Bovine Tuberculosis in Latin American Countries. Current situation and recommendations
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Summary

The relevance of bovine tuberculosis (BTB) infection in cattle varies among different Latin American and Caribbean countries, affecting especially dairy cattle. BTB has decreased considerably in countries where control and surveillance activities are conducted. The increasing food demand due to the world global growth emphasizes the importance of control and eradication programs (CEP) for zoonotic diseases in the Region, in which several countries are producers and exporters of meat and dairy products. BTB control and its eventual eradication will have a positive impact both on the economy and on the public health in these countries.

BTB is transmitted to man by ingestion of contaminated milk and dairy products, or by respiratory route. In infected areas, rural inhabitants, especially children and slaughterhouse workers, constitute the main population at risk. Main aspects considered relevant for the strengthening of national CEP are: sustainable financial and technical support, standardized methods and reagents for the tuberculin test in cattle, efficient systems of veterinary inspection in slaughterhouses and meat processing plants, epidemiological tracing, slaughter of infected animals detected and trace-back to the farm, and public information on the problem. In the present report the analysis and conclusions from a workshop on BTB in Latin America and the Caribbean , sponsored by OIE, are presented.

Keywords

Bovine tuberculosis – Latin America - *Mycobacterium bovis* – Tuberculin test –
Veterinary inspection.

Introduction

Tuberculosis infection in bovines exists in most of the countries in the Latin American Region (LAC), with variable relevance and especially concentrated in dairy cattle. Control and surveillance activities are performed in all LAC countries; and several of these are already achieving the eradication phase (Cuba, Costa Rica, Panama, Uruguay) (10,12, 13, 16, 19, 20, 28, 30, 31, 34-37, 39, 41, 42, 50, 51, 53).

Bovine tuberculosis (BTB) limits livestock production, and affects their quality. The etiological agent, *Mycobacterium bovis*, is transmitted to humans through contaminated milk and dairy products, being the source of primary tuberculosis (TB) in children and infants (mainly extra pulmonary forms: miliary, meningeal, or lymphatic nodes- localized). The respiratory route is the transmission origin of pulmonary TB cases in exposed adults, like slaughterhouse and meat processing industry workers (2, 5, 14, 15, 27, 40).
The world global growth in the demand for food contributes to emphasize the importance of control and eradication programs for zoonotic diseases in our Region, where several countries are relevant producers and exporters of meat and dairy products. BTB control and its eventual eradication will have a positive impact both on the economy and on the public health in these countries.

Taking all this issues into consideration, this Workshop, sponsored by OIE, held at the 3rd Latin American Congress of Zoonoses, focused on identifying the priorities for National BTB Control and Eradication Programs (CEP), to formulate recommendations for these Programs on basic subjects such as the infection diagnosis in cattle, its standardization and quality assurance, the veterinary inspection in slaughterhouses and meat processing plants, cattle tracing, epidemiological surveillance, and on the scientific and technological research priorities for the Region in this field.

Based on information from the public health laboratories in LAC (15), an estimation was made on the importance of TB due to M. bovis in humans in relation to TB due to M. tuberculosis (human tubercle bacillus), and on the need for a better coordination between human and animal health fields, to achieve an integral control of TB in the Region.

Conclusions and Recommendations

**Tuberculosis in cattle**

*The following decisions and actions are required in order to strengthen BTB control and eradication campaigns:*

1. Public policies involving local approaches and strategies for the strengthening of CEP, or their implementation in countries where these programs are not yet working. These policies mainly imply an adequate budget, human and material resources, as well as public financial resources destined to promote and improve cattle production and to compensate farmers from costs involved in herd cleaning or depopulation. These compensation and promotion systems tend to eliminate problems in advance, and stimulate the participation in the Program.


3. Legal obligation to report any suspected or confirmed case of the cattle disease to the relevant authorities.

4. Cooperation and coordination between CEP, universities, institutions for agricultural and livestock research and development, cattle producers, veterinarians associations, and many other organisms linked to derived industries, especially of meat and dairy products. This cooperation can be expressed in different financial and technical ways of support.

5. Programs for certification of veterinarians in the BTB field. Diffusion of information on BTB addressed to technicians, professional groups and institutions involved in TB control. Coordination
with public health institutions to improve the transmission of information on BTB to health workers and to the community.

6. Standardized diagnostic methods and reagents, especially those for the tuberculin test (Tub-test).

7. Effective regulation, organization and continuous quality assurance of the veterinary inspection in slaughterhouses and meat processing plants.

8. Abattoir surveillance, cattle identification and tracing systems, to attain an effective trace-back investigation to the farm of infection origin, in cases of animals with TB lesions detected at slaughterhouse inspection.

9. Epidemiologic investigation systems to detect contact-neighbor herds where BTB infection could have been expanded from the farm of origin.

*Diagnosis of tuberculous infection in cattle. Critical factors to take into consideration in the production and quality control of tuberculin PPD products.*

The intradermal tuberculin test is the standard method for detection of BTB infection in cattle. It involves measuring skin thickness, injecting the purified protein derivative (PPD) into the measured area and measuring any subsequent swelling at the site of injection three days later. This test, which has been practiced since more than a century in BTB control campaigns all over the world, is still largely used for field testing. This is due to several characteristics like *(a)*: the high sensitivity of the single test with bovine PPD *(11, 38)*; *(b)*: the excellent specificity of the comparative test with bovine and avian PPD; *(c)*: an early diagnosis of BTB infection, that allows elimination of infected animals in the pre-excretory phase from the herd; *(d)*: the particularly low cost of production in relation to other diagnostic methods in use for bacterial infections.

This last reason also explains that the Tub-test is still used use in industrialized as well as in developing countries *(6, 7, 43, 49)*.

In the chapter on *Bovine tuberculosis*, in the OIE *Terrestrial Manual, 2008* *(49)* requirements for tuberculin-PPD production, quality control and use in the field are described. The *M. bovis* and *M. avium* strains used for this production, seed management, preservation, culture methods, PPD manufacture, in process and final batch controls, potency test in guinea pigs and the standardization in cattle are described in detail. After production and quality control tests are performed, an additional control test must be carried out on each batch, by an officially recognized organism, completely independent from the producing laboratory. This control includes bioassays in guinea pigs to evaluate the activity of the product *versus* an International Reference, a bio-safety assay, and a test for absence of secondary effects.

PPD from *M. bovis* is the only reagent produced in the Region destined to the intradermal tuberculin
test in cattle. This is because its relatively high potency and maximum specificity in relation to the weight in proteins, as compared to previously produced tuberculins (3, 4, 49). However, more recently, results obtained in bio-assays showed disparity in the uniformity and quality between avian and bovine PPD, according to the information supplied from different references labs. While for the avian PPD the specificity and potency by weight unit resulted notably constant, it was not the case for the bovine PPD, in which the specificity -and most particularly the potency by weight- varied for different tested products and origins. In fact, potency of different bovine PPD batches analyzed ranged between 0 International Units (IU) to 40,950 UI /mg. It would mean that if applied in a 1 mg/ml dose, most of these PPD products will not fulfill the requirement of 2000 UI per dose. Even though the potency of these PPD products could be increased till a certain limit by increasing the protein concentration per dose, this increase would imply a deficit in the specificity (5, 49). This variability in the quality of PPD can also be the origin of variations in the evaluation results for the \textit{gamma} interferon test (IFN-\(\gamma\)), in countries where those PPD reagents are used for the intradermal Tub-test and in the \textit{in vitro} IFN-\(\gamma\) assay as well. These differences were demonstrated in experimentally infected cattle (Bovine Tuberculosis: Schiller I., Waters R., Vordermeier M., Palmer M., Egnumi T., Hardegger R., Kyburz A., Raebel A. & Oesch B. Effects of culture conditions and tuberculin source on IFN-\(\gamma\) production in whole blood cultures. 5th International Conference on Emerging Zoonoses, November 15-19, 2007, Limassol, Chypre).

This information is highly relevant for the standardization of tests used by the CEP in different countries. Discrepancies in the final quality of PPD products are apparently due to the different production methods followed, to differences in bio-assays carried out for the quality control, as well as to the use of different sub-strains, or different methods for seed management of the \textit{M. bovis} strain employed in the production. More detailed investigations are needed in order to determine the role of each of these factors in the final quality of bovine PPD.

\textit{Bovine PPD. Current situation in Latin American and Caribbean countries. Recommendations.}

There are several official and private PPD producer laboratories in the LAC countries, Even though documents containing standard procedures (SOPs) and manuals are available, in certain cases it should be desirable a re-edition and update, with the advice of international experts(OIE, PAHO/WHO).

Until now, a Regional network system for the standardization of production methods and quality assurance does not exist. Thus, the knowledge on the actual situation concerning PPD reagents (relative potency of batches in use in different countries, and bio-equivalents ) is quite incomplete. Thus, the organization of this network is recommended.

It is also suggested to strengthen the cooperation among the International Reference Laboratories (OIE) in projects for evaluation and quality assurance trials for PPD or other biological reagents. In this aspect, there are several valuable experiences in the human tuberculosis field (Global Network of Supranational Reference Laboratories) (48).
PPD reagents with appropriated and similar potency should be used in the different countries of the Region, in order to obtain comparable results in the Tub-test. For that purpose it is necessary:

- To employ the same *M. bovis* standard strain (ANS), preferably from the same origin (International Reference Laboratory), and with the same seed management system.

- To employ a Standard (Reference) bovine PPD batch to establish the relative potency of batches produced in different laboratories.

- Countries producing or importing PPD, should have a national quality control system, independent of the producers. Besides, it is also recommended that an International Reference Laboratory (OIE) could play the role of Regional Reference, receiving and controlling PPD samples submitted by the countries, at least once every 2 years. In case that the potency of a batch does not fulfill the international quality requirements, the entire production and quality assurance system should be revised. In the meantime, and until the problem has been overcome, the corresponding CEP in that country should get provision of a quality guaranteed bovine PPD (external source).

- A bovine PPD batch should be identified and designed as Regional Reference. To this aim, National Reference Batches should be evaluated versus the International Standard for bovine PPD, in an OIE Reference Laboratory. Aliquots of this Regional Reference batch should be easily available to the corresponding national laboratories to be employed as Reference in the evaluation of PPD batches produced locally.

- Each vial of a PPD batch previously approved by the national authority, should have easily readable, potency data and the expiry date on the label.

- OIE, through their Reference Laboratories, should regularly provide the corresponding national authorities with a list of the PPD producers that comply with the officially recognized standards and quality assurance conditions. These PPD products could be obtained and used by the countries CEP if there were not local production, or in emergency situations.

On these bases, the national CEP could count on adequate provision of bovine PPD batches with a quality assurance system, either locally produced or imported from abroad.

New diagnostic tests. Interferon gamma assay (IFN-γ).

During the last 15 years, the gamma-interferon (IFN-γ) assay has been increasingly applied to the diagnosis of BTB infection in cattle, especially in developed countries. Results obtained in different experimental evaluations of IFN-γ against the Tub-test have shown multiple variations. These differences can be related to the potency of the bovine PPD used as well as in the method adopted for the Tub-test. In fact, it has been generally observed that the single cervical, or the caudal fold test performed with bovine PPD, attain a higher sensitivity than the comparative test, performed with avian and bovine PPD. The inverse situation was observed in relation to the specificity.

Among other variables that can influence these comparisons are the interpretation criteria used for the Tub-test or for the IFN-γ assay, the epidemiological status of the herd and its management, the
presence of other environmental mycobacteria, and the booster effect of previous Tub-tests performed (17, 24, 33, and 45).

It is usually accepted that the IFN-γ assay is more sensitive and less specific than the comparative Tub-test. In order to improve the specificity, two individual M. tuberculosis complex-specific antigens, ESAT-6 and CFP-10, were recently included in the assay (1). However, it was observed that, even though the specificity of the assay increased with the inclusion of these antigens, its sensitivity decreased.

The relatively high cost of the IFN-γ assay is an additional obstacle to adopt this diagnostic assay in our Region for the extensive field testing screening. It could be adopted, if the costs resulted affordable, as an adjunct to the Tub-test. This could facilitate the early removal of infected animals, and increase the global diagnostic sensitivity, in areas and situations where the comparative Tub-test (with avian and bovine PPD) is employed. On the other hand, due to its relatively higher specificity in relation to the caudal fold test, in eradication areas where this test is the standard, the IFN-γ assay could be applied as a second confirmatory test.

With this strategy the waiting period of at least 6 weeks to perform a new series of Tub-tests could be avoided.

But the major advantage of IFN-γ consists in that it is an in vitro assay. Thus, it can be performed with an identical methodology in different laboratories by trained technicians, and the interpretation of results can be completely standardized. The assay interpretation is more objective than that of Tub-test. Additional advantages are the possibility of repeating the assay as many times as necessary to confirm a result, what is not the case for the Tub-test, and also its apparent capacity of detecting BTB infection earlier.

Main disadvantages are its cost, and the short interval required between the moment the sample is obtained in the field and that of its incubation with the antigen in the lab.

In the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals, it is said -referred to the blood based tests in general-, that due to the cost and the more complex nature of these laboratory-based assays, they are usually used as ancillary tests to confirm or refute the results of an intradermal skin test. Even though the IFN-γ assay is there described in detail, the specific setting where it could be applied is not defined. It is recommended, before taking a decision, to evaluate the assay versus the Tub-test in a field trial, in the region or country where it is intended to be adopted. For doing so, the relative sensitivity should be determined in animals with post-mortem confirmed BTB infection, with M. bovis isolated by culture. On the other hand, the relative specificity should be determined in free herds, situated in BTB free areas. In these evaluation studies, the bovine PPD used in the intradermal test should be one complying with the international requirements of quality assurance (46, 49).

*Diagnostic Laboratories for BTB in Animal Health*

*It is recommended:*
- To strengthen of diagnostic laboratories for animal disease at the country level, and their integration in national networks.

- To constitute of a Regional network composed of National BTB Reference Laboratories in Animal Health. This network should count on the advice and cooperation of International Agencies (PAHO/WHO, OIE).

**Bovine tuberculosis (BTB) in humans**

*Main causes that contribute to the risk of infection and disease by M. bovis in the human population are:*

- The presence of BTB infection in bovines, and the contact of humans with cattle (especially relevant in rural population, slaughterhouse and meat processing plants workers, veterinarians and laboratory technicians, among others).

- The development of livestock and derived industries without simultaneous BTB control programs and bio-safety measures.

*The relevance of BTB in humans in the Region.*

The certainty in the diagnosis of TB, human or bovine, is only achieved by the isolation of the bacillus and the species identification, on the basis of the corresponding phenotypic and molecular tests. As *M. bovis* has suffered a mutation that inactivated the enzyme pyruvate kinase, this species is unable to use glycerol as a sole carbon source (21). As a result, *M. bovis* can only be isolated *in vitro* by using special media containing pyruvate or other special carbon sources (i.e. Stonebrink medium). To confirm the species identification, phenotypic or genetic tests should be carried out on the isolates. Following these procedures, the frequency of TB due to *M. bovis*, in relation to the total TB cases confirmed by culture, can be established (32).

However, in the LAC Region, the bacteriological diagnosis of pulmonary TB is usually based on the finding of acid fast bacilli in the sputum microscopy. This is a rapid and low cost method, that can be performed in the local health centers, and that is highly specific for mycobacteria. It detects the infectious pulmonary TB cases, in order to be treated, thus interrupting the chain of transmission of the disease in the community. Treatment regimens have an initial phase, consisting usually of four drugs: isoniazid, rifampicin, pyrazinamid (PZA) and ethambutol, that have a similar efficacy for *M. tuberculosis* and for *M. bovis*, even though *M. bovis* is inherently resistant to PZA. On account of this similar efficacy, achievement of a differential diagnosis between these two mycobacterium species is not considered a priority in public health (32, 47).

For the direct diagnosis of TB, culture is much more sensitive than microscopy, but it is also slower in rendering results, and much more expensive than microscopy. For these reasons, the Regional Standards reserve the use of culture to confirm cases of childhood, extra-pulmonary TB, suspected pulmonary disease with sputum negative microscopy, or to perform drug susceptibility tests (i.e. when multi-drug resistance is suspected in a patient).

In this situation, the relative importance of BTB in humans can only be acknowledged through specially designed surveys in which, during a period of time all samples are inoculated in culture
media where both *M. tuberculosis* and *M. bovis* can grow, and the respective isolates are then submitted to differential tests, to determine the species status. This type of survey have been performed in several reference laboratories, and as a result, valuable information on the relative importance of *M. bovis* in public health could be collected. According to it, the relationship between frequency of BTB in man and the infection status in cattle could be documented in several countries.

According to this information, the relative importance of *M. bovis* in relation to *M. tuberculosis* as a source of disease in the Region seems to be very low, usually less than one per cent. However, it could be the origin of primary TB, frequently extra-pulmonary forms, in children and infants, due to ingestion of contaminated –un-pasteurized, not boiled- milk. Additionally, slaughterhouse and rural workers in BTB infected areas are at risk of aerosol-borne pulmonary disease (5, 9, 14, 15, 18, 22, 25-27, 37, 40, 47).

Thus, public health services and their TB control programs should direct the search of TB cases due to *M. bovis* in particular among these two groups at risk. In addition, adequate information should be transmitted to these groups on specific prevention, protection and bio-safety.

**Several study results on *M. bovis* isolation in humans.**

The existence of TB due to *M. bovis* in humans has been recently documented in four countries in the Region: Argentina, Brazil, Ecuador and Venezuela. In addition, epidemiologic links between BTB cases diagnosed in the USA and consumption of a cheese produced with raw milk in Mexico, have been demonstrated (9, 22, 25, 27). On the other hand, in Uruguay, Colombia and Dominican Republic, even though systematic laboratory research was made using the appropriate methods, no *M. bovis* isolates from human specimens could be obtained (15).

The percentage of cases caused by *M. bovis* –in relation to the total TB cases diagnosed- has been the highest in Argentina. These results could be due to a long term and continual investigation, especially performed in reference laboratories situated in Buenos Aires and in Santa Fe, as well as to a history of relatively high rate of infection in cattle. Pulmonary disease due to *M. bovis* predominated among slaughterhouse workers.

A complete register of BTB cases diagnosed there from 1977 to now is available at the National Institute for Respiratory Disease (INE E. Coni), in Santa Fe, where a total of 2485 pulmonary TB cases were bacteriologically confirmed between 1988 and 2006. The percentage of *M. bovis* cases decreased steadily during this period from 2.7% found between 1988 and 1993, 1.7% for 1994-99, to 1.3% for 2000-06. Approximately a 70% of the BTB patients had a history of working with cattle, most of them as slaughterhouse workers. These decreasing percentages of BTB cases in 18 years could be related to the progress achieved in the control and eradication program (SENASA) and also to the improvement in the sanitary and hygiene conditions in the food industry (14, 15, and 37) during the period.

In the A. Cetrángolo Laboratory (Muñiz Hospital, and Vacarezza Institute, Buenos Aires University), a total of 5550 TB cases were bacteriologically confirmed in HIV (-) patients, in the period 2000-2006. *Mycobacterium bovis* was isolated in 0.22 per cent of these cases. In the same period, the percentage of *M. bovis* cases among 1400 HIV (+)/AIDS TB patients was 0.57 per cent (15, 18).
Bovine TB in humans decreases with:

- Appropriate hygienic measures in slaughterhouses, meat processing plants, and in dairy industry establishments.
- Boiling of milk and industrial pasteurization performed with quality assurance.
- Adequate protection of workers at risk (especially to prevent transmission by respiratory route).
- Improvement in the TB control, in the animal and in the human health fields.
- Adequate information to the population at risk, on the mechanism of TB transmission, the preventive measures to be taken, the symptoms and signs of the disease, its treatment and cure.
- Cleaning-up sources of disease (i.e. infected herds, or facilities), and performing the corresponding epidemiological investigation.

The role of basic research in the Region

Several relevant research contributions on BTB recently made in the Region could find application in the CEP (1, 8, 10, 23, 26, 29, 36, 44, 52-55).

To develop and strengthen research projects on BTB it is required:

- To obtain financial support from the corresponding public and private institutions, to those evaluated projects on subjects of possible interest for the control of BTB in the Region.
- To facilitate the access –via internet- to updated information from the CEP, and to publications related to these programs (i.e. National CEP, International Agencies websites).
- On the basis of the already existent cooperation, to establish a Latin American network for research on bovine tuberculosis.

Some research projects currently under way or programmed in the Region concern:

- Systematic molecular typing of *M. bovis* strains isolated from animals.
- Epidemiological analysis and trace-back of zoonotic TB human cases. Molecular analysis of *M. bovis* strains and their comparison with genotypes of isolations from bovines.
- Design of a screening test to detect *M. bovis* contamination in milk tanks (in bulk) for dairy establishments.
- Standardization of ADN amplification assays, and their evaluation in field conditions.
- Development of software for electronic identification, record keeping, trace-back systems and other epidemiological surveillance projects.
- Experimental vaccine development.

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