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AEEMA-RFSA MEETING - MARCH 18th, 2014: BOVINE TUBERCULOSIS - RESULTS OF RECENT APPLIED RESEARCH WORK DONE IN FRANCE

Particular features of screening and diagnosis of bovine tuberculosis

N. Keck, J-L. Moyen, E. Gueneau & Maria-Laura Boschioli

The emergence of tuberculosis outbreaks in certain geographical areas of France has led to strengthening of surveillance and management methods. They have to rely on adapted herd screening methods and *post-mortem* diagnostic tests. However, certain epidemiological, immunological and pathogenic characteristics of the disease can constrain performance of the available tests. An early detection of the infection in live animals is possible with cellular mediated immunology (CMI) based tests, such as intradermal tuberculin test and the gamma interferon test. The use of specific antigens of *Mycobacterium tuberculosis* complex species has enhanced the specificity of the latter, which however still needs to be improved. The antibody-based assays are generally considered as poorly sensitive, although they can improve detection of animals with particular infectious status which are not recognized by CMI-based tests, such as anergic animals. Direct diagnosis with samples taken at the slaughterhouse combines nowadays molecular and bacteriological techniques, as well as histologic examination, in order to improve diagnostic sensitivity and reduce the infection confirmation delays. The diagnosis of bovine tuberculosis remains extremely challenging, as there is currently no single test able to identify all infected animals, and the performance estimates for the tests can be dependent upon local epidemiological patterns within the studied animal population. Consequently, a combination of approaches is needed to achieve an adequate level of diagnosis, in order to improve either the sensitivity (parallel testing) or the specificity (serial testing). The conditions in which screening and diagnostic tests can be associated and their results interpreted for the implementation of management measures should be defined according to scientific data coming from different epidemiological situations. The easier and less costly use of certain sophisticated techniques open interesting perspectives for the future, requiring being evaluated beforehand by research studies. They will lead to the development of diagnostic schemes and to the adaptation of the laboratory network to these evolutions.

Bovine tuberculosis confirming tests assessment: comparison of PCR, histology and culture

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Bacteriology and histopathology are the most commonly used tests for official confirmatory diagnosis of bovine tuberculosis (bTB) in cattle. In most countries, PCR is also increasingly being used because it allows a fast diagnosis. This test can be applied as a supplement to or a replacement for current bTB confirmatory diagnostic tests but its characteristics have first to be evaluated. This study was designed to estimate and compare sensitivities and specificities of bacteriology, histopathology and PCR under French field conditions, in the absence of a gold standard using latent class analysis. The studied population amounted to 5,211 animals from

whom the samples were subjected to bacteriology and PCR (LSI VetMAX™ *Mycobacterium tuberculosis* Complex PCR Kit, Life Technologies). Samples from 697 of these animals were also subjected to histopathology. Bayesian models were developed, allowing for dependence between bacteriology and PCR, while assuming independence from histopathology. The sensitivity of PCR was higher than that of bacteriology (on average 87.7% [82.5-92.3%] versus 78.1% [72.9-82.8%]) while specificity of both tests was found to be very high (on average 97.0% for PCR [94.3-99.0%] and 99.1% for bacteriology [97.1-100.0%]). Histopathology was at least as sensitive as PCR (on average 93.6% [89.9-96.9%]) but less specific than the two other tests (on average 83.3% [78.7-87.6%]). These results suggest that PCR has the potential to replace bacteriology to confirm bTB diagnosis on samples collected from suspected cattle.

Assessment of the sensitivity of tests for the diagnosis of bovine tuberculosis: serial association of skin test and gamma-interferon test

Anne Praud & Barbara Dufour

The aim of this study was to assess the accuracy of a testing suspicion scheme associating skin tests and gamma interferon test assay (IFN) in Côte-d'Or between 2009 and 2012, and to compare its sensitivity to the sensitivity of the scheme recommended by European Union. The sensitivities of single and comparative intradermal skin tests (SIT and SICCT, respectively) and IFN were estimated using a latent class model implemented through a Bayesian approach. Then, the sensitivities of both serial schemes (SICCT-IFN and SIT-SICCT) were estimated. No significant difference of sensitivity could be showed between the Côte-d'Or protocol (serial testing scheme with SICCT and IFN) and the E.U. protocol (serial testing scheme with SIT and SICCT). Nevertheless, this protocol engenders some biases: the interpretation of the results should thus be cautious.

First assessment of the use of serology for the diagnosis of bovine tuberculosis in France

J-L. Moyen, E. Gueneau, N. Keck, Hélène Gares & Maria-Laura Boschioli

Bovine tuberculosis (bTB) diagnosis relies mainly on skin test (ST), which shows good intrinsic characteristics that can however be significantly hampered by field performing conditions and cross reactions. The difficulties with the interferon gamma test are related to its very complicated logistics and its high cost, which constrains its use to particular contexts. Serology is largely employed in prophylactic campaigns against many animal diseases. It is rapid, practical and quite affordable. Its use in beef or in bullfighting herds is simpler than for ST which needs two veterinary interventions at a 3-days interval. However, sensitivity for mycobacteria's diagnosis is reduced and antibody high titers are detected too late after infection which makes its use impossible for an early detection of bTB infection. New specific antigens of the *M. tuberculosis* complex (MTC) species have led to promising serologic tools. The first assays performed in different countries where bTB is enzootic have shown good results (Se: 60-90%). This has led to first field evaluation in France with the IDEXX serologic kit *M. bovis*. Specificity and sensitivity tests have been conducted in Camargue, Côte-d'Or and in Dordogne. Specificity is good and homogeneous among different breeds, regions and laboratories (96.9 to 99.7%). In contrast, sensitivity is acceptable in bullfighting animals with lesion (average 50%) but disappointing in milk or beef cattle (0 to 30%). The very low inside-herd prevalence, associated to the early detection of infected herds could explain those

results, which contrast with those obtained in other countries. Additional tests were performed in animals from infected herds previously tested by IDT, in order to take advantage of a possible anamnestic response. Preliminary results, even though not numerous, are quite encouraging (Se: 50-60%) and close to those obtained by other countries when a similar use was employed. Nonetheless, they have to be confirmed and validated in a more important number of animals. Serology could be used in animals previously skin tested as a complementary tool to cellular mediated immunology' based tests, in order to increase detection sensitivity in infected herds, particularly for anergic animals.

Analysis of epidemiological data produced by field control in France

J-J. Bénet & Barbara Dufour

Epidemiological studies by trainees, have been conducted over the past 10 years using data produced by the health plan against bovine tuberculosis in France. The first type of study, descriptive, confirmed the perception of local actors, namely the low number of infected cattle per herd (up to 3 in 71% of cases after discovery of a cattle with Tb lesions at the slaughterhouse, or 66% of cases upon detection by a skin test). But the exploitation of data collected in 1988 showed that the corresponding percentages were not significantly different. Geographical distribution was localized in some areas where some risk factors have been identified (number of animals per farm, age of the reformed animals and fragmentation of pastures). The second type of study focused on quality testing. The sensitivities of the single and comparative skin tests at herd level were calculated, respectively, 0.95 and 0.90. Very large differences between counties were observed in rates of herds in which at least one bovine had a nonspecific reaction to skin test, from 5% up to 40% or more. They corresponded to minimal individual specificity differences (from 0.993 up to 0.9997), herd size significantly amplifying their effect. The comparison of tuberculin injection devices has served to legitimize the use of new syringes in place of the traditional one. A protocol alternative to the measurement of skin fold before injection has been studied (J3 measure either slightly forward or on the other side of the neck): change of injection site introduced an additional variability, which alter the result of 6 out of the 19 concerned cattle, leading to pursue this study. The discussion serves to highlight the implications of these findings from the perspective of the design of the disease control.

Comparison by molecular methods of *Mycobacterium bovis* strains, isolated from bovine herds and wild animals

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Genotyping of *Mycobacterium bovis* strains, responsible of the tuberculosis in mammals, provides valuable information for studying dynamics of this zoonotic disease and better understanding its complexity. In France, the total collection of animal *M. bovis* strains, from wildlife and livestock, identified from 1978 to 2011, were genotyped by spoligo-typing and VNTR typing. Fluctuations in genetic variability, depending on time and geographical localization, were observed with a decrease in the genetic diversity during the past 10 years. However, some strains which are widespread in the whole French territory tend to persist. Three of them, represent more than 50% of the global strains identified: SB0120, SB0134 and

the F4-family. VNTR typing is very useful for highlighting their important genotypic diversity and strong regionalization of some strains. Besides, a better genotyping characterization had shown that the same type of strains can be identified both in wildlife and cattle. This observation proves the existence of multi-host *M. bovis* transmission systems. To conclude, molecular characterization tools in bovine tuberculosis have an important role to complete classical epidemiologic surveys. They permit to improve outbreak investigations, to implement new surveillance schemes, and to evaluate the efficacy of national control programs.

Impact of change of cattle population structure on bovine tuberculosis in France between 1965 and 2000

M. El Amine Bekara, Lamiae Azizi, J-J Bénet & B. Durand

We analysed the spatiotemporal variations in bovine tuberculosis (bTB) incidence between 1965 and 2000 in France at the department level. Using a Bayesian space-time model, we studied the association between changes in bTB incidence and changes in structure of the cattle population and in herd management practices. Southern France remained a high-risk area over the period analysed. Four factors were found to be associated with an increase in the risk of bTB: average herd density and size, percentage of dairy cows in the cattle population, and percentage of permanent grassland in cultivated areas. Our results suggest that, besides mandatory detection and control procedures, other trends (professionalization and specialization) played a significant role in reducing the risk of bTB in France between 1965 and 2000.

Study of neighbourhood between bovine herds on 2010 by network analysis

Laure Dommergues, Séverine Rautureau, E. Petit & Barbara Dufour

France attained “Officially Tuberculosis-Free” status in 2001. However, the Côte-d’Or department (a French administrative unit) has since seen an increase in bovine tuberculosis (bTB) cases, with 35% of cases attributed to neighbourhood contamination. The aim of this field study was to investigate the risk factors of neighbourhood contacts in an area affected by bTB in 2010, through the use of social network methods. Contacts were weighted, as not all types of contact carried the same risk of bTB transmission. We attributed 95% of the contacts to direct contact in pasture or contact with wild boars or badger latrines. Most of the contact types were correlated but none was sufficient in itself to account for all contacts between one particular farm and its neighbours. Contacts with neighbours therefore represent a challenge for the implementation or improvement of on farm control measures.

Quantification of the transmission risk of bovine tuberculosis by cattle trades

Aurore Palisson, J-J. Bénet & B. Durand

France became officially bovine Tuberculosis (bTB) free in 2001. But there are about 100 cases every year, some of them due to cattle trade. A retrospective cohort study was performed to quantify the risk for a herd to be infected by cattle bough from herds later detected as infected. The hazard, represented by a cattle, was formalized using the time spent in the selling herd and the time between sale and disease report in that herd. Purchases of cattle which lived in infected herds during the sixth or the first years before disease report were bTB breakdown risk factors, with adjusted OR of 2.89 [1.54-5.44] and 4.44 [1.01-19.54]

respectively. These results are consistent with the disease pathogenesis. Some herds may show a fast within-herd transmission. Therefore, the most dangerous animals would be those sold during the year prior bTB report. On the contrary, the disease may show a slow within-herd transmission and animals sold six years before bTB report could be infected.

Detection of *Mycobacterium bovis* in environment: first results of research done in Côte-d'Or

Elodie Barbier & A. Hartmann

Since year 2000, an increasing number of cases of bovine tuberculosis were found in cattle in Côte-d'Or. These are caused Wild animals Infected by *Mycobacterium bovis* found near contaminated farms were considered as the likely source of contamination. We assume that contamination between wild and domestic animals may occur indirectly, by environmental factors (soil, water, faeces). To quantify the prevalence of *M. bovis* in the environment and to evaluate its role in indirect transmission of the disease between species, we developed molecular detection tools. These are adapted to complex environmental matrices (soil, water, faeces), and are both specific and sensitive. They allowed us to analyse numerous samples collected in 2013. Two samples gave slight positive results.

Role of wildlife in the *Mycobacterium bovis* multi-host system in Côte-d'Or: assessment of the risk of spillback to cattle and the risk of reservoir constitution

Ariane Payne, J. Hars, Céline Fossot, Élodie Barbier, A. Hartmann, Maria-Laura Boschioli, É. Guéneau, Barbara Dufour & Émmanuelle Gilot-Fromont

Our objective was to investigate the role of the different wild populations involved in the *M. bovis* multi-host system of Côte-d'Or. In this region, bovine tuberculosis has been reoccurring in cattle since 2002 and has also been found in badgers, wild boar, red deer and foxes. To do so, we have assessed different risk factors. These include infection rate, ability to shed *M. bovis*, populations' densities and level of indirect contact between these wild populations and cattle. In this paper, we have focused on the excretion capacity and the estimation of the level of contact by using remote surveillance in farms. Our results suggest that, in this study site, badgers, wild boar and red deer may be able to spillback the infection to cattle. Nevertheless, further studies are required to confirm these hypotheses and to investigate whether some of these wild populations can act, individually as reservoirs. It might also be the case that, taken jointly, these wild populations could constitute a maintenance community.

Using a serological tool to assess the exposure of wild boar to *Mycobacterium bovis*

Céline Richomme, Mariana Boadella, Aurélie Courcoul, B. Durand, A. Drapeau, Y. Corde, J. Hars, Ariane Payne, A. Fediaevsky & Maria Laura Boschioli

The Eurasian wild boar (*Sus scrofa*) is increasingly considered as a significant actor in the epidemiology of animal tuberculosis (TB). Therefore, monitoring TB in wild boar becomes a key tool in the development of comprehensive control schemes for this disease. To estimate the exposure of free-living wild boars to the *Mycobacterium tuberculosis* complex (MTC) in France, a bovine-purified protein derivative (PPD) based ELISA was used to test 2,080 archived serum samples from hunter-harvested animals in 58 French "départements". Two cut-off values were used for diagnostic interpretation: 0.2, recommended by the manufacturer

(specificity: 96.4%; sensitivity: 72.6%), and 0.5 (specificity: 100%; sensitivity: 64%). A good consistence between seropositive wild boar and TB outbreaks in cattle was found, especially at the 0.5 cut-off value (the mean distance to the nearest cattle TB outbreak was 13km for seropositive wild boars). Our results provide a first description of the global seroprevalence and geographic distribution of MTC contact in wild boars in France but they also suggest that the ELISA could be used as a first screening tool to conduct TB surveillance in wild boars at a population level, to be completed, in case of seropositivity, by investigations combining pathology and culture.