.1128/CVI.00150-09 PMID: 19571108

75. Coad M, Hewinson RG, Clifford D, Vordermeier HM, Whelan AO. Influence of skin testing and blood storage on interferon-gamma production in cattle affected naturally with Mycobacterium bovis. The Veterinary record. 2007; 160(19):660–2. Epub 2007/05/15. PMID: 17496274.

76. Lopes LB, Alves TM, Stynen APR, Mota PM, Leite RC, Lage AP. Parameter estimation and use of gamma interferon assay for the diagnosis of bovine tuberculosis in Brazil. Pesquisa Veterinária Brasileira. 2012; 32(4):279–83.

77. Ryan T, Buddle B, De Lisle G. An evaluation of the gamma interferon test for detecting bovine tuberculosis in cattle 8 to 28 days after tuberculin skin testing. Research in veterinary science. 2000; 69(1):57–61. https://doi.org/10.1053/rvsc.2000.0386 PMID: 10924395

78. Sears PM, Paape MJ, Pearson RE, Gwazdauskas FC. Comparison between tail vein and jugular vein cannulation in cattle. Journal of dairy science. 1978; 61(7):974–9. https://doi.org/10.3168/jds.S0022-0302(78)83676-5 PMID: 701538

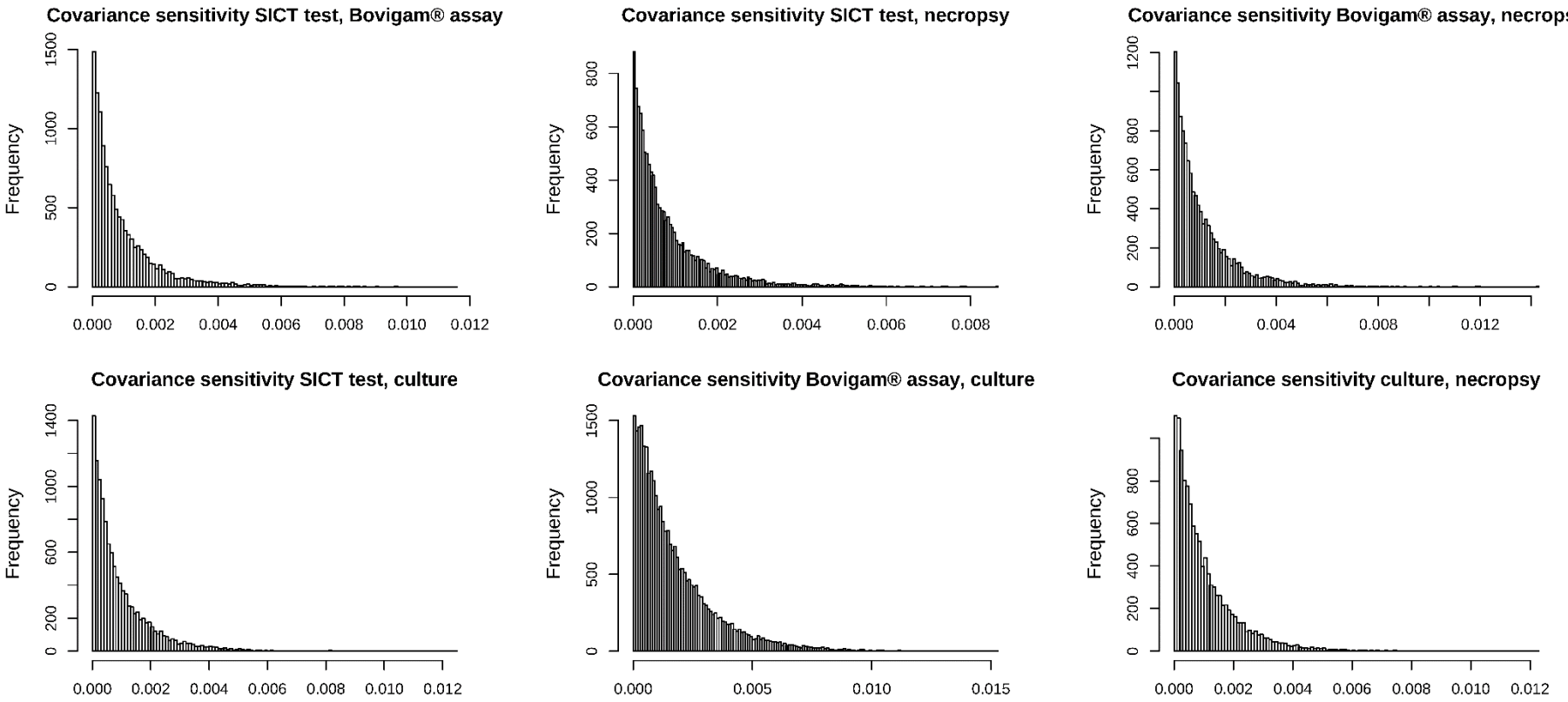
79. Rothel JS, Corner LA, Wood PR. Bovine tuberculosis: immunodiagnosis. 1993. In: Australian Standard Diagnostic Techniques for Animal Diseases [Internet]. East Melbourne, Vic: CSIRO. Available from: http://www.agriculture.gov.au/SiteCollectionDocuments/animal/ahl/ASDT-bovine_tuberculosis.pdf.

80. Böttcher J, Janowetz B, Moser H, Winter P, Alex M, Meier N. Diagnosis of BHV-1Infection in the presence of maternally derived antibodies. 1st Congress of the European Association of Veterinary Laboratory Diagnosticians; Lelystad2010.

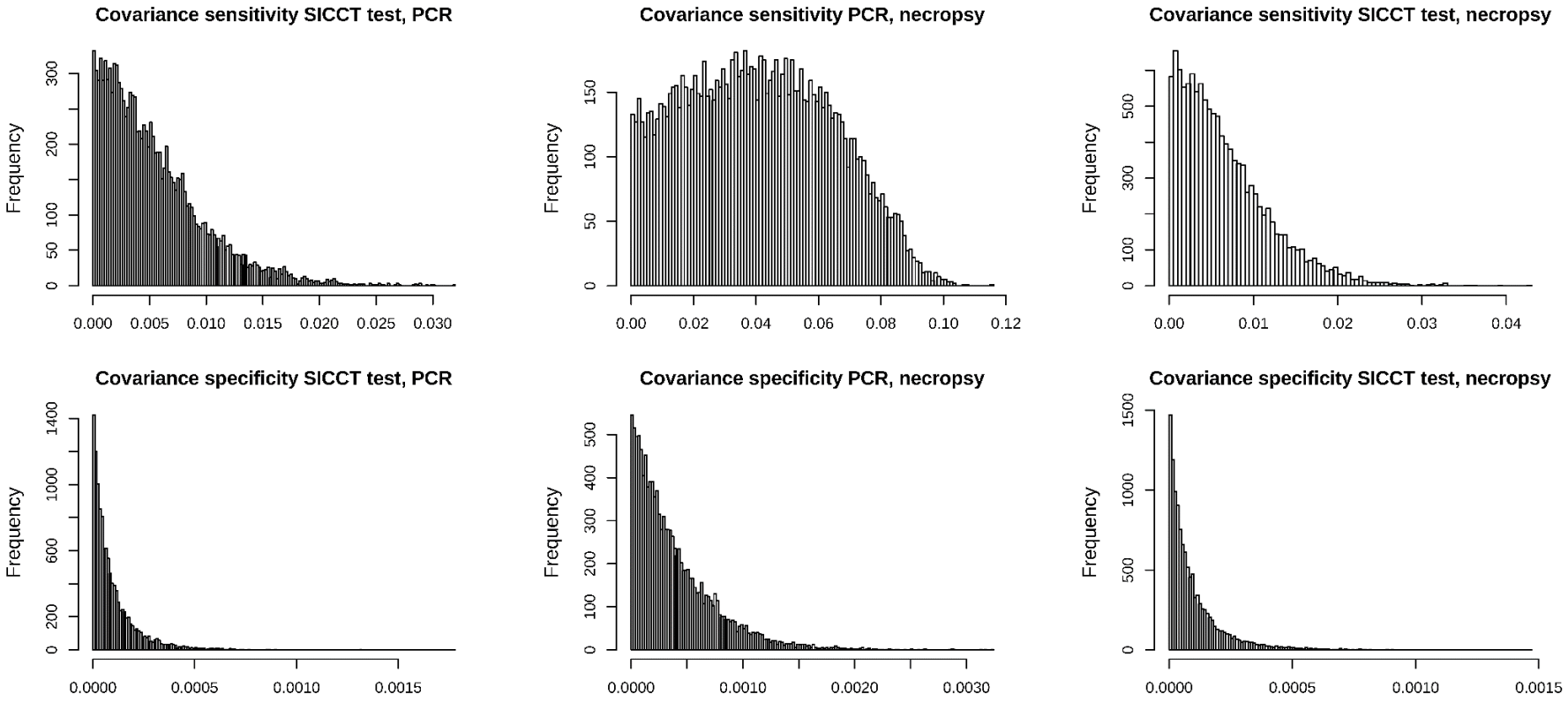
81. Aagaard C, Govaerts M, Meikle V, Gutiérrez-Pabello JA, McNair J, Andersen P, et al. Detection of bovine tuberculosis in herds with different disease prevalence and influence of paratuberculosis infection on PPDB and ESAT-6/CFP10 specificity. Preventive Veterinary Medicine. 2010; 96(3–4):161–9. https://doi.org/10.1016/j.prevetmed.2010.06.007 PMID: 20630607

82. BOVIGAM® 2G TB Kit 2016. Available from: https://www.thermofisher.com/order/catalog/product/63330?ICID=search-product.

S1 Fig: Histograms from the covariances of the sensitivities between the different tests, considered from the four-test dataset (n=175) tested with SICT test [standard interpretation; no prior information], Bovigam® assay [cut−off=0.1], culture [sp=100%] and necropsy



S2 Fig: Histograms from the covariances of the sensitivities and specificities between the different tests, considered from the three-test dataset (n=389) tested with SICCT test [standard interpretation; prior information], PCR and necropsy



S1 Text: Bayesian latent-class model code for four diagnostic tests

```
############################################################
##Definition of the variables in the model
############################################################

var p[N], q[N,8], pr[N], L[N],checks[N,16];

#N <- observations
# p <- individual samples
# q <- different combinations of test results
# pr <- prevalence
# s <- test sensitivities
# c <- test specificities
#  cs <- conditional dependency between tests sensitivities
#  cc <- conditional dependency between tests specificities
# mobokupa <- data set name


############################################################
## Modelling the different probabilities of combinations of tests results
############################################################

model {

  for(i in 1:N){

  q[i,1]<-pr[i]*(s1*s2*s3*s4+cs12+cs13+cs23+cs14+cs24+cs34)+(1-pr[i])*((1-c1)*(1-c2)*(1-
c3)*(1-c4)+cc12+cc13+cc23+cc14+cc24+cc34);
  q[i,2]<-pr[i]*(s1*s2*s3*(1-s4)+cs12+cs13+cs23-cs14-cs24-cs34)+(1-pr[i])*((1-c1)*(1-
c2)*(1-c3)*c4+cc12+cc13+cc23-cc14-cc24-cc34);
  q[i,3]<-pr[i]*(s1*s2*(1-s3)*s4+cs12-cs13-cs23+cs14+cs24-cs34)+(1-pr[i])*((1-c1)*(1-
c2)*c3*(1-c4)+cc12-cc13-cc23+cc14+cc24-cc34);
  q[i,4]<-pr[i]*(s1*s2*(1-s3)*(1-s4)+cs12+cs13-cs23-cs14-cs24+cs34)+(1-pr[i])*((1-c1)*(1-
c2)*c3*c4-cc12+cc13-cc23-cc14-cc24+cc34);
  q[i,5]<-pr[i]*(s1*(1-s2)*s3*s4-cs12+cs13-cs23+cs14-cs24+cs34)+(1-pr[i])*((1-c1)*c2*(1-
c3)*(1-c4)-cc12+cc13-cc23+cc14-cc24+cc34);
  q[i,6]<-pr[i]*(s1*(1-s2)*s3*(1-s4)-cs12+cs13-cs23-cs14+cs24-cs34)+(1-pr[i])*((1-
c1)*c2*(1-c3)*c4-cc12+cc13-cc23-cc14+cc24-cc34);
  q[i,7]<-pr[i]*(s1*(1-s2)*(1-s3)*s4-cs12-cs13+cs23+cs14-cs24-cs34)+(1-pr[i])*((1-
c1)*c2*c3*(1-c4)-cc12-cc13+cc23+cc14-cc24-cc34);
  q[i,8]<-pr[i]*(s1*(1-s2)*(1-s3)*(1-s4)-cs12-cs13+cs23-cs14+cs24+cs34)+(1-pr[i])*((1-
c1)*c2*c3*c4-cc12-cc13+cc23-cc14+cc24+cc34);
  q[i,9]<-pr[i]*((1-s1)*s2*s3*s4-cs12-cs13+cs23-cs14+cs24+cs34)+(1-pr[i])*(c1*(1-c2)*(1-
c3)*(1-c4)-cc12-cc13+cc23-cc14+cc24+cc34);
```

```
  q[i,10]<-pr[i]*((1-s1)*s2*s3*(1-s4)-cs12-cs13+cs23+cs14-cs24-cs34)+(1-pr[i])*(c1*(1-
c2)*(1-c3)*c4-cc12-cc13+cc23+cc14-cc24-cc34);
  q[i,11]<-pr[i]*((1-s1)*s2*(1-s3)*s4-cs12+cs13-cs23-cs14+cs24-cs34)+(1-pr[i])*(c1*(1-
c2)*c3*(1-c4)-cc12+cc13-cc23-cc14+cc24-cc34);
  q[i,12]<-pr[i]*((1-s1)*s2*(1-s3)*(1-s4)-cs12+cs13-cs23+cs14-cs24+cs34)+(1-pr[i])*(c1*(1-
c2)*c3*c4-cc12+cc13-cc23+cc14-cc24+cc34);
  q[i,13]<-pr[i]*((1-s1)*(1-s2)*s3*s4+cs12-cs13-cs23-cs14-cs24+cs34)+(1-pr[i])*(c1*c2*(1-
c3)*(1-c4)+cc12-cc13-cc23-cc14-cc24+cc34);
  q[i,14]<-pr[i]*((1-s1)*(1-s2)*s3*(1-s4)+cs12-cs13-cs23+cs14+cs24-cs34)+(1-
pr[i])*(c1*c2*(1-c3)*c4+cc12-cc13-cc23+cc14+cc24-cc34);
  q[i,15]<-pr[i]*((1-s1)*(1-s2)*(1-s3)*s4+cs12+cs13+cs23-cs14-cs24-cs34)+(1-
pr[i])*(c1*c2*c3*(1-c4)+cc12+cc13+cc23-cc14-cc24-cc34);
  q[i,16]<-pr[i]*((1-s1)*(1-s2)*(1-s3)*(1-s4)+cs12+cs13+cs23+cs14+cs24+cs34)+(1-
pr[i])*(c1*c2*c3*c4+cc12+cc13+cc23+cc14+cc24+cc34);

############################################################
## Check and correct potential errors of probabilities exceeding (0,1) bounds
############################################################

  checks[i,1]<- s1*s2*s3*s4+cs12+cs13+cs23+cs14+cs24+cs34;
  checks[i,2]<- (1-c1)*(1-c2)*(1-c3)*(1-c4)+cc12+cc13+cc23+cc14+cc24+cc34;
  checks[i,3]<- s1*s2*s3*(1-s4)+cs12+cs13+cs23-cs14-cs24-cs34;
  checks[i,4]<- (1-c1)*(1-c2)*(1-c3)*c4+cc12+cc13+cc23-cc14-cc24-cc34;
  checks[i,5]<- s1*s2*(1-s3)*s4+cs12-cs13-cs23+cs14+cs24-cs34;
  checks[i,6]<- (1-c1)*(1-c2)*c3*(1-c4)+cc12-cc13-cc23+cc14+cc24-cc34;
  checks[i,7]<- s1*s2*(1-s3)*(1-s4)+cs12+cs13-cs23-cs14-cs24+cs34;
  checks[i,8]<- (1-c1)*(1-c2)*c3*c4-cc12+cc13-cc23-cc14-cc24+cc34;
  checks[i,9]<- s1*(1-s2)*s3*s4-cs12+cs13-cs23+cs14-cs24+cs34;
  checks[i,10]<- (1-c1)*c2*(1-c3)*(1-c4)-cc12+cc13-cc23+cc14-cc24+cc34;
  checks[i,11]<- s1*(1-s2)*s3*(1-s4)-cs12+cs13-cs23-cs14+cs24-cs34;
  checks[i,12]<- (1-c1)*c2*(1-c3)*c4-cc12+cc13-cc23-cc14+cc24-cc34;
  checks[i,13]<- s1*(1-s2)*(1-s3)*s4-cs12-cs13+cs23+cs14-cs24-cs34;
  checks[i,14]<- (1-c1)*c2*c3*(1-c4)-cc12-cc13+cc23+cc14-cc24-cc34;
  checks[i,15]<- s1*(1-s2)*(1-s3)*(1-s4)-cs12-cs13+cs23-cs14+cs24+cs34;
  checks[i,16]<- (1-c1)*c2*c3*c4-cc12-cc13+cc23-cc14+cc24+cc34;
  checks[i,17]<- (1-s1)*s2*s3*s4-cs12-cs13+cs23-cs14+cs24+cs34;
  checks[i,18]<- c1*(1-c2)*(1-c3)*(1-c4)-cc12-cc13+cc23-cc14+cc24+cc34;
  checks[i,19]<- (1-s1)*s2*s3*(1-s4)-cs12-cs13+cs23+cs14-cs24-cs34;
  checks[i,20]<- c1*(1-c2)*(1-c3)*c4-cc12-cc13+cc23+cc14-cc24-cc34;
  checks[i,21]<- (1-s1)*s2*(1-s3)*s4-cs12+cs13-cs23-cs14+cs24-cs34
  checks[i,22]<- c1*(1-c2)*c3*(1-c4)-cc12+cc13-cc23-cc14+cc24-cc34;
  checks[i,23]<- (1-s1)*s2*(1-s3)*(1-s4)-cs12+cs13-cs23+cs14-cs24+cs34;
  checks[i,24]<- c1*(1-c2)*c3*c4-cc12+cc13-cc23+cc14-cc24+cc34;
  checks[i,25]<- (1-s1)*(1-s2)*s3*s4+cs12-cs13-cs23-cs14-cs24+cs34;
  checks[i,26]<- c1*c2*(1-c3)*(1-c4)+cc12-cc13-cc23-cc14-cc24+cc34;
```

```
checks[i,27]<- (1-s1)*(1-s2)*s3*(1-s4)+cs12-cs13-cs23+cs14+cs24-cs34;
checks[i,28]<- c1*c2*(1-c3)*c4+cc12-cc13-cc23+cc14+cc24-cc34;
checks[i,29]<- (1-s1)*(1-s2)*(1-s3)*s4+cs12+cs13+cs23-cs14-cs24-cs34;
checks[i,30]<- c1*c2*c3*(1-c4)+cc12+cc13+cc23-cc14-cc24-cc34;
checks[i,31]<- (1-s1)*(1-s2)*(1-s3)*(1-s4)+cs12+cs13+cs23+cs14+cs24+cs34;
checks[i,32]<- c1*c2*c3*c4+cc12+cc13+cc23+cc14+cc24+cc34;


valid[i]<- step(s1+c1-1.0)*step(s2+c2-1.0)*step(s3+c3-1.0)*step(s4+c4-1.0)*

        step(1-checks[i,1])*step(checks[i,1])*
        step(1-checks[i,2])*step(checks[i,2])*
        step(1-checks[i,3])*step(checks[i,3])*
        step(1-checks[i,4])*step(checks[i,4])*
        step(1-checks[i,5])*step(checks[i,5])*
        step(1-checks[i,6])*step(checks[i,6])*
        step(1-checks[i,7])*step(checks[i,7])*
        step(1-checks[i,8])*step(checks[i,8])*
        step(1-checks[i,9])*step(checks[i,9])*
        step(1-checks[i,10])*step(checks[i,10])*
        step(1-checks[i,11])*step(checks[i,11])*
        step(1-checks[i,12])*step(checks[i,12])*
        step(1-checks[i,13])*step(checks[i,13])*
        step(1-checks[i,14])*step(checks[i,14])*
        step(1-checks[i,15])*step(checks[i,15])*
        step(1-checks[i,16])*step(checks[i,16])*
        step(1-checks[i,17])*step(checks[i,17])*
        step(1-checks[i,18])*step(checks[i,18])*
        step(1-checks[i,19])*step(checks[i,19])*
        step(1-checks[i,20])*step(checks[i,20])*
        step(1-checks[i,21])*step(checks[i,21])*
        step(1-checks[i,22])*step(checks[i,22])*
        step(1-checks[i,23])*step(checks[i,23])*
        step(1-checks[i,24])*step(checks[i,24])*
        step(1-checks[i,25])*step(checks[i,25])*
        step(1-checks[i,26])*step(checks[i,26])*
        step(1-checks[i,27])*step(checks[i,27])*
        step(1-checks[i,28])*step(checks[i,28])*
        step(1-checks[i,29])*step(checks[i,29])*
        step(1-checks[i,30])*step(checks[i,30])*
        step(1-checks[i,31])*step(checks[i,31])*
        step(1-checks[i,32])*step(checks[i,32]);
```

```
#########################################################
## Contribution to the likelihood for each observation
#########################################################

  L[i]<- equals(valid[i],1)*(
          equals(mobokupa [i,2],1)*equals(mobokupa[i,3],1)*equals(mobokupa [i,4],1)*equals
(mobokupa [i,6],1)*q[i,1]
        + equals(mobokupa [i,2],1)*equals(mobokupa[i,3],1)*equals(mobokupa [i,4],1)*equals
(mobokupa [i,6],0)*q[i,2]
        + equals(mobokupa [i,2],1)*equals(mobokupa[i,3],1)*equals(mobokupa [i,4],0)*equals
(mobokupa [i,6],1)*q[i,3]
        + equals(mobokupa [i,2],1)*equals(mobokupa[i,3],1)*equals(mobokupa [i,4],0)*equals
(mobokupa [i,6],0)*q[i,4]
        + equals(mobokupa [i,2],1)*equals(mobokupa[i,3],0)*equals(mobokupa [i,4],1)*equals
(mobokupa [i,6],1)*q[i,5]
        + equals(mobokupa [i,2],1)*equals(mobokupa[i,3],0)*equals(mobokupa [i,4],1)*equals
(mobokupa [i,6],0)*q[i,6]
        + equals(mobokupa [i,2],1)*equals(mobokupa[i,3],0)*equals(mobokupa [i,4],0)*equals
(mobokupa [i,6],1)*q[i,7]
        + equals(mobokupa [i,2],1)*equals(mobokupa[i,3],0)*equals(mobokupa [i,4],0)*equals
(mobokupa [i,6],0)*q[i,8]
        + equals(mobokupa [i,2],0)*equals(mobokupa[i,3],1)*equals(mobokupa [i,4],1)*equals
(mobokupa [i,6],1)*q[i,9]
        + equals(mobokupa [i,2],0)*equals(mobokupa[i,3],1)*equals(mobokupa [i,4],1)*equals
(mobokupa [i,6],0)*q[i,10]
        + equals(mobokupa [i,2],0)*equals(mobokupa[i,3],1)*equals(mobokupa [i,4],0)*equals
(mobokupa [i,6],1)*q[i,11]
        + equals(mobokupa [i,2],0)*equals(mobokupa[i,3],1)*equals(mobokupa [i,4],0)*equals
(mobokupa [i,6],0)*q[i,12]
        + equals(mobokupa [i,2],0)*equals(mobokupa[i,3],0)*equals(mobokupa [i,4],1)*equals
(mobokupa [i,6],1)*q[i,13]
        + equals(mobokupa [i,2],0)*equals(mobokupa[i,3],0)*equals(mobokupa [i,4],1)*equals
(mobokupa [i,6],0)*q[i,14]
        + equals(mobokupa [i,2],0)*equals(mobokupa[i,3],0)*equals(mobokupa [i,4],0)*equals
(mobokupa [i,6],1)*q[i,15]
        + equals(mobokupa [i,2],0)*equals(mobokupa[i,3],0)*equals(mobokupa [i,4],0)*equals
(mobokupa [i,6],0)*q[i,16]
       ) +(1-equals(valid[i],1)) *(1e-14);
```

```
#############################################################
## Ensure the probabilities are always less than 1
#############################################################

##Since in a Bernoulli density an observation of 1 has a likelihood of p[i]
      p[i] <- L[i] / 1;## divided by a constant just to ensure all p's <1
      ones[i] ~ dbern(p[i]);
  }

 ############################################################
## Definition of model priors
############################################################

  prc~dbeta(1,1);
  c1~dbeta(1,1);          # Specificity SICT test
  c2~dbeta(1,1);          # Specificity Bovigam® assay
  c3<-1;                  # Specificity culture fixed
  c4~dbeta(1,1);          # Specificity necropsy
  s1~dbeta(1,1);          # Sensitivity SICT test
  s2~dbeta(1,1);          # Sensitivity Bovigam® assay
  s3~dbeta(1,1);          # Sensitivity culture
  s4~dbeta(1,1);          # Sensitivity necropsy

## Covariance terms

  cs12<-0;
  cs13<-0;
  cs23<-0;#~dbeta(1,1);
  cs14<-0;
  cs24<-0;
  cs34<-0;
  cc12<-0;
  cc13<-0;
  cc23<-0;
  cc14<-0;
  cc24<-0;
  cc34<-0;

  logL<-sum(log(p[1:N]));
    }
```

S1 Table: Number of test result combinations in the four-test dataset (n=175), the inconclusive test results of the SICT test once considered as negative (standard interpretation) and once as positive (severe interpretation)

SICT test	Bovigam® assay[b]	Culture	Necropsy	No of animals SICT test as standard interpretation	No of animals SICT test as severe interpretation
+	+	+	+	8	9
+	+	+	-	0	2
+	+	-	+	0	1
+	+	-	-	38	149
+	-	+	+	1	1
+	-	+	-	0	0
+	-	-	+	0	0
+	-	-	-	1	8
-	+	+	+	1	0
-	+	+	-	2	0
-	+	-	+	1	0
-	+	-	-	115	4
-	-	+	+	0	0
-	-	+	-	0	0
-	-	-	+	0	0
-	-	-	-	8	1
Total				175	175

[b] cut-off: 0.1

S2 Table: Number of test result combinations in the three-test dataset (n=389), the inconclusive test results of the SICCT test once considered as negative (standard interpretation) and once as positive (severe interpretation)

SICCT test	PCR	Necropsy	No of animals [SICCT test as standard interpretation]	No of animals [SICCT test as severe interpretation]
+	+	+	26	47
+	+	-	3	6
+	-	+	8	13
+	-	-	26	282
-	+	+	24	3
-	+	-	3	0
-	-	+	5	0
-	-	-	294	38
Total			389	389

S3 Table: DIC, prevalence and diagnostic test accuracies of different models, without and with covariances of the sensitivities between the different tests, considered from the dataset (n=175) tested with SICT test [standard interpretation, uninformative priors], Bovigam® assay [cut-off=0.1], culture [sp=100%] and necropsy

Model	DIC	Prevalence (95% CI)	SICT test (95% CI)		Bovigam® assay (95% CI)		Culture (95% CI)		Necropsy (95% CI)	
			se	sp	se	sp	se	sp	se	sp
1	393.7	7.7 (4.2-12.3)	70.3 (44.9-90.5)	75.8 (68.8-82.2)	95.7 (91.3-99.2)	6.9 (3.6-11.1)	88.9 (65.5-99.7)	fixed at 100	76.8 (51.6-94.4)	99.0 (96.8-100)
2	395.0	8,0 (4,3-12,8)	71,3 (47,2-90,3)	75.9 (69.2-82.2)	94.8 (90.4-98.3)	7.4 (4.0-11.8)	81.1(56.1-97.2)	fixed at 100	70.9 (46.9-90.3)	99.0 (96.9-100)
3	394.6	8.1 (4.4-13.1)	70.1 (46.7-89.1)	75.9 (69.0-82.2)	94.7 (90.2-98.4)	7.5 (4.0-11.8)	81.3 (57.6-97.1)	fixed at 100	70.1 (46.1-90.1)	99.0 (96.9-100)
4	394.4	8.1 (4.3-12.9)	69.0 (45.8-87.9)	76.0 (69.3-82.2)	94.7 (90-3-98.4)	7.4 (4.1-11.7)	79.9 (53.9-96.8)	fixed at 100	72.4 (48.6-90.9)	99.0 (97.0-100)
5	393.6	7.8 (4.2-12.4)	66.5 (43.6-86.8)	75.8 (69.1-82.1)	94.9 (90.6-98.4)	7.3 (4.0-11.5)	87.4 (65.1-98.5)	fixed at 100	72.6 (48.6-91.4)	99.0 (96.9-100)
6	394.4	7.9 (4.3-12.6)	65.6 (42.8-86.0)	75.9 (67.0-82.2)	94.8 (90.4-98.4)	7.4 (4.0-11.7)	81.8 (57.6-97.2)	fixed at 100	77.0 (52.6-93.9)	99.0 (96.9-100)
7	393.6	8.0 (4.3-12.9)	65.0 (42.2-85.6)	75.9 (69.0-82.3)	94.7 (90.3-98.4)	7.4 (4.1-11.8)	82.3 (58.0-97.1)	fixed at 100	75.4 (51.0-92.7)	99.0 (97.0-100)

Model 1: no covariances
Model 2: covariance sensitivity SICT test, Bovigam® assay
Model 3: covariance sensitivity SICT test, culture
Model 4: covariance sensitivity SICT test, pathology
Model 5: covariance sensitivity Bovigam® assay, culture
Model 6: covariance sensitivity Bovigam® assay, pathology
Model 7: covariance sensitivity culture, pathology
CI, credibility interval
se, sensitivity
sp, specificity

S4 Table: Dichotomized test results of the Bovigam® assay for two different cut-offs

	Positive		Negative		Total
Cut-off	n	%	n	%	n
>0.1	166	97.1	5	2.9	171
>0.2	46	26.9	125	73.1	171

S5 Table: Positive and negative predictive values of the SICT test, Bovigam® assay, culture [sp=100%] and necropsy calculated from the prevalence and diagnostic test accuracies obtained from the models of Table 1.

Test	PPV %	NPV %	based on model
SICT test [standard interpretation]	19.51	96.83	1
SICT test [severe interpretation]	8.07	96.37	4
Bovigam® assay [cut-off = 0.1]	7.90	95.06	1
Bovigam® assay [cut-off = 0.2]	8.54	94.25	3
Culture	-	99.08	1
Necropsy	86.50	98.08	1

PPV, positive predictive value; NPV, negative predictive value

S6 Table: DIC, prevalence and diagnostic test accuracies of different models, without and with covariances of the sensitivities and specificities between the different tests, considered from the dataset (n=389) tested with SICCT test [standard interpretation; prior information], PCR and necropsy

Models	DIC	Prevalence (95% CI)	SICCT test (95% CI)		PCR (95% CI)		Necropsy (95% CI)	
			se	sp	se	sp	se	sp
1	756.4	17.2 (13.4-21.4)	57.5 (46.5-68.1)	91.5 (88.4-94.2)	80.6 (69.1-90.6)	99.1 (97.5-100)	90.7 (80.7-98.0)	99.1 (97.2-100)
2	756.6	17.4 (13.6-21.5)	57.3 (46.6-67.6)	91.5 (88.4-94.1)	79.1 (67.4-89.0)	99.1 (97.6-100)	89.5 (79.8-96.7)	99.2 (97.4-100)
3	756.3	17.5 (13.7-21.7)	57,1 (46.3-67.6)	91.5 (88.3-94.2)	79.6 (68.3-89.3)	99.3 (97.8-100)	88.1 (77.9-96.1)	99.1 (97.2-100)
4	750.7	19.8 (14.6-26.5)	57.8 (48.0-67.6)	92.8 (89.2-96.3)	70.6 (52.0-86.0)	99.0 (97.4-99.9)	78.4 (58.6- 93.7)	98.9 (96.8-100)
5	758.9	16.7 (12.9-20.8)	58.1 (47.1-68.6)	91.4 (88.3-94.1)	82.1 (70.4-92.0)	98.6 (96.7-99.7)	92.0 (82.1-98.8)	98.5 (96.4-99.7)
6	758.9	16.7 (12.9-20.9)	58.2 (47.3-69.1)	91.4 (88.2-98.7)	82.1 (70.6-92.1)	98.6 (96.8-99.7)	91.8 (82.2-98.7)	98.5 (96.3-99.8)
7	757.8	17.0 (13.1-21.2)	58.0 (47.1-68.7)	91.2 (88.0-94.0)	81.2 (69.9-90.9)	98.8 (97.1-99.8)	91.1 (81.4-98.1)	98.7 (96.9-99.8)

Model 1: no covariances
Model 2: covariance sensitivity SICCT test, PCR
Model 3: covariance sensitivity SICCT test, pathology
Model 4: covariance sensitivity PCR, pathology
Model 5: covariance specificity SICCT test, PCR
Model 6: covariance specificity SICCT test, pathology
Model 7: covariance specificity PCR, pathology
CI, credibility interval
se, sensitivity
sp, specificity

S7 Table: Positive and negative predictive values of the SICCT test, PCR and necropsy calculated from the prevalence and diagnostic test accuracies obtained from the models of Table 3.

Test	PPV %	NPV %	based on model
SICCT Test [standard interpretation]	66.46	89.91	3
SICCT Test [severe interpretation]	16.62	92.72	5
PCR	94.57	93.17	3
Necropsy	94.62	94.88	3

PPV, positive predictive value; NPV, negative predictive value

S8 Table: Test results of the Bovigam® assay from five officially approved laboratories; the results of the time point with the best accordance of the proportion of positive test results is shown

Cow no	Laboratory 1	Laboratory 2	Laboratory 3	Laboratory 4	Laboratory 5
1	pos	pos	pos	pos	neg
2	pos	neg	neg	pos	neg
3	pos	pos	pos	pos	pos
4	neg	neg	neg	neg	neg
5	pos	pos	pos	pos	neg
6	pos	pos	pos	pos	pos
7	pos	pos	pos	pos	neg
8	pos	pos	pos	pos	pos
9	neg	pos	pos	n.a.	neg
10	neg	pos	pos	n.a.	pos
11	pos	pos	pos	n.a.	neg
12	neg	pos	pos	pos	pos
13	pos	pos	pos	neg	pos
14	neg	pos	pos	neg	pos
15	pos	pos	pos	pos	pos
16	neg	neg	pos	pos	pos
17	pos	neg	pos	neg	neg
18	pos	pos	pos	pos	pos
19	pos	pos	pos	pos	pos
20	pos	pos	pos	pos	pos
21	neg	neg	neg	pos	neg

Pos, positive; neg, negative; n.a., not analyzable

S9 Table: Test results of the Bovigam® assay from five officially approved laboratories. The assayed blood was taken at two time points respectively from two differing localizations.

cow no	Laboratory 1				Laboratory 2				Laboratory 3				Laboratory 4				Laboratory 5			
	V.j. / V.s.a.		*V.j. / V.c.m.*		*V.j. / V.s.a.*		*V.j. / V.c.m.*		*V.j. / V.s.a.*		*V.j. / V.c.m.*		*V.j. / V.s.a.*		*V.j. / V.c.m.*		*V.j. / V.s.a.*		*V.j. / V.c.m.*	
	V.j.	*V.s.a.*	*V.j.*	*V.c.m.*	*V.j.*	*V.s.a.*	*V.j.*	*V.c.m.*	*V.j.*	*V.s.a.*	*V.j.*	*V.c.m.*	*V.j.*	*V.s.a.*	*V.j.*	*V.c.m.*	*V.j.*	*V.s.a.*	*V.j.*	*V.c.m.*
1	neg	neg	pos	pos	pos	neg	pos	neg	pos	pos	pos	neg	pos	pos	pos	neg	pos	neg	neg	neg
2	neg	pos	pos	pos	pos	neg	neg	neg	neg	neg	neg	neg	neg	pos	pos	pos	pos	neg	neg	neg
3	pos	pos	pos	pos	pos	pos	pos	neg	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos
4	neg	n.a.	neg	pos	neg	neg	neg	neg	n.a.	n.a.	n.a.	n.a.	neg	neg	neg	neg	neg	neg	neg	neg
5	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos	neg	pos
6	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos	neg	pos	pos	neg	pos	?	pos	pos
7	pos	neg	pos	neg	pos	neg	pos	neg	pos	pos	pos	neg	pos	pos	pos	pos	neg	neg	neg	neg
8	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos
9	neg	neg	neg	pos	n.a.	pos	pos	pos	pos	pos	pos	pos	pos	neg	n.a.	neg	neg	neg	neg	pos
10	pos	pos	neg	pos	pos	pos	pos	pos	pos	pos	pos	pos	neg	neg	n.a.	neg	pos	pos	pos	pos
11	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos	neg	neg	n.a.	neg	pos	?	neg	pos
12	pos	neg	neg	neg	pos	pos	pos	pos	pos	pos	pos	pos	neg	neg	pos	pos	pos	neg	pos	pos
13	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos	neg	pos	pos	pos	pos	pos
14	pos	pos	neg	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos	neg	pos	pos	pos	pos	pos
15	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos
16	neg	pos	neg	pos	pos	pos	neg	pos	pos	pos	pos	pos	pos	pos	pos	neg	pos	neg	pos	pos
17	pos	pos	pos	pos	pos	neg	neg	neg	pos	pos	pos	pos	neg	pos	neg	pos	pos	neg	neg	neg
18	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos
19	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos	?	pos	pos
20	pos	pos	pos	neg	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos	neg	pos	pos
21	neg	pos	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	pos	pos	neg	neg	neg	neg	neg

V.j., V. jugularis; V.s.a., V. subcutanea abdominis; V.c.m., V. caudalis mediana; pos, positive; neg, negative; ?, inconclusive; n.a., not analyzable

IV. DISCUSSION

The detection of bovine TB cases during routine abattoir inspections in Germany led to the implementation of a new bovine TB control program in November 2012. Within this program the SICCT test was performed as primary test and the Bovigam® assay was performed for the first time in Germany as a field test. Animals that were diagnosed positive with the SICCT test or the Bovigam® assay were slaughtered and further examinations were performed post mortem. A post mortem diagnosis was also performed for animals that were, within 6 weeks, twice diagnosed as inconclusive[6]. This approach refers to the test and slaughter policy which is used in many countries for the achievement or maintenance of the OTF status (Good, M., 2011; Humblet, M. F. et al., 2009; Ryan, T. J. et al., 2006; Strain, S. A. J. et al., 2011b).

1. Publication 1

Since 1997, with reaching the OTF status, the regular nationwide intradermal tuberculin testing was replaced by routine abattoir inspections for the control of bovine TB (Gerstmair, E.-M., 2011). As a result, German practitioners only perform the tuberculin test occasionally and a lot of young veterinarians have no or only minor experience with the execution of the test and the interpretation of the results. For the success of a control program a correct execution and interpretation of the tuberculin tests is essential, since this can reduce false positive or false negative test results (Humblet, M.-F. et al., 2011; Schiller, I. et al., 2011). The aim of the first study was the analysis of farm-animal practitioners' current knowledge on the tuberculin tests technique. The majority of the veterinarians, which completed the questionnaire about the performance and their experience with the intradermal tuberculin test, execute the test with regard to the Commission Regulation (EC) no. 1226/2002 of 8 July 2002 amending Annex B to Council Directive 64/432/EEC. The veterinarians that stated the least accordance with the Commission Regulation do not work in the Allgäu Region or examined only single animals. As a consequence of increased bovine TB cases in Bavaria a one-time

[6] Tuberkulose-Verordnung in der Fassung der Bekanntmachung vom 12. Juli 2013 (BGBl. I S. 2445, 2014 I S. 47), die zuletzt durch Artikel 2 der Verordnung vom 17. Mai 2017 (BGBl. I S. 1253) geändert worden ist

tuberculosis control program was already performed during 2007 to 2009 in the Allgäu Region (Gerstmair, E.-M., 2011). As the veterinarians, which were not working in this region, were not involved in the nationwide tuberculosis control program of 2007/2009, they might have less experience in the correct execution of the intradermal tuberculin test. The least concordance with the required standards was observed for the monitoring of the results. This might be due to lack of knowledge for the need of correct interpretation and monitoring of the intradermal tuberculin test. An improper interpretation of the intradermal tuberculin test can lead to false negative or false positive test results and will have a consequence on the success of a control program (Collins, J. D., 2006; Humblet, M.-F. et al., 2011).

The fact that 19 out of 42 veterinarians also test animals that were recently treated with inflammation inhibitors might be due to lack of awareness that an anti-inflammatory treatment previous to the intradermal tuberculin test could alter the results (Doherty, M. L. et al., 1995). Another unconsciousness is the influence of a *F. hepatica* infection on the diagnosis on bovine TB. The majority of the contributing veterinarians seems not to be aware of the issue that an infection with *F. hepatica* leads to an anti-inflammatory state and cattle that was experimentally co infected with *F. hepatica* was more often diagnosed as negative than animals not infected with liverflukes (Claridge, J. et al., 2012; Flynn, R., J. et al., 2007).

The communication between farmers and veterinarians was stated as more difficult during the control program implemented in 2012 than during the nationwide intradermal tuberculin testing which was performed until 1997. Because of changes in the council directive and the withdrawal of the Bovigam® assay during the year 2013 the uncertainty of the veterinarians and farmers with regard to the control program of bovine tuberculosis had increased[7,8] (Anonymous, 2013, 2014a). This might have led to a more difficult farmer-veterinarian relation and indicates the importance of information and communication for both sides. As the involvement of the farmer as stakeholder is crucial for the success of control

[7] Erste Verordnung zur Änderung der Tuberkulose-Verordnung (1. RindTbVÄndV [k.a.Abk.]) V. v. 14.03.2013 BAnz AT 15.03.2013 V1; aufgehoben durch Artikel 1 V. v. 12.07.2013 BGBl. I S. 2442 Geltung ab 16.03.2013

[8] Zweite Verordnung zur Änderung der Tuberkulose-Verordnung (2. RindTbVÄndV k.a.Abk.) V. v. 12.07.2013 BGBl. I S. 2442 (Nr. 39); Geltung ab 20.07.2013, abweichend siehe Artikel 4

programs a good farmer-veterinarian communication is an essential requirement (Collins, J. D., 2006; Cowie, C. E. et al., 2015). The subjective feeling of some veterinarians towards more complicated handling of the intradermal tuberculin test and a stricter council directive could have been provoked through the frequent discussion on usage of a sterile cannula for every animal and the correct injection site. This was also content of a proceeding which led to even more attention and uncertainty[9] (Anonymous, 2015; 2014; 2015).

Although most of the veterinarians affirm that they perform the intradermal tuberculin test correct, the education of veterinary students on the correct performance of the intradermal tuberculin tests is indispensable with the view to future control programs. In addition, a uniform approach based on actual literature and directive lectured in universities can lead to less uncertainty and discrepancies amongst the veterinarians confronted with such control programs. Within this study a guide for the execution of the intradermal tuberculin tests was designed and could help for the improvement of a standardized method.

2. Publication 2

During the bovine TB control program contradictory test results, especially related to the Bovigam® assay were recognized by the persons involved in this program. Due to these discrepancies the application of the Bovigam® assay was terminated and the determination of the test characteristics became of a greater interest. The aim of the second study was the estimation of sensitivities and specificities from the tests used within the bovine TB control program which was implemented in Germany in 2012. Due to absence of a true gold standard with 100% sensitivity and 100% specificity the test accuracies were estimated with a latent class approach used within a Bayesian model. Bayesian latent class approaches are increasingly used in medical and veterinary science (Courcoul, A. et al., 2014; Hartnack, S. et al., 2013; Hartnack, S. et al., 2014; Narad, M. E. et al., 2017). To the best of our knowledge a latent class analysis has never been used before for the estimation of test characteristics for bovine TB diagnostic tests in Germany.

The estimated test characteristics of the SICT test were 70.3% [44.9-90.5%] for sensitivity and 75.8% [68.8-82.2%] for specificity. The SICCT test had an

[9] VGH München, Beschluss v. 03.07.2014 – 20 CS 14.1032

estimated sensitivity of 57.8% [48.0-67.6%] and an estimated specificity of 92.8% [89.2-96.3%]. With regard to the studies evaluated from de la Rua-Domenech et al. (de la Rua-Domenech, R. et al., 2006) our estimated test characteristics stand in line with already published data. However, the estimated values are close to the lower limit. This could be due to the inaccuracy in the execution and interpretation of the intradermal tuberculin tests performed by German practitioners during the bovine TB control program implemented in 2012 (Pucken, V.-B. et al., 2015). Especially false negative results can occur by incorrect administration, reading and recording of the test and will lead to a decrease in sensitivity (Humblet, M.-F. et al., 2011). In comparison to the SICT test the SICCT test had a higher estimated specificity as false positives due to the infection of other mycobacteria are already reduced (Rolle, M. et al., 2011). With the increased specificity the sensitivity of the SICCT test decreases (Brenner, H. et al., 1997).

For the Bovigam® assay we assessed the highest sensitivity of all tests (95.7% [91.3-99.2%]), though also by far the lowest specificity (6.9% [3.6-11.1%]). In comparison to the values found in literature the estimated sensitivity stands in line with other studies (Álvarez, J. et al., 2012; de la Rua-Domenech, R. et al., 2006). Nevertheless, such a low specificity has never before been estimated for the Bovigam® assay. Even by setting the cut off to a higher value and thus making the Bovigam® assay more specific and less sensitive the specificity increased only to 23.5% [17.4-30.3%], which still is too low to be a suitable field test in a bovine TB control program (Good, M., 2011). The estimated low specificity could be due to various reasons affecting the host and the test himself. The factors which affects the host and can influence the outcome of the Bovigam® assay could be a previous performed intradermal tuberculin test, the breed, an infection with *Mycobacterium avium* subspecies *paratuberculosis* (MAP) or an infection with *F. hepatica* (Amos, W. et al., 2013; Barry, C. et al., 2011; Flynn, R., J. et al., 2007; Schiller, I. et al., 2010b). The current bovine TB outbreak could be mainly attributed to three different molecular types of *M. caprae* (Domogalla, J. et al., 2013). This might have had an impact on the outcomes of the Bovigam® assay as it was developed for the detection of *M. bovis* infections (Wood, P. R. et al., 2001). Although nothing of the mentioned influences were until now stated for a decrease of the Bovigam® assays specificity. However the estimated low inter- and intralaboratory agreements estimated with Cohen's kappa could not be explained

by factors affecting only the host. In this context factors affecting the test itself should be considered to be responsible for the estimated low agreements. These factors could be a delay in processing the blood sample, differing injection sites for taking the blood and differing concentration and potency of PPDs (Böttcher, J. et al., 2010; Cagiola, M. et al., 2004; Gormley, E. et al., 2004). Looking at the results of Cohen's kappa the transportation time as well as the experience in Bovigam® assay performance seems to have an influence, because the best agreements were estimated for the laboratories with the shortest transportation time and the most experience in performing the Bovigam® assay. However also between and within these laboratories only a substantial agreement was estimated. A more detailed discussion on the possible reasons for the estimated test characteristics of the Bovigam® assay can be found in the second publication (Pucken, V.-B. et al., 2017).

The PCR had a sensitivity of 70.6% [52.0-86.0] and a specificity of 99.0% [97.4-99.9]. A high specificity can be achieved with the decision of *M. bovis* and *M. caprae* specific primers and due to the fact that only the evidence of both target genes leads to a positive test result (Gerstmair, E.-M., 2011). In our study dependence between the sensitivity of the PCR and necropsy seemed to be the most likely. It was already reported by Parra et al. that with the appearance of NVL, which is correlated to the accuracy of the pathological examination and stage of disease, the sensitivity of the PCR decreases (Parra, A. et al., 2008). Owing to the fact that for the PCR examination only 1 gram of tissue is normally used, false negative results can additionally occur due to inhomogeneous distribution of mycobacteria in the examined organs. To overcome this problem larger sample volumes can be processed to concentrate mycobacterial DNA and therefore sensitivity of the PCR will increase (Fell, S. et al., 2016). In comparison to the PCR a slightly higher sensitivity could be estimated for necropsy (78.4% [58.6-93.7]), which correlates on the number of tissues examined and on the occurrence of NVL (Corner, L. et al., 1990; Norby, B. et al., 2004). As the pathological examinations were performed at different places (pathology of the Bavarian Health and Food Safety Authority, carcass disposal plants) and by different veterinarians a variation between the examiners might be probable as different persons might differ in the focuses on the tissue to be examined. Some examiner, which were involved in this tuberculosis control program, reported that

the majority of affected lymph nodes were the mesenteric lymph nodes and the lymph nodes of the intestines (Dr. Johann Mages, personal communication, May 14th, 2014). The initial infection of bovine TB starts with an inflammatory process at the organ of the portal of entry. Furthermore the regional lymph node is affected due to lymphogenic spread. This primary focus together with the lesion in the regional lymph node is called primary complex (Menzies, F. D. et al., 2000). An aerogenous infection will lead to a primary focus in the lymph nodes of the head, the mediastinal lymph nodes and the bronchial lymph node which corresponds to the normal presence of lesions (Corner, L. A., 1994). Unfortunately the information which lymph nodes were mostly affected and collected for the further examination were not sufficiently documented during the bovine TB control program. Though cumulative occurrence of the primary focus in the intestinal and mesenteric lymph nodes indicates an infection through the gastrointestinal tract by swallowing contaminated food. The transmission of *M. caprae* provoked by red deer during pasture was discussed as one of the main transmission routes responsible for the increase in bovine TB cases in cattle alongside the alps (Boenchendorf, J. A. D., 2016; Müller, M. et al., 2014). As contaminated salt lick stones and shared water sources are likely for transmission, these route of infection might be possible and could explain the subjective perception of intestinal and mediastinal lymph nodes being more affected (Anonymous, 2009a; Payne, A. et al., 2016). Shared water sources were already reported to be significantly associated with bovine TB (Marsot, M. et al., 2016). The estimated specificities of the PCR and necropsy are 99.0% [97.4-99.9%] and 98.9% [96.8-100%] respectively 99.9% [96.8-100%], which made the occurrence of false positive due to this examination methods improbable. For the culture the highest sensitivity, in comparison to the other post mortem examinations, was estimated (88.9% [65.5-99.7%]). This makes the culture still a good diagnostic tool but clearly shows that it is not a perfect test with 100% test accuracy. Therefore estimated sensitivities and specificities referring to the culture as gold standard will be over- or underestimated (Hartnack, S. et al., 2012).

3. Conclusion

The success of a bovine TB control program depends on several factors as occurrence of a wildlife host, the correct identification of infected or uninfected animals and the involvement of all stakeholders (Collins, J. D., 2006; Corner, L.

A. L. et al., 2011; Good, M., 2011; Waddington, K., 2012). For the involvement of all stakeholders, especially the herd owners, dialogue and risk communication is crucial (Collins, J. D., 2006; Cowie, C. E. et al., 2015). Hence diagnostic tests with high specificities are important, as a frequent culling of animals that are later diagnosed as negative will lead to dissatisfaction and uncertainty on the owner site. This might lead to poor communication between the persons that test the animals and the herd owner, as experienced by some veterinarians involved in the bovine TB program (Pucken, V.-B. et al., 2015). Due to the fact that for the Bovigam® assay an extreme low specificity was estimated, some herd owners were confronted with animals that have been slaughtered but were later on diagnosed as bovine TB negative. This may have caused mistrust in the premortal tests and test execution and might be the cause of the discussion about injection site of the intradermal tuberculin tests and changing of cannula after each animal[10] (Anonymous, 2013; 2014; 2015). Hence a uniform execution of the tests used within a bovine TB control program is crucial and a guideline like published with the first paper might help practitioners for a more standardized execution of the intradermal tuberculin tests. Not only high specificities are important for the success of a bovine TB control program. Also high sensitivities of the premortal tests are important, as false negative tested animals will stay in the herd and infect others (Skuce, R. A. et al., 2011). If a single positive animal determines the status of the herd as positive, herd level sensitivity (HSe) will increase even with imperfect diagnostic tests on the animal level (Good, M., 2011). This made the SICCT test with regard to the estimated sensitivities and specificities a suitable test for future control programs in the Allgäu Region. The decision to terminate the Bovigam® assay as a diagnostic test during the bovine TB control program was, with regard to the estimated test characteristics and agreements, appropriate and reasonable. However no prognoses could be made for the application of the Bovigam assay in future control programs as a new Bovigam® assay was developed during the last years (Anonymous, 2016a). For post mortem confirmation of bovine TB the culture is still an essential diagnostic tool with a high sensitivity and 100% specificity. As sensitivity and specificity depend on each other there has to be always a decision made between higher sensitivity or higher specificity for tests being used in a bovine TB control program. This has always to be evaluated with regard to contribution of stakeholders, compromise of

[10] VGH München, Beschluss v. 03.07.2014 – 20 CS 14.1032

OTF status, available tests and experience in test performance of the persons involved in the control program.

V. SUMMARY

The success of bovine TB control programs is important for international trade and public health. Since Germany reached the EU status OTF in 1996 bovine TB control was done at routine abattoir inspections. After the diagnosis of bovine TB positive cattle during such routine inspections, a one-time surveillance program was implemented in Germany with the intradermal tuberculin tests and the Bovigam® assay as premortal tests and necropsy, PCR and bacterial culture as postmortal tests.

In this study first farm-animal practitioners' current knowledge on the execution of the intradermal tuberculin tests was reviewed with regard to literature and current legislation. A questionnaire was developed and send out to farm-animal practitioners. Additionally the current and previous versions of the corresponding legislations and related literature was reviewed. To standardize the execution of the intradermal tuberculin test a hand out was developed with the aim towards a more uniform approach. Second, the test characteristics of the diagnostic tests used within this bovine TB surveillance program were assessed with a latent class analysis used within a Bayesian approach.

Predominantly the participating farm-animal practitioners' performed the intradermal tuberculin test with regard to the Commission Regulation (EC) no. 1226/2002 of 8 July 2002 amending Annex B to Council Directive 64/432/EEC. However for the control of test results the least accordance existed. This was particularly monitored for farm-animal practitioners' that did not work in the Allgäu Region and were thus not severely affected with the control of bovine TB and regular performance of intradermal tuberculin testing. A more uniform approach in the execution and interpretation of the intradermal tuberculin test is desirable as uncertainties among the stakeholders might be removed. The guideline for the correct performance of the tuberculin skin test, which was designed during this study, might be supportive.

The estimated test specificities ranged between 75.8% [68.8-82.2%] and 99.0% [96.8-100%] for the SICT test, SICCT test, PCR and necropsy. For the Bovigam® assay an extreme low specificity of 6.9% [3.6-11.1%] and the highest sensitivity of

95.7% [91.3-99.2%] were assessed. For the other tests the sensitivities ranged from 57.8% [48.0-67.6%] to 88.9% [65.5-99.7%].

Except of the specificity of the Bovigam® assay all estimated test results stand in line with already published data. Dependence of the sensitivity of the PCR and necropsy seems to be true for the estimates of the latent class analysis and can be justified as number and kind of examined lymph nodes and the occurrence of NVL in the examined cattle has an impact on the outcome of the PCR. The estimated low specificity of the Bovigam assay might be explained by several factors affecting the host and the test itself. With regard to the fact that in the meantime a new Bovigam® assay was developed no conclusion can be made for the test accuracy in future control programs.

VI. ZUSAMMENFASSUNG

Die erfolgreiche Bekämpfung der Rindertuberkulose ist bedeutsam für den internationalen Handel und die öffentliche Gesundheit. Seitdem Deutschland, im Jahr 1996, den Status "amtlich frei von Tuberkulose" erreicht hat, dient die amtliche Fleischuntersuchung am Schlachthof zur Tuberkuloseprävention. Jedoch führte das wiederholte Auftreten von Tuberkulose positiven Tieren zu einer flächendeckende Tuberkulinisierung, mit dem Fokus auf den Regionen entlang der Alpenkette. Dabei wurden der Tuberkulin-Hauttest und der Bovigam® Test als Nachweis am lebenden Tier eingesetzt. Die pathologische Untersuchung, die PCR und die bakterielle Kultur dienten zum postmortalem Nachweis der Tuberkulose.

In dieser Forschungsarbeit wurde das Wissen von Praktikern in Bezug auf die Durchführung der Tuberkulin-Hauttests im Vergleich zur Literatur und der aktuellen Gesetzlage ermittelt. Zu diesem Zweck wurde ein Fragebogen entwickelt und an Praktiker verteilt. Ergänzend wurden aktuelle und frühere Versionen der entsprechenden Gesetzestexte sowie die zugehörige Literatur gesichtet. Um ein einheitlicheres Verfahren der Tuberkulinisierung zu erreichen wurde im Rahmen dieser Studie ein Leitfaden entwickelt. Außerdem wurden mit Hilfe einer latenten Klassenanalyse die Sensitivitäten und Spezifitäten der eingesetzten diagnostischen Verfahren ermittelt.

Die innerhalb dieser Studie befragten Praktiker halten sich größtenteils, bei der Durchführung des Tuberkulin-Hauttest, an die Verordnung (EG) Nr. 1226/2002 der Kommission vom 8.Juli 2002 zu Änderung von Anhang B der Richtlinie 64/432/EWG. Jedoch weichen viele Praktiker bei der Kontrolle der Ergebnisse von den Vorgaben der Verordnung (EG) Nr. 1226/2002 ab. Insbesondere sind dies die Tierärzte, die nicht in der Region Allgäu arbeiten und somit in geringerem Maße von der Rindertuberkulose betroffen sind. Ein einheitliches Vorgehen bei der Durchführung und dem Ablesen der Ergebnisse ist jedoch wünschenswert, da dadurch Unsicherheiten bei den Interessenvertretern beseitigt werden könnten. Der Leitfaden, der während dieser Studie entworfen wurde, könnte dieses unterstützen.

Die ermittelten Spezifitäten der eingesetzten diagnostischen Verfahren lagen zwischen 75.8% [68.8-82.2%] und 99.0% [96.8-100%] für den Intrakutan-Monotest, den Intrakutan-Simultantest, die PCR und die pathologische

Untersuchung. Für den Bovigam® Test wurde eine extrem niedrige Spezifität von 6.9% [3.6-11.1%] und die höchste Sensitivität von 95.7% [91.3-99.2%] ermittelt. Bei den übrigen diagnostischen Verfahren waren die ermittelten Sensitivitäten zwischen 57.8% [48.0-67.6%] und 88.9% [65.5-99.7%].

Mit Ausnahme der Spezifität des Bovigam® Tests liegen alle ermittelten Werte im Bereich von schon publizierten Daten. Eine Abhängigkeit zwischen der Sensitivität von der PCR und der pathologischen Untersuchung konnte innerhalb dieser latenten Klassenanalyse ermittelt werden. Dies kann damit begründet werden, dass Anzahl und Art der untersuchten Lymphknoten und das Auftreten von nicht makroskopisch sichtbaren Veränderungen (non visible lesions) einen Einfluss auf das Ergebnis der PCR haben können. Die ermittelte niedrige Spezifität für den Bovigam® Test könnte an vielen Faktoren liegen, die den Test selber, oder das infizierte Tier betreffen. Da mittlerweile eine neue Version des Bovigam® Tests entwickelt wurde lässt sich keine Aussage darüber treffen, wie genau und fehlerfrei der Test in zukünftigen Kontrollprogrammen abschneiden würde.

VII. REFERENCES

Álvarez, J., Perez, A., Bezos, J., Marqués, S., Grau, A., Saez, J. L., et al. (2012). Evaluation of the sensitivity and specificity of bovine tuberculosis diagnostic tests in naturally infected cattle herds using a Bayesian approach. *Veterinary Microbiology, 155*(1), 38-43. doi:http://dx.doi.org/10.1016/j.vetmic.2011.07.034

Amos, W., Brooks-Pollock, E., Blackwell, R., Driscoll, E., Nelson-Flower, M., & Conlan, A. J. K. (2013). Genetic Predisposition to Pass the Standard SICCT Test for Bovine Tuberculosis in British Cattle. *PloS one, 8*(3), e58245. doi:10.1371/journal.pone.0058245

Anonymous. (2009a). *Bovine Tuberculosis*. Retrieved from Iowa: http://www.cfsph.iastate.edu/Factsheets/pdfs/bovine _ tuberculosis.pdf

Anonymous. (2009b). *TB-Situation in Bayern – TBC-Projekt Allgäu.* Paper presented at the Symposium: Rindertuberkulose und Blauzungenkrankheit in Mitteleuropa, Bozen.

Anonymous. (2012). *Standard Operating Procedure for OIE Registration of Diagnostic Kits, Guide and Administrative Forms* Paris: World Organisation for Animal Health (OIE) Retrieved from http://www.oie.int/doc/ged/D12069.PDF.

Anonymous. (2013). TBC - Bekämpfungspflicht in Bayern. *Ig-gesunde-tiere.de.* Retrieved from www.Ig-gesunde-tiere.de

Anonymous. (2014a). Rinder-TBC im Allgäu: Zwangsuntersuchungen bringen Landwirte auf die Barrikaden. *Augsburger Allgemeine*. Retrieved from http://www.augsburger-allgemeine.de/bayern/Zwangsuntersuchungen-bringen-Land wirte-auf-die-Barrikaden-id31270477.html

Anonymous. (2014b). *Rindertuberkulose im Oberallgäu: die Fakten*. Retrieved from http://www.oberallgaeu.org/Rindertuberkulose _ im _ Oberallgaeu _ die _ Fakten.html.

Anonymous. (2015). Tuberkulose des Rindes: Empfehlung zur Tuberkulin-Injektionsstelle [Press release]. Retrieved from http://www.pei.de/SharedDocs/Downloads/tieraerzte/tuberkulose-rind-empfehlungen-tuberkulin.pdf;jsessionid=C3C9D879F2BB9528DAF15F7C9C77CBB7.1_cid354?__blob=publicationFile\& v=4

Anonymous. (2016a). BOVIGAM® 2G TB Kit. Retrieved from: https://www.thermofisher.com/order/catalog/product/63330?ICID=search-product

Anonymous. (2016b). *Global tuberculosis report 2016*. Retrieved from Geneva: http://www.who.int/tb/publications/global _ report/en/

Anonymous. (2017a). Bovine tuberculosis (*Mycobacterium bovis* and *Mycobacterium caprae*)Official Collection of Methods (pp. 1-22). Jena: Friedrich-Loeffler-Institute Retrieved from https://www.fli.de/de/publikationen/amtliche-methodensammlung/.

Anonymous. (2017b). *Terrestrial Animal Health Code*. Retrieved from http://www.oie.int/en/international-standard-setting/terrestrial-code/access-online/

Anonymous. (2017c). TSIS - TierSeuchenInformationsSystem. Retrieved from: https://tsis.fli.de/Reports/AnimalHealth.aspx

Anonymous. (2017d). Tuberkulose der Rinder (*Mykobakterium bovis* und *Mykobakterium caprae*) *Official Collection of Methods*. Greifswald - Insel Riems: Friedrich-Loeffler-Institute.

Aranaz, A., Liébana, E., Gómez-Mampaso, E., Galán, J. C., Cousins, D., Ortega, A., et al. (1999). Mycobacterium tuberculosis subsp. caprae subsp. nov.: A taxonomic study of a new member of the Mycobacterium tuberculosis complex isolated from goats in Spain. *International Journal of Systematic Bacteriology, 49*(3), 1263-1273. doi:10.1099/00207713-49-3-1263

Barry, C., Corbett, D., Bakker, D., Andersen, P., McNair, J., & Strain, S. (2011). The effect of Mycobacterium avium complex infections on routine Mycobacterium bovis diagnostic tests. *Veterinary medicine international, 2011*, 7 pages. doi:10.4061/2011/145092

Bayes, F. R. S., & Price, R. (1763). An essay towards solving a problem in the doctrine of chances. By the late Rev. Mr. Bayes, F.R.S. communicated by Mr. price, in a letter to John Canton, A.M.F.R.S. *Philosophical Transactions*, 370-418. doi: 10.1098/rstl.1763.0053

Berkvens, D., Speybroeck, N., Praet, N., Adel, A., & Lesaffre, E. (2006). Estimating Disease Prevalence in a Bayesian Framework Using Probabilistic Constraints. *Epidemiology, 17*(2), 145-153 doi:10.1097/01.ede.0000198422 .64801.8d

Bisping, W. (1998). Zur Geschichte der Bekämpfung der Rindertuberkulose - Beiträge aus dem Institut für Mikrobiologie und Tierseuchen der Tierärztlichen Hochschule Hannover. *Berl Munch Tierarztl Wochenschr, 111*(10), 362-367.

Boenchendorf, J. A. D. (2016). *Netzwerkanalyse zur bovinen Tuberkulose im bayerischen Voralpenraum.* Ludwig-Maximians Universität München, München. Retrieved from https://edoc.ub.uni-muenchen.de/20614/

Bolstad, W. M., & M., C. J. (2007). *Introduction to Bayesian statistics*. New Jersey: John Wiley & Sons.

Bossuyt, P. M., Reitsma, J. B., Bruns, D. E., Gatsonis, C. A., Glasziou, P. P., Irwig, L. M., et al. (2015). STARD 2015 – An updated list of essential items for reporting diagnostic accuracy studies. doi: 10.1136/bmj.h5527

Böttcher, J., Janowetz, B., Moser, H., Winter, P., Alex, M., & Meier, N. (2010). *Diagnosis of BHV-1 Infection in the presence of maternally derived antibodies*. Paper presented at the 1st Congress of the European Association of Veterinary Laboratory Diagnosticians, Lelystad.

Brenner, H., & Gefeller, O. (1997). Variation od Sensitivity, Specificity, Likelihood Ratios and Predictive Values with Disease Prevalence. *Statistics in Medicine, 16*(9), 981-991. doi:10.1002/(SICI)1097-0258(19970515)16:9<981:: AID-SIM510>3.0.CO;2-N

Cagiola, M., Feliziani, F., Severi, G., Pasquali, P., & Rutili, D. (2004). Analysis of possible factors affecting the specificity of the gamma interferon test in tuberculosis-free cattle herds. *Clinical and Diagnostoc Laboratory Immunology, 11*(5), 952-956. doi:10.1128/cdli.11.5.952-956.2004

Cambau, E., & Drancourt, M. (2014). Steps towards the discovery of Mycobacterium tuberculosis by Robert Koch, 1882. *Clinical Microbiology and Infection, 20*(3), 196-201. doi:10.1111/1469-0691.12555

Claridge, J., Diggle, P., McCann, C. M., Mulcahy, G., Flynn, R., McNair, J., et al. (2012). Fasciola hepatica is associated with the failure to detect bovine tuberculosis in dairy cattle. *Nature Communications, 3*, 853. doi: 10.1038/ncomms1840

Collins, J. D. (2006). Tuberculosis in cattle: Strategic planning for the future. *Veterinary Microbiology, 112*(2–4), 369-381. doi: 10.1016/j.vetmic.2005.11.041

Corner, L., Melville, L., McCubbin, K., Small, K. J., McCormick, B. S., Wood, P. R., et al. (1990). Efficiency of inspection procedures for the detection of tuberculous lesions in cattle. *Aust Vet J, 67*. doi:10.1111/j.1751-0813. 1990.tb03020.x

Corner, L. A. (1994). Post mortem diagnosis of Mycobacterium bovis infection in cattle. *Veterinary Microbiology, 40*(1–2), 53-63. doi:10.1016/0378-1135(94) 90046-9

Corner, L. A., Melville, L., McCubbin, K., Small, K. J., McCormick, B. S., Wood, P. R., et al. (1990). Efficiency of inspection procedures for the detection of tuberculous lesions in cattle. *Aust Vet J, 67*(11), 389-392. doi:10.1111/j.1751-

0813.1990.tb03020.x

Corner, L. A. L. (2006). The role of wild animal populations in the epidemiology of tuberculosis in domestic animals: How to assess the risk. *Veterinary Microbiology, 112*(2–4), 303-312. doi: 10.1016/ j.vetmic.2005.11.015

Corner, L. A. L., Murphy, D., & Gormley, E. (2011). Mycobacterium bovis Infection in the Eurasian Badger (Meles meles): the Disease, Pathogenesis, Epidemiology and Control. *Journal of Comparative Pathology, 144*(1), 1-24. doi: 10.1016/j.jcpa.2010.10.003

Courcoul, A., Moyen, J. L., Brugere, L., Faye, S., Henault, S., Gares, H., et al. (2014). Estimation of sensitivity and specificity of bacteriology, histopathology and PCR for the confirmatory diagnosis of bovine tuberculosis using latent class analysis. *PloS one, 9*(3), e90334. doi:10.1371/journal.pone.0090334

Cousins, D. V. (2001). Mycobacterium bovis infection and control in domestic livestock. *Rev Sci Tech, 20*(1), 71-85.

Cousins, D. V. (2009). Bovine tuberculosisManual of Diagnostic Tests and Vaccines for Terrestrial Animals 2014 (pp. 1-16). Paris: World Organisation for Animal Health. Retrieved from http://www.oie.int/international-standard-setting/terrestrial-manual/access-online/.

Cowie, C. E., Gortázar, C., White, P. C. L., Hutchings, M. R., & Vicente, J. (2015). Stakeholder opinions on the practicality of management interventions to control bovine tuberculosis. *The Veterinary Journal, 204*(2), 179-185. doi: 10.1016/j.tvjl.2015.02.022

de la Rua-Domenech, R., Goodchild, A. T., Vordermeier, H. M., Hewinson, R. G., Christiansen, K. H., & Clifton-Hadley, R. S. (2006). Ante mortem diagnosis of tuberculosis in cattle: a review of the tuberculin tests, gamma-interferon assay and other ancillary diagnostic techniques. *Research in veterinary science, 81*(2), 190-210. doi:10.1016/j.rvsc.2005.11.005

Doherty, M. L., Bassett, H. F., Quinn, P. J., Davis, W. C., & Monaghan, M. L. (1995). Effects of dexamethasone on cell-mediated immune responses in cattle sensitized to Mycobacterium bovis. *Am J Vet Res, 56*(10), 1300--1306.

Dohoo, I., Martin, W., & Stryhn, H. (2009). *Veterinary epidemiologic research*. Charlottetown, Canada.

Domogalla, J., Prodinger, W. M., Blum, H., Krebs, S., Gellert, S., Mller, M., et al. (2013). Region of difference 4 in alpine Mycobacterium caprae isolates indicates three variants. *J Clin Microbiol, 51*(5), 1381--1388. doi:10.1128/JCM.02966-12

Enøe, C., Georgiadis, M. P., & Johnson, W. O. (2000). Estimation of sensitivity and specificity of diagnostic tests and disease prevalence when the true disease state is unknown. *Preventive Veterinary Medicine, 45*(1–2), 61-81. doi: 10.1016/S0167-5877(00)00117-3

Faye, S., Moyen, J.-L., Gares, H., Benet, J.-J., Garin-Bastuji, B., & Boschiroli, M.-L. (2011). Determination of decisional cut-off values for the optimal diagnosis of bovine tuberculosis with a modified IFNγ assay (Bovigam®) in a low prevalence area in France. *Veterinary Microbiology, 151*(1–2), 60-67. doi:10.1016/j.vetmic.2011.02.026

Fell, S., Bröckl, S., Büttner, M., Rettinger, A., Zimmermann, P., & Straubinger, R. K. (2016). Two alternative DNA extraction methods to improve the detection of Mycobacterium-tuberculosis-complex members in cattle and red deer tissue samples. *BMC Microbiology, 16*(1), 213. doi:10.1186/s12866-016-0816-2

Flynn, R., J., Mannion, C., Golden, O., Hacariz, O., & Mulcahy, G. (2007). Experimental Fasciola hepatica Infection Alters Responses to Tests Used for Diagnosis of Bovine Tuberculosis. *Infect Immun, 75*(3), 1373--1381. doi:10.1128/IAI.01445-06

Francis, J., Seiler, R. J., Wilkie, I. W., Oboyle, D., Lumsden, M. J., & Frost, A. J. (1978). Sensitivity and specificity of various tuberculin tests using bovine PPD

and other tuberculins. *Veterinary Record, 103*(19), 420-425.

Gerstmair, E.-M. (2011). *Validierung molekularbiologischer und immunologischer Nachweisverfahren für die Tuberkulose bei Rindern und Tuberkulosemonitoring beim Rotwild.* Ludwig-Maximians Universität München, München. Retrieved from https://edoc.ub.uni-muenchen.de/13467/1/ Gerstmair_Eva_Maria.pdf

Good, M. (2011). *The Tuberculin Test and its Role in the Strategic Management and Eradication of Tuberculosis in Cattle.* (Dissertation), Utrecht University, Utrecht.

Gormley, E., Doyle, M. B., McGill, K., Costello, E., Good, M., & Collins, J. D. (2004). The effect of the tuberculin test and the consequences of a delay in blood culture on the sensitivity of a gamma-interferon assay for the detection of Mycobacterium bovis infection in cattle. *Vet Immunol Immunopathol, 102*(4), 413-420. doi:10.1016/j.vetimm.2004.08.002

Grange, J. M., & Yates, M. D. (1994). Zoonotic aspects of Mycobacterium bovis infection. *Veterinary Microbiology, 40*(1-2), 137-151.

Hartnack, S., Budke, C. M., Craig, P. S., Jiamin, Q., Boufana, B., Campos-Ponce, M., et al. (2013). Latent-Class Methods to Evaluate Diagnostics Tests for Echinococcus Infections in Dogs. *PLOS Neglected Tropical Diseases, 7*(2), e2068. doi:10.1371/journal.pntd.0002068

Hartnack, S., Nathues, C., Nathues, H., Grosse Beilage, E., & Lewis, F. I. (2014). Estimating diagnostic test accuracies for Brachyspira hyodysenteriae accounting for the complexities of population structure in food animals. *PloS one, 9*(6), e98534. doi:10.1371/journal.pone.0098534

Hartnack, S., & Torgerson, P. (2012). The accuracy of the Single Intradermal Comparative Skin Test for the diagnosis of Bovine Tuberculosis estimated from a systematic literature search. *J Mycobac Dis, 2*(120), 2161-1068. doi:10.4172/2161-1068.1000120

Held, J. (2014). Keine sterile Kanüle für Rinder TBC-Test nötig. Retrieved from http://www.wir-sind-tierarzt.de/2014/12/keine-sterile-kanuele-fuer-rinder-tbc-test-noetig/

Hofmann, W., Bostedt, H., & Bardella, I. (2005). *Rinderkrankheiten: Innere und chirurgische Erkrankungen* (Vol. 8044): UTB.

Homeier-Bachmann, T., Reimer, N., & Schell, S. (2016). Tiergesundheitsjahresbericht 2015.

Hui, S., & Walter, S. (1980). Estimating The Error Rates of Diagnostic-Tests. *Biometrics, 36*, 167 - 171.

Hui, S. L., & Walter, S. D. (1980). Estimating the Error Rates of Diagnostic Tests. *Biometrics, 36*(1), 167-171. doi:10.2307/2530508

Hui, S. L., & Zhou, X. H. (1998). Evaluation of diagnostic tests without gold standards. *Statistical Methods in Medical Research, 7*(4), 354-370. doi:10.1177/096228029800700404

Humblet, M.-F., Walravens, K., Salandre, O., Boschiroli, M., Gilbert, M., Berkvens, D., et al. (2011). Monitoring of the intra-dermal tuberculosis skin test performed by Belgian field practitioners. *Research in veterinary science, 91*(2), 199-207.

Humblet, M. F., Boschiroli, M. L., & Saegerman, C. (2009). Classification of worldwide bovine tuberculosis risk factors in cattle: a stratified approach. *Vet Res, 40*(5), 50. doi:10.1051/vetres/2009033

Hunermund, G., & Kropp, R. (2006). The battle against cattle tuberculosis in Germany. [Die Bekampfung und Ausrottung der Rindertuberkulose in Deutschland.]. *Pneumologie, 60*(12). doi:10.1055/s-2006-944284

Jungi, T. W. (2000). *Klinische Veterinärimmunologie*: Enke.

Koch, O., & Selzer, P. M. (2012). Biologie der Mykobakterien und neue molekulare Targets. *Pharmazie in unserer Zeit, 41*(1), 19-26. doi:10.1002/pauz.201100449

Köhler, H., Hoffmann, B., Moser, I., & Möbius, P. (2013). *Novel probe-based real-time PCR for rapid diagnosis of bovine tuberculosis in tissue samples with suspect lesions.* Paper presented at the 16th International Symposium of the World Association of Veterinary Laboratory Diagnosticians, Berlin.

Köhler, H., Moser, I., & Menge, C. (2012). *Tuberkulose des Rindes: wieder ein Thema?!* Paper presented at the AfT/TiHo -Symposium Hannover.

Lesaffre, E., Speybroeck, N., & Berkvens, D. (2007). Bayes and diagnostic testing. *Veterinary Parasitology, 148*(1), 58-61. doi: 10.1016/j.vetpar.2007.05.010

Marsot, M., Béral, M., Scoizec, A., Mathevon, Y., Durand, B., & Courcoul, A. (2016). Herd-level risk factors for bovine tuberculosis in French cattle herds. *Preventive Veterinary Medicine, 131*(Supplement C), 31-40. doi: 10.1016/j.prevetmed.2016.07.006

Menzies, F. D., & Neill, S. D. (2000). Cattle-to-Cattle Transmission of Bovine Tuberculosis. *The Veterinary Journal, 160*(2), 92-106. doi: 10.1053/tvjl.2000.0482

Michel, A. L., Müller, B., & van Helden, P. D. (2010). Mycobacterium bovis at the animal–human interface: A problem, or not? *Veterinary Microbiology, 140*(3–4), 371-381. doi: 10.1016/j.vetmic.2009.08.029

Monaghan, M. L., Doherty, M. L., Collins, J. D., Kazda, J. F., & Quinn, P. J. (1994). The tuberculin test. *Veterinary Microbiology, 40*(1–2), 111-124. doi: 10.1016/0378-1135(94)90050-7

Morris, R. S., Pfeiffer, D. U., & Jackson, R. (1994). The epidemiology of Mycobacterium bovis infections. *Veterinary Microbiology, 40*(1–2), 153-177. doi:10.1016/0378-1135(94)90053-1

Moser, I., Köhler, H., & Menge, C. (2014). Die Tuberkulose des Rindes–überraschend wieder oder immer noch präsent? *Tierärztliche Praxis Großtiere, 4*(2014).

Müller, M., Hafner-Marx, A., Ehrlein, J., Ewringmann, T., Ebert, U., Weber, B., et al. (2014). *Pathomorphologische Veränderungen bei der Tuberkulose des Rotwildes* (Vol. 4).

Narad, M. E., Treble-Barna, A., Peugh, J., Yeates, K. O., Taylor, H. G., Stancin, T., et al. (2017). Recovery trajectories of executive functioning after pediatric TBI: a latent class growth modeling analysis. *The Journal of head trauma rehabilitation, 32*(2), 98-106.

Norby, B., Bartlett, P. C., Fitzgerald, S. D., Granger, L. M., Bruning-Fann, C. S., Whipple, D. L., et al. (2004). The sensitivity of gross necropsy, caudal fold and comparative cervical tests for the diagnosis of bovine tuberculosis. *Journal of Veterinary Diagnostic Investigation, 16*(2), 126-131.

O'Reilly, L. M., & Daborn, C. J. (1995). The epidemiology of Mycobacterium bovis infections in animals and man: A review. *Tubercle and Lung Disease, 76, Supplement 1*(0), 1-46. doi: 10.1016/0962-8479(95)90591-X

Parra, A., García, N., García, A., Lacombe, A., Moreno, F., Freire, F., et al. (2008). Development of a molecular diagnostic test applied to experimental abattoir surveillance on bovine tuberculosis. *Veterinary Microbiology, 127*(3), 315-324.

Payne, A., Chappa, S., Hars, J., Dufour, B., & Gilot-Fromont, E. (2016). Wildlife visits to farm facilities assessed by camera traps in a bovine tuberculosis-infected area in France. *European Journal of Wildlife Research, 62*(1), 33-42.

doi:10.1007/s10344-015-0970-0

Pritchard, D. G. (1988). A century of bovine tuberculosis 1888–1988: Conquest and controversy. *Journal of Comparative Pathology, 99*(4), 357-399. doi: 10.1016/0021-9975(88)90058-8

Prodinger, W. M., Eigentler, A., Allerberger, F., Schönbauer, M., & Glawischnig, W. (2002). Infection of Red Deer, Cattle, and Humans with Mycobacterium bovis subsp. caprae in Western Austria. *J Clin Microbiol, 40*(6), 2270-2272. doi:10.1128/jcm.40.6.2270-2272.2002

Pucken, V.-B., Götz, F., Mansfeld, R., Moder, S., Sauter-Louis, C., Straubinger, R. K., et al. (2015). Der Tuberkulin-Hauttest: Literatur, Richtlinie und Umsetzung in der Praxis. *Tierärztliche Praxis Großtiere*(2015-07-02 00:00:00). doi:10.15653/TPG-141145

Pucken, V.-B., Knubben-Schweizer, G., Döpfer, D., Groll, A., Hafner-Marx, A., Hörmansdorfer, S., et al. (2017). Evaluating diagnostic tests for bovine tuberculosis in the southern part of Germany: A latent class analysis. *PloS one, 12*(6), e0179847. doi:10.1371/journal.pone.0179847

Rabenau, H. F., Kessler, H. H., Kortenbusch, M., Steinhorst, A., Raggam, R. B., & Berger, A. (2007). Verification and validation of diagnostic laboratory tests in clinical virology. *J Clin Virol, 40*(2), 93 - 98.

Rastogi, N., Legrand, E., & Sola, C. (2001). The mycobacteria: an introduction to nomenclature and pathogenesis. *Revue Scientifique Et Technique-Office International Des Epizooties, 20*(1), 21-54.

Reddington, K., O'Grady, J., Dorai-Raj, S., Niemann, S., van Soolingen, D., Barry, T., et al. (2011). A Novel Multiplex Real-Time PCR for the Identification of Mycobacteria Associated with Zoonotic Tuberculosis. *PloS one, 6*(8), e23481. doi:10.1371/journal.pone.0023481

Rodríguez, J. G., Fissanoti, J. C., Del Portillo, P., Patarroyo, M. E., Romano, M. I., & Cataldi, A. (1999). Amplification of a 500-Base-Pair Fragment from Cultured Isolates of Mycobacterium bovis. *J Clin Microbiol, 37*(7), 2330-2332.

Rodriguez, J. G., Mejia, G. A., Del Portillo, P., Patarroyo, M. E., & Murillo, L. A. (1995). Species-specific identification of Mycobacterium bovis by PCR. *Microbiology, 141*(9), 2131-2138. doi:10.1099/13500872-141-9-2131

Rolle, M., Mayr, A., Selbitz, H.-J., Truyen, U., & Valentin-Weigand, P. (2011). *Tiermedizinische Mikrobiologie, Infektions und Seuchenlehre*. Stuttgart: Enke Verlag.

Ryan, T. J., Livingstone, P. G., Ramsey, D. S. L., de Lisle, G. W., Nugent, G., Collins, D. M., et al. (2006). Advances in understanding disease epidemiology and implications for control and eradication of tuberculosis in livestock: The experience from New Zealand. *Veterinary Microbiology, 112*(2–4), 211-219. doi: 10.1016/j.vetmic.2005.11.025

Sattelmair, H. (2005). *Die Tuberkulose des Rindes-ein Beitrag zur Geschichte der Haustierkrankheiten.* Freie Universität Berlin, Berlin. Retrieved from http://www.diss.fu-berlin.de/diss/servlets/MCRFileNodeServlet/FUDISS_derivate_000000001736/00_Sattelmair_00.pdf

Schiller, I., Oesch, B., Vordermeier, H. M., Palmer, M. V., Harris, B. N., Orloski, K. A., et al. (2010a). Bovine tuberculosis: a review of current and emerging diagnostic techniques in view of their relevance for disease control and eradication. *Transbound Emerg Dis, 57*(4), 205-220. doi:10.1111/j.1865-1682.2010.01148.x

Schiller, I., RayWaters, W., Vordermeier, H. M., Jemmi, T., Welsh, M., Keck, N., et al. (2011). Bovine tuberculosis in Europe from the perspective of an officially tuberculosis free country: Trade, surveillance and diagnostics. *Veterinary Microbiology, 151*(1–2), 153-159. doi: 10.1016/j.vetmic.2011.02.039

Schiller, I., Vordermeier, H. M., Waters, W. R., Whelan, A. O., Coad, M., Gormley, E., et al. (2010b). Bovine tuberculosis: effect of the tuberculin skin test on in vitro interferon gamma responses. *Vet Immunol Immunopathol, 136*(1-2), 1-11. doi:10.1016/j.vetimm.2010.02.007

Sewpersadh, M. (2012). *Differences between genotypic and phenotypic resistance in MDR-TB strains in South Africa.* Faculty of Health Science, University of the Witwatersrand, Johannesburg.

Shinnick, T. M., & Good, R. C. (1994). Mycobacterial taxonomy. *European Journal of Clinical Microbiology and Infectious Diseases, 13*(11), 884-901. doi:10.1007/BF02111489

Skuce, R. A., Allen, A. R., & McDowell, S. W. J. (2011). *Bovine Tuberculosis (TB): A Review Of Cattle-To-Cattle Transmission, Risk Factors And Susceptibility*. Retrieved from

Strain, S. A. J., McNair, J., & McDowell, S. W. J. (2011a). *Bovine tuberculosis: a review of diagnostic tests for M. bovis infection in badgers*. Retrieved from https://www.daera-ni.gov.uk/sites/default/files/publications/dard/afbi-literature-review-tb-review-diagnostic-tests-badgers.pdf

Thoen, C. O., Steele, J. H., & Gilsdorf, M. J. (2006). *Mycobacterium bovis Infection in Animals and Humans*: Wiley.

Toft, N., Jørgensen, E., & Højsgaard, S. (2005). Diagnosing diagnostic tests: evaluating the assumptions underlying the estimation of sensitivity and specificity in the absence of a gold standard. *Preventive Veterinary Medicine, 68*(1), 19-33. doi: 10.1016/j.prevetmed.2005.01.006

Vacek, P. M. (1985). The Effect of Conditional Dependence on the Evaluation of Diagnostic Tests. *Biometrics, 41*(4), 959 - 968. doi: 10.2307/2530967

Waddington, K. (2012). To Stamp Out “So Terrible a Malady”: Bovine Tuberculosis and Tuberculin Testing in Britain, 1890–1939. *Medical History, 48*(1), 29-48. doi:10.1017/S0025727300007043

Wagner, A. (2015). Tierarztfrust über praxisuntaugliche TBC-Test-Empfehlung Retrieved from http://www.wir-sind-tierarzt.de/2015/02/praxisuntaugliche-tbc-test-empfehlung/

Walter, S. D., & Irwig, L. M. (1988). Estimation of test error rates, disease prevalence and relative risk from misclassified data: a review. *Journal of Clinical Epidemiology, 41*(9), 923-937. doi: 10.1016/0895-4356(88)90110-2

Wards, B. J., Collins, D. M., & de Lisle, G. W. (1995). Detection of Mycobacterium bovis in tissues by polymerase chain reaction. *Veterinary Microbiology, 43*(2–3), 227-240. doi:10.1016/0378-1135(94)00096-F

Wood, P. R., & Jones, S. L. (2001). BOVIGAM: an in vitro cellular diagnostic test for bovine tuberculosis. *Tuberculosis (Edinburgh, Scotland), 81*(1), 147-155. doi:10.1054/tube.2000.0272

Zellner, G. (2013). *Untersuchungsprogramm "Rindertuberkulose in den Landkreisen der Alpenkette"*. München.

VIII. ACKNOWLEDGEMENTS

First of all I would like to thank my thesis advisor Prof. Dr. Gabriela Knubben-Schweizer for all her help and support. Without your belief in me I would never have made it so far. Thanks for all your help, the numerous supportive talks and your trust!

Second I would like to thank my supervisor Dr. Sonja Hartnack for giving me so much support with the statistics. The latent class analysis would never have happened without your knowledge! Thank you for sharing this and giving me an inside view into latent class analysis and Bayesian approach. Additionally I would like to thank you for the eye-opening talks about epidemiology, animal welfare and ethics.

In addition I would like to thank all the people that were involved in this project. R. K. Straubinger for his support, his advice and the additional data of the Bovigam® assay. D. Döpfer and A. Groll for their help with the latent class analysis. A. Hafner-Marx, P. Zimmermann and S. Hörmansdorfer for sharing the data of the diagnostic tests and explaining me all the abbreviations and things to respect. M. Büttner for his support. C. Sauter-Louis for giving me the excellent book on R and her help. The staff members of the veterinary authorities from Ober- and Ostallgäu, especially F. Götz and J. Mages, for all the skin test results, the chance to get an inside view into animal disease control, and the experience of swimming in the Forggen-Lake. O. Stadler and W. Schuh for the great pictures for the hand out and for having tee after a hard carcass disposal plant day.

Further I would like to thank all the staff member and peers from the Clinic for Ruminants with Ambulatory and Herd Health Services in Oberschleissheim. You gave me such a great time! In particular I would like to thank Julia for sharing this bovine TB experience, the great talks that we had and for winning a new friend! I would like to thank Chrissy for making the start so much nicer and for sharing a coffee with me at the end of the world! Furthermore, I would like to thank Freddy for giving me support when I needed it and having always an sympathetic ear. And Susanne for her smile and giving me always a warm welcome.

A very special thank-you goes to my family! Without your help I would never have reached all of this! Thanks for giving me the opportunity to realize my dream

and for standing always behind me!

And thank you Frank for accepting me always like I am. For giving me enjoyable breaks and the try to give us a home.

At last I would like to thank all my friends for their patience and support! Thank you Rupert for all the talks and the correction! Thank you Sophie, Melissa, Falk, Eike, Maya, Mitra, Hannah and Isolde for supporting me the last years through every event that happened!