Report of the WHO working group on zoonotic tuberculosis (*Mycobacterium bovis*), with the participation of FAO

Mainz, Germany, 14 June 1994
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Report of the WHO working group on zoonotic tuberculosis (*Mycobacterium bovis*), with the participation of FAO
1. INTRODUCTION

The participants (see Annex 1) were welcomed by Dr F.-X. Meslin, Chief, VPH, on behalf of Dr H. Nakajima, Director-General of the World Health Organization. Dr Meslin expressed gratitude to Professor Schliesser for his valuable collaboration in organizing this meeting and to the Convenors of the 28th World Conference of the International Union against Tuberculosis and Lung Disease (IUATLD) for the excellent facilities provided to the Working Group.

The purpose and scope of the meeting were as follows:

■ to review the activities of the working group with special attention to the work carried out by participating institutions in developing and developed countries;

■ to strengthen or establish collaborative research in diagnosis, prevention, control, epidemiology, surveillance and reporting systems as well as the public health consequences of zoonotic aspects.

Professor C. Thoen was elected as Chairman, Drs J. Berrada, J.M. Grange, J. Haagsma and Professor Th. Schliesser as Moderators and Dr C. Daborn served as Rapporteur.

1.1 WHO’s activities in the field of zoonotic tuberculosis

The Veterinary Public Health unit (VPH) has been particularly interested in the public health significance of Mycobacterium bovis infection in humans and animals as well as the safety of food of animal origin with regard to contamination by M. bovis. In this connection, WHO has been continuing its efforts: i) in the worldwide surveillance of animal tuberculosis, jointly with the Food and Agriculture Organization (FAO) and the Office International des Epizooties (OIE) (15); and ii) research in epidemiology, standardization of diagnostic methods and reagents and measures for prevention/control, including food hygiene, with special reference to intersectoral collaboration.

VPH has recently organized and coordinated a working group team comprising an international group of experts. Their subjects are the epidemiology, public health, control and research of animal tuberculosis. A previous meeting of the working group was convened in conjunction with the IUATLD Conference on Animal Tuberculosis in Africa and the Middle-East, Cairo, 28-30 April 1992 (15). A later meeting was convened in Geneva, 15 November 1993, to discuss with the teams working in Tanzania and Zambia, to promote their projects and to engender international cooperation and support (16).
1.2 FAO’s activities in this field

Several FAO-executed field projects, aimed at strengthening national veterinary services have addressed infectious disease problems, including animal tuberculosis control. There are a lack of projects dealing specifically with bovine tuberculosis; a situation which reflects the lack of demand for assistance in this field by Member Countries. However, FAO has reconsidered its position and has listed tuberculosis as a priority infectious disease. In collaboration with other concerned regional or international organizations, FAO’s activities aim to: i) encourage applied research into the development of a suitable, simple serological test; ii) sustain scientific institutes currently involved in the field of vaccine development; iii) assist Member Countries in conducting epidemiological surveys and; iv) test and apply new strategies for tuberculosis control.

2. AFRICA

2.1 Egypt

As a consequence of early studies indicating that bovine tuberculosis (BTB) was present in Egypt (6.9% to 26.5% of reactors to the tuberculin test in various areas and 11.1% carcasses with suspected tuberculous lesions) the Egyptian Veterinary Services of the Ministry of Agriculture started, in 1981, a control programme in dairy cattle and buffaloes, and within 10 years succeeded in reducing the level of infection among the large farms to a prevalence of 2.6%. This national programme started in three governorates of the Suez Canal and was extended in 1986 to all governorates. In 1993, a total of 97,959 cattle and 61,670 buffaloes were tested. The proportion of positive reactors was 0.25% for cattle and 0.11% for buffaloes. The overall proportion of positive animals was 0.19%.

The national control programme for tuberculosis in Egypt is based on the application of the test and slaughter policy in dairy buffaloes and cattle. The tuberculin used is the single intradermal test using purified protein derivatives (PPDs) prepared locally from a M. tuberculosis strain.

Infected herds remain under temporary quarantine. Reactors should be slaughtered and owners compensated. Decrees and rules regulating compensation are periodically revised to meet the breeders’ needs and to encourage testing. Public health authorities are informed which farms have tuberculin positive animals so that examination of the workers and measures on milk produced by such farms can be undertaken. Disinfection procedures and other necessary measures related to the disease are
conducted under supervision of the official veterinary authorities.

Imported cattle must be certified tuberculosis-free by the veterinary authorities and the Egyptian Embassy in the exporting country. In addition, tuberculin testing should be carried out while the cattle are undergoing quarantine in Egypt prior to their movement to the importer’s farm and any reactors should be slaughtered.

The PPDs for the tuberculin tests are prepared locally by the Serum and Vaccine Production Institute at Abbassia and mainly used by the General Organization for Veterinary Services (GOVS) after routine potency and quality control tests have been performed on all batches. This institute has a specialized department responsible for the isolation and identification of mycobacteria from animal tissues.

2.2 Morocco

A survey on BTB was conducted at five major abattoirs of the country as well as on five selected herds (dairy and autochthonous) in 1990 and 1991. The survey included tuberculin skin testing, post-mortem examinations, histopathological and mycobacteriological (isolation and identification of mycobacteria) examinations as well as serological investigations using an ELISA test.

The results of this survey showed that BTB is widespread among Moroccan cattle and emphasized the need for implementing a control programme.

Recently, a research project to assess the importance of human tuberculosis due to \textit{M. bovis} has been initiated with the National Institute for Hygiene. This includes epidemiological investigations as well as the use of improved procedures and appropriate media for the isolation and identification of \textit{M. bovis} from human patients. Results from this project are not yet available.

2.3 South Africa

The results from recent surveys of African buffalo in the Natal game reserves and Kruger National Park (KNP) will be used to establish the specificity of ELISA and -IFN assays versus the tuberculin test.

A collaborative research programme by the Onderstepoort Veterinary Institute (OVI) and a South African university on the use of PCR for specimens from buffalo to diagnose tuberculosis has been established. Data are not yet available.

Collaborative work between OVI, New Zealand and UK institutions on DNA fingerprinting of isolates from African buffalo and cattle is progressing. It is hoped that the results will enable the source of investigated outbreaks to be established.
There are plans to investigate possible public health consequences of the KNP tuberculosis outbreaks. Tuberculin testing of all workers of the KNP involved in culling African buffalo (eg. veterinarians, game wardens, abattoir and tannery workers) has been planned.

Concerning international cooperation, preliminary contact on animal tuberculosis research and control were established with neighbouring countries during a recent meeting held at OVI in March 1994 (6).

2.4 Tanzania

The Bovine Tuberculosis in the Tropics research programme, funded by the Overseas Development Administration (ODA), UK, is broadly divided into field and laboratory components.

The following results have been achieved to date:

i) Molecular Test Development. Polymerase Chain Reaction (PCR) assays have been developed to detect: a) M. tuberculosis complex and b) M. tuberculosis only. These two assays have been adapted to run simultaneously in a single tube (Table 1). Recent work at the Moredun Research Institute (Edinburgh), utilising the Restriction Fragment Length Polymorphism (RFLP) technique, has demonstrated apparent strain similarities between Tanzanian M. bovis human and cattle isolates.

ii) Isolation of mycobacteria from slaughter cattle in Tanzania. Small calcified lesions in the lymph nodes were found in approximately 30% of 1 000 slaughter cattle. From the culture of lesions the myco-bacteria laboratory of the Sokoin University of Agriculture (SUA) reported that 32 were positive for M. bovis and one was positive for M. tuberculosis. Nine subcultures from the first of the SUA isolates (reported as 8 M. bovis and 1 M. tuberculosis) were examined at the City Hospital, Scottish

Table 1. Combined (Multiplex) PCR technique that detects M. tuberculosis complex organisms and differentiates M. tuberculosis from other members of the group.

<table>
<thead>
<tr>
<th></th>
<th>IS986</th>
<th>IS1081</th>
<th>MPB70</th>
<th>mtp40</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. tuberculosis</td>
<td>26</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>M. bovis</td>
<td>20</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>M. africanum</td>
<td>2</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Others</td>
<td>5</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

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Mycobacterium Reference Laboratory (SMRL) and identified as 5 *M. bovis* and 4 *M. tuberculosis*. Initial RFLP findings at the Moredun Research Institute (MRI), Edinburgh confirmed the diagnosis as made by the SMRL. These preliminary results suggest that *M. tuberculosis* infection in cattle may be as much of a problem as *M. bovis* infection in humans.

iii) *Culture results from human extra-pulmonary (cervical lymphadenitis) cases.*

Table 2 summarizes the results of the reported isolation made by the SUA laboratory from some human extra-pulmonary tuberculosis biopsy samples.

Table 2. Reported isolation made by the SUA laboratory from some human extra-pulmonary tuberculosis biopsy samples (Arusha Region, March 1994).

<table>
<thead>
<tr>
<th>Occupation</th>
<th>No. of cases</th>
<th>Special Isolated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Livestock keepers</td>
<td>4</td>
<td>2 <em>M. bovis</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 Negative</td>
</tr>
<tr>
<td>Small farmers</td>
<td>6</td>
<td>2 <em>M. tuberculosis</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 <em>M. bovis</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 Negative</td>
</tr>
<tr>
<td>Children</td>
<td>3</td>
<td>2 <em>M. tuberculosis</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 <em>M. bovis</em></td>
</tr>
<tr>
<td>Occupation not given</td>
<td>6</td>
<td>3 <em>M. tuberculosis</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 Negative</td>
</tr>
<tr>
<td>Totals</td>
<td>19</td>
<td>7 <em>M. tuberculosis</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td>4 <em>M. bovis</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td>8 Negative</td>
</tr>
</tbody>
</table>

Report of the WHO working group on zoonotic tuberculosis (*Mycobacterium bovis*), with the participation of FAO
Although the numbers of samples cultured are as yet small, it is possible to report the first isolation of *M. bovis* from human patients in Tanzania. More human samples are being collected and cultured. Genetic “fingerprinting” of the *M. bovis* and *M. tuberculosis* isolates has commenced at the MRI and attempts will be made to isolate matched strains from cattle in the patient’s village of origin.

### 3. AUSTRALASIA

#### 3.1 Australia

The following are some of the activities carried out at the Australian Reference Laboratory for Bovine Tuberculosis:

i) **Diagnostic methods.** A Multiplex PCR method is being used for rapid identification of *M. tuberculosis* complex, *M. avium* and *M. intracellulare*. The test is performed in a single tube and results can be obtained within 4-5 hours.

The Multiplex PCR is being used for the rapid confirmation of suspect cases of tuberculosis outbreaks in cattle. This technique has also been useful in cases where *M. avium* was proved to be the cause of generalised tuberculosis in cattle.

ii) **Prevention and control.** Australia achieved the target of Impending Freedom (IF = no known infected herds) from Tuberculosis in December 1992. However, as expected, some breakdowns occurred during the 1993 season. Most breakdowns are handled by de-stocking affected properties. The γ-IFN used in conjunction with the tuberculin test may be used in Western Australia to test herds to freedom so that valuable breeding stock are not lost.

iii) **Epidemiology, surveillance and reporting systems.** A Granuloma Submission Programme aimed at submitting all granulomas detected at abattoirs to the laboratory for diagnosis commenced in July 1992. The success of this programme varies between States but has increased the detection of tuberculosis in carcasses at abattoirs.

DNA fingerprinting is being applied to *M. bovis* isolates from different geographical locations in Western Australia using pTBN12, IS6110 and DR-r probes.

DNA fingerprinting and SDS/ PAGE and western blotting have been used to study *M. tuberculosis* complex organisms isolated from captive seals, a seal trainer and wild seals and...
have determined that tuberculosis is endemic in three species of seals along the southern coasts of Australia. This study may be extended to study the source of tuberculosis in seals along the coasts of South America.

Some work has been done using random amplified polymorphic DNA analysis for typing M. tuberculosis complex isolates, particularly M. bovis, using random 10 primers and some from within the IS6110 element.

3.2 New Zealand

i) Ag Research Institute, Wallaceville Animal Research Centre, Upper Hutt

The Infectious Diseases Group of the Ag Research carry out basic research on BTB, ranging from immunological investigations of pathogenesis in cattle and possums to the molecular biological search for virulence factors and the development of an improved tuberculosis vaccine. This emphasis has been given to basic research because more than a quarter of New Zealand’s land area contains wild animals potentially infected with animal tuberculosis. In this situation, control strategies based on cattle testing and slaughter, even when coupled with poisoning of wild animals and movement control of farmed animals, have only limited effectiveness.

a) Culturing. All culturing for animal isolates of mycobacteria in New Zealand is performed by the Infectious Diseases Group. Approximately 1,500 isolates of M. bovis and 300 isolates of M. avium are identified from 3,500 submitted samples each year.

b) Polymerase Chain Reaction (PCR). A sensitive test has been developed and this has been carefully compared to culture using tissue samples submitted for diagnosis. The PCR test has the advantages of providing a much faster result (2 days versus 6 weeks) and the ability to detect the presence of M. bovis in samples even when organisms have become non-viable for culture. However, the PCR test has disadvantages: it is not as sensitive as culture, it does not always detect those samples that contain small numbers of organisms, and it is susceptible to artifactual positive results due to cross-contamination and negative results due to enzyme inhibition. Avoidance of these artifacts requires a high level of skill and good laboratory facilities.
c) **γ-IFN test.** This has been investigated as an alternative to the currently used skin test. While interferon testing is more sensitive than skin testing, the costs and inconvenience of the method outweigh this advantage and it is unlikely to be used in the future except possibly as an ancillary test to complement skin testing.

d) **DNA fingerprinting.** This has been performed for almost ten years and has yielded a large amount of useful epidemiological information. Commercial analyses are performed on a limited basis for clients from other countries. The technique is expensive (US$ 150/sample) and technically difficult to perform.

e) **Public Health.** New Zealand has approximately 300 cases of human tuberculosis per year of which less than 5% are identified as *M. bovis*. So far, very few human isolates of *M. bovis* have been typed, but the establishment of a system for typing such isolates is expected by 1995.

ii) **Deer Research Laboratory (DRL), University of Otago, Dunedin**

The DRL is involved in researching improved tuberculosis diagnostic methods for farmed deer, and to chart patterns of immunity following infection with virulent *M. bovis* or vaccination with BCG. To complement these studies it has developed an experimental challenge (virulent *M. bovis*) model to evaluate disease transmission and protective immunity following vaccination.

a) **Diagnosis.** DRL has developed a blood test (BT) for animal tuberculosis which monitors specific humoral (ELISA) and cellular responses (lymphocyte transformation - LT) to *M. bovis* antigens. This method is 96% sensitive and 98.6% specific for the diagnosis of tuberculosis in deer. Composite tests which monitor cellular and humoral immunity are complementary and together they give excellent sensitivity and specificity. However, the disadvantage of BT is that it is technically complex and consequently expensive to carry out. In New Zealand BT has been proven to be cost-effective when used on skin test positive animals where it can accurately confirm reactivity due to *M. bovis* in infected animals. Its high level of specificity allows it to salvage animals with non-specific mycobacterial sensitization without compromising disease diagnosis.

An alternative approach is to use the single intradermal skin
test (ST) and ELISA in combination. The combined tests give a sensitivity of 95\% whereas individually the ST has a sensitivity of 82\% and the ELISA 85\%. ELISA reactivity is significantly enhanced by prior skin testing. Blood samples for ELISA should therefore be obtained 10–20 days post skin test injection of PPD.

Because deer are uniquely susceptible to mycobacteria in general, up to 90\% of skin test reactivity is due to non-specific sensitization. To circumvent problems of poor specificity and false positive ST reactivity, the comparative cervical test (CCT) has been used as an ancillary test for ST-positive animals. While CCT has a high level of specificity (98.5\%) its sensitivity is only 65\% under field conditions. This introduces a significant risk that CCT will miss *M. bovis* infected animals when disease is present at low prevalence.

b) Diagnostic antigens. Studies have been carried out to evaluate the diagnostic potential of different types of PPD, native culture supernatant antigens, fractionated proteins and isolated peptides. Generally PPD has been proved to be superior to any other antigen currently available. Considerable differences in the potency of PPD obtained from different countries (Australia, NZ, UK and USA), have been observed even though each batch is supposed to contain equivalent units of PPD. Generally, diseased deer show a highly specific response to PPD-B (*M. bovis* in vitro), with relatively low non-specific reactivity to PPD-A (*M. avium*). By contrast, vaccinated animals show highly cross reactive responses to PPD. Diseased or vaccinated animals show a highly heterogeneous response to fractionated antigen from *M. bovis* and filtrates of early cultures of *M. tuberculosis* principally containing secreted peptide antigens. While isolated peptides, such as MPB70, give highly specific responses in diseased animals they are generally less sensitive as diagnostic antigens than PPDs. BCG vaccinated animals show very low levels of reactivity to MPB70 which allows distinctions to be made between vaccination and disease.

c) Experimental infection with virulent *M. bovis*. An experimental model for virulent *M. bovis* challenge of deer to mimic natural infection has been developed. Three routes of challenge have been evaluated; *intranasal* aerosol, *intratracheal* injection and *intratracheal* injection. The *intranasal* route provided variable responses while *intra-tra-
cheal inoculation tended to produce lung abscesses unlike anything found in naturally infected deer. The intratonsillar route has been chosen as most appropriate because it produces lymphadenitis, especially in the draining retropharyngeal lymph node, similar to that found in naturally infected deer, where the retropharyngeal lymph nodes are the commonest site for pathology. Bacterial numbers and histopathology seen with intratonsillar challenge are similar to that found in natural tuberculous in deer. Infection can be established in 50-80% of experimentally challenged animals with doses as low as 10 colony forming units (cfu’s) of virulent M. bovis per inoculum. Disease severity is increased by concurrent treatment of challenged animals with slow-release corticosteroids (dexamethazone). Protection against experimental challenge develops following vaccination, so the model can be used to evaluate protective efficacy of tuberculosis vaccines.

c) Vaccination with BCG. Extensive studies have been carried out over the past four years, initially to monitor vaccine safety and patterns of immune reactivity in deer vaccinated with different formulations of BCG. BCG is safe for use in deer and does not produce any pathology or adverse effects. Live BCG in saline produces cross-reactive cellular responses to mycobacterial antigens, without any humoral reactivity. Live BCG in oil adjuvant produces M. bovis specific cellular and antibody reactivity. Killed BCG in oil produces high levels of cellular and antibody reactivity specific for M. bovis and provokes inflammatory reactivity following skin testing with PPD. This latter reaction is similar to the response found in naturally diseased deer. It remains to be confirmed whether live BCG or killed BCG produce a protective response in vaccinated deer.

Recently, studies on vaccine efficacy in deer vaccinated with live BCG at different doses have been carried out. Moderate (5x10^7) or low dose (5x10^6) of live BCG protected against virulent experimental challenge with 100 cfu of M. bovis. High dose vaccine (5x10^8) had lower efficacy than the lower doses. Non-vaccinated control animals had significantly higher levels of disease (75%) than that found in any of the vaccinated groups (Table 3).
Table 3. Protection using BCG vaccination in experimentally infected deer.

<table>
<thead>
<tr>
<th>BCG dose</th>
<th>5x10^4</th>
<th>5x10^5</th>
<th>5x10^6</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Animals</td>
<td>5</td>
<td>20</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>No. Diseased</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>% Diseased</td>
<td>0</td>
<td>5%</td>
<td>25%</td>
<td>75%</td>
</tr>
</tbody>
</table>

While the numbers of animals in this experiment are small it nonetheless provides evidence for the protective efficacy of BCG.

Studies are currently underway to monitor patterns of immunity and unequivocal evidence for protection in larger groups (30) of animals exposed to optimal doses of live BCG. These will be compared with responses and protective levels in animals or in deer vaccinated with a killed BCG in oil vaccine and non-vaccinated controls. Cytokine levels (IL-2, IL-4, IL-10, IL-12, γ-INF and TNFa) will be monitored in these animals to provide evidence of TH1 vs TH2 cell activation. Further work is underway to select animals with innate susceptibility and resistance to determine how innate responses affect impact on the ability of animals to develop acquired immunity.
4. LATIN AMERICA

The bovine population in Latin America and the Caribbean (L.A. and C.), amount to nearly 322 million heads in 37 countries and political units, while both USA and Canada have 111 million heads.

According to recent information 24 out of 37 countries in L.A. and C. (64.9%) present a very low prevalence of BTB or are already free of this infection. Their cattle population attain 83.5 million heads, 26.0% of all cattle in this Region (1a, 3).

Prevalence would be higher than 0.5% in the remaining 238 million heads situated in 13 countries. In some of these countries the real epidemiological situation is scarcely known, while in others BTB is qualified as enzootic. (Table 4).

Table 4. BTB infection in cattle: Condition in 37 L.A. and C. countries

<table>
<thead>
<tr>
<th>Sub Region or country</th>
<th>Countries</th>
<th>Cattle population (in thousands)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. Total</td>
<td>* No. Free (%) or very low prevalence**</td>
</tr>
<tr>
<td>The Caribbean</td>
<td>17</td>
<td>151 (88.2)</td>
</tr>
<tr>
<td>Central America</td>
<td>7</td>
<td>52 (71.4)</td>
</tr>
<tr>
<td>Mexico</td>
<td>1</td>
<td>1 (100)</td>
</tr>
<tr>
<td>South America</td>
<td>12</td>
<td>43 (33.3)</td>
</tr>
<tr>
<td>TOTAL</td>
<td>37</td>
<td>24 (64.9)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>Free or very low prevalence</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>The Caribbean</td>
<td>9139</td>
<td>5449 (59.0)</td>
<td></td>
</tr>
<tr>
<td>Central America</td>
<td>10557</td>
<td>7305 (69.2)</td>
<td></td>
</tr>
<tr>
<td>Mexico</td>
<td>32054</td>
<td>26300 (82.0)</td>
<td></td>
</tr>
<tr>
<td>South America</td>
<td>270000</td>
<td>44485 (16.5)</td>
<td></td>
</tr>
<tr>
<td>TOTAL</td>
<td>321750</td>
<td>83569 (26.0)</td>
<td></td>
</tr>
</tbody>
</table>

* Less than 0.5% of reactors among cattle tuberculin tested
** Meat cattle, Northern States of Mexico

1 Antigua, Bahamas, Barbados, Bermuda, British V.I., Cuba, Dominica, Grenada, Guadalupe, Jamaica, Montserrat, Saint Kitts/Nevis, Saint Lucia, Saint Vincent and the Grenadines and Trinidad-Tobago.

2 Belize, Costa Rica, Honduras, Nicaragua and Panama.

3 Colombia, Suriname, Uruguay and Venezuela.

Since these data on tuberculin testing as well as those on sanitary inspection of slaughterhouses originated from different types of samples, the figures do not necessarily reflect the actual status of infection and disease. However, the following sections underline the most relevant control and elimination activities carried out in L.A. and C.
Detection of tuberculous infection in live cattle is based exclusively on the tuberculin skin test. Sensitivity and specificity of these methods have been assessed in the conditions of Argentina and Uruguay (Table 5).

| Table 5. Sensitivity and Specificity of tuberculin test³⁰, ⁷, ¹⁴ & ³ |
|-------------------------|-------------------------|
|                         | SENSITIVITY             | SPECIFICITY             |
| Caudal                  | 0.75 - 0.82             | 0.96 - 0.99             |
| Simple Cervical         | 0.86 - 0.96             | 0.70 - 0.76             |
| Comparative             | 0.72 - 0.78             | 0.92 - 0.99             |

The Instituto Panamericano de Proteccion de Alimentos y Zoonosis (INPPAZ), Buenos Aires, Argentina, of the Panamerican Health Organization (WHO/PAHO) prepares and distributes reference bovine and avian PPDs to the countries of the Region. Tuberculin PPD is produced in eight Latin American countries (Argentina, Brazil, Uruguay, Paraguay, Venezuela, Cuba, Dominican Republic and Mexico).

An ELISA test for detection of IgG against *M. bovis* was developed and assessed in cooperation between INPPAZ and several Argentine institutions¹³. According to these studies sensitivity for detecting BTB in further bacteriologically confirmed cases was 74% and specificity 94%.

In a comparison between results obtained with a γ-IFN assay and ELISA for IgG anti *M. bovis* an inverse relationship was observed. Localized lesions seem to be associated with higher γ-IFN and low specific antibody levels, and extended lesions inversely¹².

The low sensitivity of this ELISA excludes its use as a single diagnostic tool in eradication campaigns. However, its high specificity as well as its low cost, and its operational advantages, would make it a valuable tool for epidemiological surveillance.

Bacteriological diagnosis of animal tuberculosis including culture is only performed in certain animal health laboratories in Latin America. These laboratories follow well-defined PAHO/WHO standard methods and procedures¹¹. Where BTB status approaches elimination, bacteriological confirmation of suspect BTB lesions, detected at slaughter, should be regularly performed.
DNA fingerprinting techniques were first applied in 1993 in a collaborative project aimed at characterizing *M. bovis* strains by RFLP in order to analyse possible associations between fingerprint patterns and the geographical origins of the strains and to evaluate the usefulness of this method for epidemiological surveillance of BTB. Results are not yet available.

There is little epidemiological information on the impact of BTB on human health in this region, mainly because the bacteriological diagnosis of human tuberculosis in L.A. and C. is generally limited to smear examination. Most of the data originates from Argentina, where a relatively high prevalence of BTB coincides with highly reliable bacteriological diagnosis. Average percentages of cases due to *M. bovis* among pulmonary tuberculosis cases are between 1% and 4% and reach 8% in extrapulmonary localizations.

### 4.1 Mexico

Mexico has 26.3 millions heads of beef cattle, 2.2 million of dual purpose cattle and 925 000 heads of specialized cattle for milk production. A total of 7 250 million litres of milk were produced in 1993, 41% of the total was marketed through informal channels (raw milk), only 20% was pasteurized and the remaining 40% was converted into milk products. Mexico has a deficit of 12 million litres of milk per day, the consequent importation of milk powder costs 371 million dollars in 1992 (9).

Livestock industry represents 33% of Agriculture’s Gross Product and 2.5% of the Gross National Product (9).

The Mexican Commission for Control and Eradication of Bovine Tuberculosis was established in September 1993 and mainly operates in the northern states bordering USA. In 1994, 1,848 herds were found to be BTB free (468,293 heads), this represents an increase of 452% from 1993. In 1994 2.4 million tuberculin tests were performed and 4,259 (0.17%) positive reactors were found. The prevalence of BTB in beef cattle was 0.5% (131,491 heads tested), in dual purpose cattle it was 12% (263,943 heads tested), and in dairy cattle it was 16% (148,196 heads) (9). According to the same report, out of the overall number of slaughtered cattle with tuberculous lesions found in USA abattoirs the percentage of cattle of Mexican origin was 62% in 1989, 66% in 1990, 77% in 1991, 81% in 1992, 84% in 1993 and 57% so far in 1994.

Exportation of cattle represents an income of 450 million dollars and BTB could have severe economic consequences, mostly in the exporting states. It has also been estimated that BTB diminishes animal production by up to 17%, therefore hidden losses could be
significant in terms of lost weight, meat and milk products.

It is customary in the country to boil milk before consumption. Unfortunately, human consumption of fresh cheese is very common. During 1991 and 1992, 15,210 human tuberculosis cases were reported and it is likely that the real figure was 30% higher. It has been estimated that 5% to 8% of these human tuberculosis cases are due to *M. bovis* and the remainder to *M. tuberculosis*.

During 1992 and 1993 the eradication programme cost 15 million dollars; however, during the same period 1.3 million heads of cattle were exported at a value of 578 million dollars. This represents the 9th most important commodity of the non-oil export.

Research into BTB has been limited and carried out on a voluntary basis in intensive dairy cattle herds within the temperate zone. In some northern states such as Hidalgo, Durango and Coahuila, the comparative tuberculin skin test is routinely carried out. Tuberculins of mammalian and avian origin are produced by the National Producer of Biological Veterinary Products and Government Plant.

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**5. NORTH AMERICA**

**5.1 Canada**

The Tuberculosis Research Interest Group of the University of Alberta in Edmonton, Canada, is working on human tuberculosis treatment and tuberculosis control programmes, as well as microbiological basic research. The laboratory handles 20,000 specimens for primary isolation annually, and acts as a reference for sensitivity testing and speciation for the Alberta and Northwest Territories population of 2.7 million. It also functions as the reference centre for an ongoing Canadian study of *M. avium* complex bacteraemia in HIV infection.

A DNA fingerprint system for *M. bovis* is being developed and will assist in the study of tuberculosis transmission from animals to humans. Present research focuses on the development of a recombinant BCG which might have potential for human as well as animal tuberculosis control. The method involves insertion of cytokine genes into BCG in an effort to augment the response to mycobacterial infection.

In 1992 a study of an epidemic of *M. bovis* in elk (red deer) in Alberta, yielded one human case which led to an extensive contact follow-up. The initial fingerprint of the *M. bovis* isolates were carried
out with the IS 6110 insertion element which has only 1-3 copies in these strains of *M. bovis*, hence, limited information was obtained from the epidemiological study. The human case had only one copy, as did all the other animals in the outbreak, whereas the index animal, with which the human case had contact, had three copies. The human contact follow-up identified 564 persons who reported contact with infected herds. These persons were grouped by occupation (veterinary surgeon and inspector, renderer, farmer, autopsy laboratory technician, tanner, abattoir worker and other). They were divided according to the classification of the herd of contact (culture positive herd, reactor herd, non-reactor herd, and other). During the course of the investigation nine persons who had been non-reactors at initial testing converted to tuberculin skin test positivity. None had reported exposure to other sources of tuberculosis. Two were veterinarians, five were renderers and two worked in the autopsy suite. When the reactor rate (including new positives as well as converters) was examined by occupation, assuming farmers represented the norm, the risk ratio was highest for veterinarians, 4.29 (95% CI, 2.06-8.91). The odds ratio for reactivity was 3.48 (95% CI, 1.73-7.02) for those exposed to culture positive animals assuming “other” herds exposure represented the norm.

5.2 USA

The Mycobacteriology Laboratory at Iowa State University (ISU) has been conducting research on the mechanisms associated with pathogenesis of *M. bovis* in animals for several years and is actively involved in the development of improved diagnostic methods for tuberculosis in animals.

Chemical and molecular techniques have been utilized to identify antigens with greater specificity for use in evaluating kinetics of immune responses of the host following exposure to pathogenic mycobacteria such as *M. bovis*, *M. tuberculosis* and *M. avium*-M. intracellulare (MAI complex). Immunomodulators have been employed to enhance specific in vitro blastogenic responses to antigens and mitogens. Recent research has shown that cell-mediated immune responses to specific mycobacterial antigens were altered in cattle experimentally exposed to mycobacteria following exposure to different strains of modified-live BVDV vaccines.

Investigations have focused on the development of ELISA for detecting antibodies in the sera of animals experimentally or naturally exposed to pathogenic mycobacteria, including *M. bovis*, *M. tuberculosis* or *M. avium* complex. Mycobacterial antigens chemically extracted from virulent strains of
mycobacteria have facilitated improved specificity in ELISA.

Collaborative studies have been established with scientists in several developing countries including Morocco, Nigeria, Turkey, Mexico and India. Evaluation of ELISA developed at ISU for use in the diagnosis of M. bovis in wild animals are currently in progress. Of particular interest the ELISA has been used successfully for detecting M. bovis-infected buffalo in Kruger National Park of South Africa.

The Mycobacteriology Laboratory at ISU is currently conducting collaborative studies with the Swedish Institute for Infectious Disease Control on M. tuberculosis complex isolates from patients in Bissau-Guinea. Pathogenicity studies have been conducted on isolates with biochemical properties intermediate between M. bovis and M. tuberculosis.

i) Diagnostic methods. Isolation and identification of mycobacteria to confirm the diagnosis of tuberculosis from pathological samples (lymph nodes, lungs, etc.) of domestic and wild animals. Mycobacteria are also isolated from environmental samples.

ii) Control of biologic products (bovine and avian tuberculin). The potency of bovine or avian tuberculin is determined in guinea pigs sensitised with M. bovis or M. avium, by comparison with a preparation calibrated in International Units.

iii) Epidemiology, surveillance and reporting system. During 1992, 442,000 cattle herds, i.e. 18,5 millions of cattle have been screened against tuberculosis. Annual prevalence of infected herds was 0,32 %, point prevalence on 31 December 1993 was 0,16 and incidence rate was 0,16 %. The rate of infected animals was 2,5 out of 10,000.

iv) Public health consequences of zoonotic aspects. Tuberculosis in domestic animals in France has been reduced to a low prevalence. However, widespread foci of infection persist. Mycobacterial infections including tuberculosis in mammals and birds still constitute a serious problems in zoos. There is no doubt that this situation indicates the existence of

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6. EUROPE

6.1 France

The following are the research activities of the mycobacteria laboratory of the Centre national d'études vétérinaires et alimentaires - Laboratoire central de recherche vétérinaires (CNEVA-LCRV):
a dangerous potential reservoir of tuberculosis to humans, farm animals and household pets.

v) Work plans and international cooperation: development of blood-based tests for the diagnosis of BTB. The antigenic specificity of several mycobacterial glycopep-tidolipids (GPL), phenolic glycolipids (PGL) and trehalose derived glycolipids (lipooligosaccharides or LOS) has been studied. These investigations clearly showed that some of these antigens were species specific and therefore were of interest both for the identification of clinical isolates and for the serodiagnosis of mycobacterial diseases. The aim of this work is to determine the humoral response in BTB using specific glycolipid antigens in ELISA in order to define the optimal conditions and parameters for early diagnosis.

6.2 Germany

A research project has been initiated to compare some *M. bovis* strains isolated in Africa and Germany by RFLP pattern. The epidemiology of BTB in Ethiopia is part of this research. Among 2,000 cattle of different farms, the proportion of reactor by comparative intradermal test was between 5.1% for crossbred animals and 0.6 - 1% for local zebus. *M. bovis* was isolated from 7 (2.9%) of 241 samples of raw milk and from 2 (2.8%) of 79 nasal swabs of reactor cows.

In cooperation with the Tuberculosis Laboratory of the National Health Research Institute, Addis Ababa, the first *M. bovis* strain was found in the sputum of a tuberculous patient in Ethiopia using Löwenstein-Jensen-medium without glycerol. A survey on slaughtered cattle in six different abattoirs confirmed the occurrence of BTB in various regions of the country. Condemnation rates for whole carcasses due to generalized tuberculosis ranges between 0.008 and 1.15%.

6.3 Italy

i) Assessorato Sanità, Piemonte region

A Regional Centre on animal tuberculosis has been created in order to continue research on BTB, with special reference to *M. bovis* infection in humans. The Centre is responsible for the collection of information on human cases due to *M. bovis* and for the promotion of specific medico-veterinary investigations.

Research on occupational risks due to contact with infected premises and slaughterhouses has been carried out with the
collaboration of the Antitubercular Service and the Veterinary Faculty in Turin.

A field study on ELISA test performance on cattle tuberculin tested and found inconclusive is the object of a joint project with the Centre d'Études Vétérinaires, Maisons d’Alfort, Paris. Results are not yet available.

ii) *Istituto Zooprofilattico Sperimentale “G. Caporale”, Teramo (IZSTe)*

A veterinary information system (VIS) has been developed by IZSTe. It generates a number of key indicators used for decision-making processes concerning control programmes on BTB, bovine and ovi-caprine brucellosis, and until 1991, foot and mouth disease.

The Abruzzi Region has 110,000 heads of cattle distributed in 10,000 herds, and 620,000 small ruminants (sheep and goats) distributed in 25,000 flocks.

The unit of concern for the VIS is the herd and not the single animal. Data concerning single animals, however, are recorded only when a problem arises.

VIS provides information to several users, such as Regional Veterinary Service, Veterinary Services of the Local Health Units (LHUs) and IZSTe Bacteriology and Serology Laboratories.

In the case of BTB, the data come directly from the LHUs and enable users to:

- know the total cattle populations and herds composition. Data on fattening cattle are gathered once per year;
- know the structure of cattle population with relation to both breeding and fattening stocks (brucellosis and tuberculosis campaigns involve only herds with breeding animals);
- know the prevalence, incidence, geographic distribution and morbidity within the herds due to BTB;
- know the time necessary to eliminate infection in a herd as well as the probability of re-infection;
- periodically perform random testing to assess the effectiveness and efficiency of control programmes and to gather more information on the animal population structure (age, sex ratio, breeds, etc.) and of possible risk factors.
With reference to BTB, a 10-year analysis has been carried out, the following section reviews its results:

a) Controlled herds and heads. The percentage of controlled herds/year ranged from 24.9% in 1985 to 44.8% in 1992. The percentage of heads controlled was 28.9% and 45.5% in 1985 and 1992, respectively. The percentage of herds and heads controlled every year is approximately the same, thus proving that there is no specific trend towards control within the largest farms.

d) Average number of animals per herd. It appears that the herd size is a risk factor for BTB. 56.2% of the outbreaks involved herds whose composition ranged from 10 to 49 heads, which represents 26.87% of the total bovine farms.

e) Morbidity. Within herds morbidity is usually low (16.3%). In 52% of the outbreaks there was only one reactor.

b) Percentage of infected herds. The percentage of herds infected (outbreaks) every year ranged from 0.47% to 1.09% for the period 1983-1989. Since 1990 this value had been increasing for a couple of years, reaching a peak of 1.89% in 1991.

c) Percentage of infected heads with relation to breeds. The incidence ranged from 0.011% to 0.033%. This estimate has been carried out once considered notified outbreaks/year, average composition of bovine herds and average morbidity in the outbreaks.

Dairy cattle are the most involved, with 78.5% of the cases between 1990 and 1992. Of the dairy cattle breeds, Holstein-Friesian cows are the most represented (45.4%). It was not possible to assess whether the breed is a risk factor because the information system does not record data on negative animals.

f) Months needed to clear infected herds. According to the national regulations the optimal period for clearing an BTB infected farm is 2.5 months. However the regional average period was 7 months, and it reached 10 months in 1988 and 1989. The elimination period seems to be too long and could represent a risk factor to farms adjacent to infected premises.

VRI does not allow more detailed analysis of the risk factors for the spread of BTB. Therefore, a study looking into the origin of BTB outbreaks and estimating the risk factors responsible for BTB spread is
currently underway. This study will also evaluate the sensitivity/specificity of the tuberculin test under local circumstances and identify the most frequent etiological agents causing non-specific reactions. The investigation is structured as a case-control study. Data are not yet available.

6.4 Spain

National data on the number of human tuberculosis cases per 100,000 people have been reported as follows: 29.7 in 1989, 19.5 in 1990, 23.1 in 1991 and 24.8 in 1992. Since mycobacteria speciation is not usually performed, it is assumed that less than 1% of cases are caused by *M. bovis*.

A BTB programme based on test and slaughter strategy covers the whole country. Some data are reported in Table 6.

<table>
<thead>
<tr>
<th>Year</th>
<th>No of tested cattle</th>
<th>% positive</th>
<th>% farms BTB free</th>
</tr>
</thead>
<tbody>
<tr>
<td>1990</td>
<td>2,952,878</td>
<td>3.7</td>
<td>no data</td>
</tr>
<tr>
<td>1991</td>
<td>3,097,096</td>
<td>3.3</td>
<td>90.8</td>
</tr>
<tr>
<td>1992</td>
<td>3,060,270</td>
<td>2.1</td>
<td>92.6</td>
</tr>
</tbody>
</table>

Significant regional differences are present. Northern regions have lower disease occurrence. According to 1992 data, the percentage of tuberculin reactors in Andalucia and Extremadura (south) were 8.1% and 6.8%, respectively, whereas, in some northern regions, such as Asturias, Cantabria and Pais Vasco the proportion of BTB reactors were 0.6, 0.4 and 0.2 respectively.

*M. bovis* causes disease in goats in Spain; however, there are no official data on caprine tuberculosis (CTB) and only part of the national goat population of more than 2 million heads is routinely tested. Some regions started a control programme based on a test and slaughter policy in 1988. Generally, CTB is under control in these areas; however, each year new flocks are found to be infected. CTB has been very difficult to eliminate from infected flocks. Yearly incidence of positive reactors of 20-30% have been recorded and, in some instances, all animals of infected flocks have been...
slaughtered to eliminate infection. Due to the habit of eating goat dairy products, CTB could have potentially severe veterinary public health consequences in Spain as well as in other Mediterranean countries where goat breeding is widespread, consumption of goat's milk products is common and the CTB situation is largely ignored.

6.5 Sweden

The Mycobacteriological Safety Laboratory, Department of Bacteriology of the Swedish Institute for Infectious Disease Control (SIIDC) is the national reference laboratory for clinical mycobacteriology. The research activities at SIIDC are focused on various public health aspects of infectious diseases, including e.g. improved clinical diagnosis of the infecting agents and epidemiological studies. SIIDC has a broad international collaboration in various public health projects. In the case of zoonosis there is a close collaboration between SIIDC and the National Veterinary Institute in Uppsala.

In the field of tuberculosis and other mycobacterial infections in animals, SIIDC could contribute mainly in areas of laboratory examination of the infecting agents, including, for example, the rapid demonstration of mycobacteria in clinical samples and epidemiological examination of strains for the study of disease transmission. SIIDC could also contribute with various reference laboratory activities, such as to keep and distribute reference strains and support to establish and control new test systems in laboratories in developing countries.

i) Diagnostic methods - demonstration and identification. The work comprises both: a) rapid culturing methods, mainly based on radiometric detection of growth in a radio-labelled substrate, and, more recently, b) rapid culture-independent methods such as the specific demonstration of mycobacterial nucleic acids by PCR or other molecular techniques, such as the MTB Direct Test from Gen-Probe.

A commercially available nucleic hybridization test system (Accu-Probe, Gen-Probe Inc. USA) is used for the identification of M. tuberculosis complex (including M. bovis) and e.g. the M. avium complex. Due to the lack of the presently available probes to separate within this complex, biochemical tests still have to be used to differentiate M. bovis from other members of the tuberculosis complex. Methods for identification of isolated mycobacteria based on the sequencing of the gene coding for the 16S-rRNA have recently been introduced for the identification of more rarely
occurring or new mycobacterial species.

ii) Prevention and control - surveillance of drug resistance. SIIDC has a research interest in mycobacterial drug resistance and could contribute in establishing solid baseline data regarding the initial resistance of clinical isolates of *M. bovis* before trials with chemotherapy, as well as monitoring the effect of chemotherapy on the resistance pattern of clinical isolates both from the individual animal and at a population basis.

iii) Epidemiology - surveillance. For epidemiological typing SIIDC is using RFLP or DNA-fingerprinting. This technique is very promising for a detailed epidemiological study of the spread of mycobacterial infections, including the transmission of *M. bovis* or *M. tuberculosis* between animals and humans.

Regarding human *M. bovis* infections in Sweden, epidemiological data for the last 10 years are summarized in Table 7 below. These data illustrate the situation in a country with no *M. bovis* infection in cattle for over 35 years, but where the laboratory examination of clinical samples from humans is optimized for the detection of *M. bovis*.

### Table 7. Culture verified cases of human *M. bovis* infection compared to tuberculosis caused by the classical human *M. tuberculosis* in Sweden 1983–1992.

<table>
<thead>
<tr>
<th>Year</th>
<th><em>M. bovis</em> isolates</th>
<th><em>M. tuberculosis</em> isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>1983</td>
<td>7</td>
<td>605</td>
</tr>
<tr>
<td>1984</td>
<td>6</td>
<td>544</td>
</tr>
<tr>
<td>1985</td>
<td>11</td>
<td>514</td>
</tr>
<tr>
<td>1986</td>
<td>9</td>
<td>501</td>
</tr>
<tr>
<td>1987</td>
<td>9</td>
<td>438</td>
</tr>
<tr>
<td>1988</td>
<td>5</td>
<td>408</td>
</tr>
<tr>
<td>1989</td>
<td>8</td>
<td>435</td>
</tr>
<tr>
<td>1990</td>
<td>10</td>
<td>441</td>
</tr>
<tr>
<td>1991</td>
<td>15</td>
<td>431</td>
</tr>
<tr>
<td>1992</td>
<td>16</td>
<td>409</td>
</tr>
</tbody>
</table>
The patients infected with *M. bovis* are mainly elderly Swedes or immigrants from regions with a high prevalence of *M. bovis* in cattle population.

Epidemiological sub-typing of human and animal clinical isolates of mycobacteria with the RFLP technique is carried out on isolates of *M. bovis* in Sweden. The results so far demonstrate that a single strain of *M. bovis* is the cause of the outbreaks of BTB in deer, and that the strains isolated from humans are not related to this strain.

### 6.6 The Netherlands

The following are the current and foreseen research activities of the Central Veterinary Institute (CVI) in Lelystad.

1. **Diagnostic methods.** Bovine and avian PPD tuberculin are produced on a large scale for national requirements and the international market. Quality testing of bovine and avian PPD tuberculin from various producers is also carried out. Some bovine PPDs from various origins, intended for use in some African countries, were found to have a low potency, ranging from only 455 to 1,267 IU per cattle dose of 0.1 ml. These potencies are much lower than 2,000 IU per cattle dose, the required minimal diagnostic dose for European Union (EU) countries. In contrast, avian PPD tuberculins from different sources had a good potency, similar to the International Standard for avian PPD tuberculin.

Countries can use their own National Reference Standard for quality testing, if this National Standard has been calibrated against the International Standard.

An ELISA test for the diagnosis of *M. bovis* infections in animals has been developed by CVI. The antigen is a KCl-extract of *M. bovis* and contains mainly cell wall proteins. The results in zoo- and wildlife animals are satisfying but for cattle the specificity has to be improved. The MPB 70 protein is very specific but sensitivity is too low. A joint research project with other EU laboratories is now working on the selection of more specific proteins with the aim to improve sensitivity by using a mixture of these proteins. The ELISA can be a complement rather than an alternative for tests based on cellular immunity and may be helpful in detecting anergic cattle.

Under Dutch conditions the IFN test had no advantage over the classical skin test. Addi-
tional disadvantages are the high costs and the fact that the examination of the blood samples must be started within 8 hours after collection.

ii) Prevention and control. The Dutch cattle population is free from BTB. Very sporadic infections of *M. bovis* may occur (0 to 3 farms per year) caused by importation of cattle or by transmission from *M. bovis* infected people to cattle. The control of BTB is based on test and slaughter strategy. DNA-fingerprinting is performed in collaboration with the National Institute of Public Health on isolated strains of *M. bovis* by RFLP and is a useful tool in epidemiology. The IS 6110 banding pattern of *M. bovis* isolates from cattle, humans, various zoo animals (lion, impala, monkeys, African antelopes, waterbucks and oryxes) and wildlife kept in parks in Saudi Arabia (oryxes and different antelopes species) have been studied.

6.7 United Kingdom

In England, BTB has been virtually eradicated by control programmes based on the test and slaughter method which achieved total coverage of the country in 1960. Reactors are occasionally encountered, especially in regions where cattle are exposed to badgers infected with *M. bovis*.

i) National Heart and Lung Institute (NHLI), London

The Department of Microbiology at the NHLI is involved in all aspects of mycobacterial disease from the educational and research point of view. The department has a particular interest in diagnostic bacteriology. The department has collaborated closely with the South East Regional Tuberculosis Centre, Dulwich, in bacteriological and epidemiological studies on human tuberculosis, including that caused by *M. bovis*.

The areas relevant to BTB, particularly in developing countries, in which collaboration could be established, are as follows:

Laboratory diagnosis of *M. bovis* disease in humans. Tests to distinguish the variants of the mammalian tuberculosis complex, including *M. bovis*, have been developed and extensively evaluated. The four-test scheme has been widely adopted but its more widespread use, together with improved and standardized culture techniques, is required for studies on the prevalence of *M. bovis* in the human population worldwide (5). Epidemiological studies on the incidence and nature of *M. bovis* disease in the human population of
South East England have been based on the above test scheme.

Human tuberculosis due to *M. bovis* in South East England is uncommon and in decline as shown in Table 8. Almost all cases in patients with English names occur in those born during the time when infection from milk occurred. About half the cases involve the lung, raising the possibility of human-to-human transmission. Two cases of HIV-related tuberculosis due to *M. bovis* in younger patients have occurred.

Table 8. Annual numbers of new cases of bacteriologically confirmed tuberculosis, including that due to *M. bovis*, in South East England, 1977-1993.

<table>
<thead>
<tr>
<th>YEAR</th>
<th>New cases of tuberculosis</th>
<th>% of total due to <em>M. bovis</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td><em>M. bovis</em></td>
</tr>
<tr>
<td>1977</td>
<td>1682</td>
<td>20</td>
</tr>
<tr>
<td>1978</td>
<td>1837</td>
<td>27</td>
</tr>
<tr>
<td>1979</td>
<td>1696</td>
<td>22</td>
</tr>
<tr>
<td>1980</td>
<td>1826</td>
<td>23</td>
</tr>
<tr>
<td>1981</td>
<td>1723</td>
<td>20</td>
</tr>
<tr>
<td>1982</td>
<td>1519</td>
<td>28</td>
</tr>
<tr>
<td>1983</td>
<td>1557</td>
<td>19</td>
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<tr>
<td>1984</td>
<td>1337</td>
<td>15</td>
</tr>
<tr>
<td>1985</td>
<td>1335</td>
<td>10</td>
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<tr>
<td>1986</td>
<td>1215</td>
<td>13</td>
</tr>
<tr>
<td>1987</td>
<td>1185</td>
<td>14</td>
</tr>
<tr>
<td>1988</td>
<td>1125</td>
<td>15</td>
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<tr>
<td>1989</td>
<td>1118</td>
<td>10</td>
</tr>
<tr>
<td>1990</td>
<td>1256</td>
<td>6</td>
</tr>
<tr>
<td>1991</td>
<td>1267</td>
<td>12</td>
</tr>
<tr>
<td>1992</td>
<td>1245</td>
<td>6</td>
</tr>
<tr>
<td>1993</td>
<td>1140</td>
<td>10</td>
</tr>
</tbody>
</table>
Studies on the impact of the HIV/AIDS pandemic on human tuberculosis. Available data, though limited, have raised the possibility of a serious impact of this pandemic on the nature and spread of *M. bovis* disease in the human population. By abrogating immunodefences, HIV facilitates human-to-human transmission of disease due to *M. bovis*. By increasing the number of infectious human beings, the risk of disease spreading to domestic and farmed animals is accentuated.

The methods suitable for control of BTB will critically depend on anthropological factors determining the perception of animals by pastoralist communities. Religious aspects must also be considered (e.g. the sacred status of the cow in Hindu communities).

New approaches to vaccination are now open to investigation and immunotherapy, based on vaccination of the already infected or even diseased animal, are fast becoming a reality. The results of the use of a heat killed environmental mycobacterium, *M. vaccae*, as an immunotherapeutic agent in humans have been very promising and indicate the need for studies in animals.

ii) Department of Agriculture for Northern Ireland, Stormont, Belfast

BTB is still an important problem in Northern Ireland with significant economic consequences for the farming community. The tuberculosis reactor incidence rate for the cattle population of approximately 1.5 million was 0.3% in 1993.

Current research is encompassed in six distinct projects: blood test development and evaluation, molecular and immunological studies on *M. bovis* and its pathogenesis in cattle.

a) Diagnostics. Improvements in this sphere remain crucial to reducing the cost of controlling/eliminating BTB. A preliminary evaluation at this laboratory, of the available alternative diagnostic tests for BTB showed the 'γ-IFN' ELISA to compare favourably with the current skin test. A large scale evaluation of this assay, employed under field conditions, has now been undertaken and an excess of 100,000 cattle have been tested in exposed and negative populations. Results are not yet available.

Development of other immunodiagnostic assays and identifying potential mycobacterial antigens for these consti-
tutes a major component of our current programme. In addition to the traditional biochemical approaches to obtain M. bovis antigens, overlapping synthetic peptides have been created to define T-cell epitopes for known antigens and recombinant antigens have also been examined. Candidate antigens are being screened for both B-cell and T-cell activity using defined monoclonal antibodies and reagents from experimentally infected cattle. In addition to lymphocyte proliferation and production of λ-IFN in tuberculous cattle, stable T-cell clones have been produced to screen for antigens producing cellular responses.

The early pathology and immunology of tuberculosis is being studied in cattle infected with M. bovis and has yielded novel information on the sequential development of lymphocyte subpopulations and of specific cytokines.

Research on PCR-based technologies for rapid confirmation of M. bovis is ongoing and direct detection of M. bovis in clinical specimens is being pursued to supplement the traditional and radiometric culture methods currently in use. Methodology using the 16S rRNA gene to rapidly identify relevant mycobacteria other than tubercle has been developed.

b) Prevention and Control. The current elimination programme is based on test and slaughter strategy. Consideration is currently being given to the feasibility of wildlife vaccination as a possible strategy for curtailing M. bovis spread to cattle.

c) Epidemiology. A reliable RFLP method has been developed for characterizing M. bovis at the subspecies level. Isolates from cattle and wildlife reservoirs are being examined for correlations between M. bovis genotypes and specific factors relevant to the epidemiology of BTB. In Northern Ireland, the recording of all cattle movement on a Unisys Mainframe Computer network linking all veterinary offices, markets and meat plants should permit maximum exploitation of this fingerprinting technology.

7. DISCUSSIONS

7.1 Diagnostic Methods

Tests that detect cellular and humoral immune responses in a tuberculous animal were discussed and the consensus was that a combination of tests, notably the skin test and an ELISA, currently pro-
vide the best results in terms of sensitivity and specificity.

The ELISA might give the most discriminative results when used on serum collected 10-20 days after a skin test with tuberculin PPD has been performed. It may also be able to detect animals with active tuberculous lesions.

The problem of the wide range of values, given by different authors, for the sensitivity and specificity of the tuberculin skin test, ELISA and γ-interferon was raised. The need to adopt standardized protocols for test evaluation trials was emphasised. This would enable a true comparison of results reported by different workers.

The need to provide guidance and training in the basic techniques of clinical diagnosis and post-mortem examinations were viewed as essential components for any tuberculosis investigation programme in developing countries.

A basic test requirement for many countries in the tropics is for the test(s) employed to provide a distinction between infected and diseased animals. It was noted that in human medicine, tests are employed to detect patients actively excreting tubercle bacilli and therefore infectious. The question was raised as to whether this approach would have a particular relevance to developing countries where control rather than eradication is seen as a more realistic first stage goal for control programmes.

The potential value of molecular techniques for the detection and diagnosis of BTB was recognised. The potential disadvantages of the PCR, with particular reference to the problem of false positive results, was noted and the technique should not be seen, at this stage, as a replacement for culture.

Experience in New Zealand from immunological studies in deer offered some challenging findings that might have fundamental implications for the diagnosis of tuberculosis in a wide range of animals.

7.2 Prevention and Control

Without doubt the test and slaughter policy, which has been used in many countries, is the most successful method of eradication. But it can only be afforded in so-called developed countries, and even then additional measures are necessary to control re-infection of tuberculosis-free herds especially from wildlife (as from opossums in New Zealand and badgers in England) or other sources.

Apart from a widespread lack of knowledge of the distribution and prevalence of the disease, lack of expertise in the laboratory and the field, laboratory equipment and general economic shortcomings, there are many other factors to be
considered when setting up and operating suitable schemes for campaigns against BTB in many developing countries. However, it should be recognized that many Latin American, Caribbean and African Countries have already set up, or are at least planning, eradication programmes. Promoting and strengthening these approaches should and must be one of the tasks of the Working Group and international organizations such as the WHO and FAO.

It was recognized that any prevention/control programme for BTB in developing countries should have clearly defined and, ideally, achievable objectives. In certain countries or areas, eradication may be both desirable and possible. It has to be recognized, however, that in many developing countries there are likely to be limited resources, inadequate infrastructures, other more pressing animal disease priorities, poorly equipped laboratories and an insufficient number of appropriately trained and/or skilled staff. In this scenario the first stage of any prevention and control programme should be targeted at reducing the general prevalence of the disease and containing any spread of infection while attention is given to remedying identified deficiencies for achieving sustainable eradication.

In particular, it was noted that epidemiological studies should precede, and have a major influence on, the design of any control programme.

The question of treatment for BTB was discussed and generally considered inappropriate and applicable in only very restricted circumstances. The main fears raised concerned the possibility of promoting the emergence of drug resistant strains, drug residues in meat leading to the development of drug resistance in consumers and the development of carrier animals. The cost of treatment in large ruminants and the problem of administration to achieve sustained therapeutic levels in wild animals was also raised.

There was considerable discussion and interest in the question of vaccination as a component of an overall tuberculosis control strategy. New Zealand and the United Kingdom are, out of necessity, considering this approach due to the reservoirs of infection in the wild animal population. The use of BCG, as described in early work conducted in the 1930s and 40s, was discussed. The problem of inducing a cross reaction with the tuberculin skin test was raised. The protective efficacy of BCG against disease due to a wide spectrum of mycobacterial pathogens including M. leprae and M. avium was noted. The possibility of heat-killed M. vaccae as a candidate vaccine for controlling tuberculosis in cattle was raised due to its non-pathogenic immunological property plus its apparent ability to convert necrotizing to healing reactions.
If a vaccine is to be approved it must be shown conclusively to be efficacious. Studies in New Zealand suggest that low dose BCG provides 100% protection and sterile immunity whilst high dose BCG can exacerbate infection. The spectre of carrier status and latent infection induced by vaccination was raised as a concern.

The possibility of identifying and utilizing naturally (genetic) resistant animals was proposed. It has been established that innate resistance to Tuberculosis is coded for by specific genes (BCG - in mice, NRAAP - in humans) which facilitate mycobacterial killing. In any population the majority of animals are likely to be resistant at some level (RR, RS or SR) but some will be uniquely susceptible (SS). In areas of high exposure risk it may be more practicable to select SS animals at low prevalence (5%) and remove such animals as uniquely susceptible, in a cost-effective culling programme, rather than use vaccines indiscriminately to generate questionable prophylactic immunity.

If vaccines are to be used it may be important to consider neonates as a special target to prevent vertical transmission to this naïve (and possibly highly susceptible) group.

7.3 Epidemiology, Surveillance and Reporting Systems

The paucity of information on the distribution and prevalence of BTB in developing countries is a general problem. This information is a prerequisite for preparing work plans and, in particular, for convincing funding agencies of the need to support such work. It is recognized that developing countries in the tropics face other more apparent animal disease problems such as tick-borne diseases, helminthiasis and viral infections such as foot-and-mouth disease. These conditions have a more readily perceived negative impact on livestock productivity and are consequently given an higher priority for action.

The public health hazards posed by BTB and other potential zoonotic infections including brucellosis, rabies and zoonotic cestodes are not well defined for most developing countries. If proven and shown to be having a significant impact, the case for re-evaluating existing animal health priorities and for justifying additional resources can be more effectively made. In this connection, the VPH unit has prepared project proposals for in-country studies (17). These have been submitted to a number of potential donor agencies and responses are awaited.

In light of the above, the need for effective surveillance and re-
porting systems coupled with targeted epidemiological studies was widely recognised. The VPH unit at WHO has recently commissioned the preparation of a working paper on surveillance methods for BTB (2). When published, this will be available as a field guide for veterinary services wishing to undertake studies on the distribution and prevalence of the disease.

It was noted that any programme for bovine tuberculosis control in developing countries would be likely to follow an “intermediate” approach which should be guided by a thorough understanding of the epidemiological factors governing the distribution and prevalence of the disease in a given country.

The importance of gaining the trust and support of the livestock owners before implementing any control programme was emphasized. A testing protocol that discriminated between lesioned and non-lesioned reactors would be a valuable development for this purpose. Such a test protocol could logically be combined with preliminary epidemiological studies.

Studies should take into consideration the different livestock systems practised and recognise that the perceived importance of BTB is likely to differ from system to system. It was generally agreed that the disease was potentially the most important in the dairy sector.

For transmission studies it will be important to adopt techniques that will detect animals actively excreting mycobacteria. This would discriminate between infected and infectious cases which could be valuable in any first stage control programme. Nasal swabs and milk (when available) would be candidate samples for such studies. However, in the design and interpretation of such studies, it should be borne in mind that intermittent excretion can occur.

Reservoirs of infection in wildlife have proved a problem for BTB control programmes in some developed countries. The presence of a wide range of potential wildlife reservoirs in developing countries, particularly Africa, was noted. Epidemiological studies would need to take cognisance of this situation and ultimately an assessment of the possible threat posed by the various wildlife species in maintaining infection will need to be made. This will be particularly important in areas where they are found in close association with domestic stock.

7.4 Public Health Consequences and Zoonotic Aspects

The historical pronouncement by Dr Robert Koch, that *M. bovis* should not be considered pathogenic to humans has persisted in some medical minds despite ample
evidence to the contrary, gathered since the days of Koch. One reason for this attitude can be found in the cultural practices of a number of medical mycobacterial diagnostic laboratories where only media containing glycerol, which inhibits the growth of some \textit{M. bovis} strains, are routinely employed. The development of a series of 4 biochemical tests that selectively differentiate between members of the \textit{M. tuberculosis} complex has materially assisted the process of achieving a reliable diagnosis \cite{5}, although it should be borne in mind that these methods are dependent on the responsible organism being successfully grown on culture in the first instance.

The question of human infection with \textit{M. bovis} has raised many contentious issues. A principal subject of debate has been the significance and prevalence of human-to-human transmission of \textit{M. bovis}. The frequency of this mode of transmission has been extremely difficult to prove or disprove because of the long interval between infection and the appearance of clinically detectable disease. This argument has been dramatically affected by the HIV/AIDS epidemic. Recent reports of HIV/AIDS patients with tuberculosis due to \textit{M. bovis} include a nosocomial outbreak in a hospital ward in Paris \cite{1}. Five contacts of HIV/AIDS patients with a multi-drug resistant strain of \textit{M. bovis} developed overt disease within 10 months of contact. The impact of HIV/AIDS in this outbreak demonstrated three epidemiologically critical factors:

i) HIV/AIDS abrogates any normal defence mechanism against \textit{M. bovis}.

ii) Human-to-human transmission is facilitated with the interval between infection and development of clinical disease dramatically reduced from several years or decades to just a few months.

iii) Multiple drug resistance is a potential problem with \textit{M. bovis} and may possibly present more of a problem than \textit{M. tuberculosis} as \textit{M. bovis} is naturally resistant to pyrazinamide.

The current HIV/AIDS epidemic in Africa and the impending spread of the epidemic through South East Asia and India gives considerable cause for concern, particularly in areas where an unknown but potentially large and widespread number of \textit{M. bovis} infected animals are kept in close association with their human owners. The facilitation of the cycle of infection of \textit{M. bovis} between animals and humans by the HIV/AIDS virus could lead to a significant amplification of the amount of infection prevalent in the two populations. Where other causal factors such as stress, malnutrition and high population densities coexist, the conditions are set for an explosive epidemic.
The problem of under-reporting *M. bovis* from human tuberculosis cases by medical laboratories was discussed. Recent surveys independently undertaken by members of this Working Group show that most laboratories routinely only use culture media containing glycerol, which suppresses the growth of some strains of *M. bovis*.

Preliminary results from a study on the zoonotic importance of *M. bovis* in Tanzania have shown that of the first 11 isolates derived from 19 human cervical lymphadenitis cases, 4 were *M. bovis* and 7 *M. tuberculosis*. In addition, 4 of the *M. tuberculosis* strains isolated from cattle at an abattoir, showed identical RFLP patterns with 5 of the 6 human strains.

In Latin America the problem of inappropriate cultural methods for *M. bovis* also exists. Current information suggests that only 1-2% of human cases are due to *M. bovis* which seems to be irrespective of HIV/AIDS status.

Results from an ongoing Swedish study in Guinea Bissau show that *M. bovis* is rarely isolated from human cases but that 3 distinct biovars with intermediate properties between human and animal strains have been isolated. The use of pyruvate-enriched, glycerol-free media was seen as critical in making these isolations.

The occupational risk of abattoir and other workers in the livestock sector contracting *M. bovis* was discussed. The cases of tuberculin conversion/infection in Canadian Elk contacts, Australian abattoir workers and possum handlers in New Zealand were raised. The isolation of a uniquely DNA profiled *M. bovis* strain from both an Australian seal trainer and his seals was reported. This isolate matched strains subsequently isolated from wild seals.

The immunogenic property of *M. bovis* as a "natural" protective vaccine for humans was discussed. This phenomenon has been reported and discussed in the literature. It was agreed that any programme to control *M. bovis*, particularly in pastoral communities, should take cognisance of this factor and, where necessary, appropriate tuberculosis control measures including BCG vaccine should be instituted.
8. CONCLUSIONS AND RECOMMENDATIONS

8.1 Diagnosis and Control

i) There is a need to devise an overall control strategy that includes conventional components such as test and slaughter, test and segregation, as well as novel approaches such as vaccination. To determine the most appropriate strategy (combination or single) it is important to conduct socioeconomic and epidemiological studies to understand the importance and ecology of the disease with particular reference to the factors influencing transmission and maintenance.

ii) It is important that diagnostic test(s) are selected and developed so that they can provide accurate information on the tuberculosis disease status of a given animal in order to gain farmer cooperation and confidence. It is likely that a combination of tests will be the best approach, probably the skin test followed by the ELISA 14 days later.

iii) Standardization of diagnostic test methods should be adopted to allow true comparison between countries and/or laboratories - including tuberculin testing, sero-diagnostics and DNA techniques.

iv) Special attention should be given to the development of an efficacious vaccine for domestic animals and, where necessary, wildlife hosts. BCG should be the starting point and pilot studies with low dose and different strains considered. An ideal vaccine should not interfere with the tuberculin test and thus some marker should be incorporated.

v) Vaccination should not be implemented as a control method in a vacuum but as part of an overall strategy tailored to a particular area. The possibility of using new approaches to vaccination, as is currently under investigation in the human field, should be pursued. It is recommended that this issue is carried forward to an expert meeting convened specifically to discuss the prospects and methodology for establishing a collaborative programme to develop a vaccine (18).

vi) An understanding of the genetic and immunological basis of resistance and susceptibility is likely to be important in selecting the most appropriate control strategy.

vii) Training for field staff, meat inspectors and public health
workers will be necessary. Production of appropriate visual aids in support of training and raising farmer/consumer awareness will also be required. Informed farmer participation, through associations etc., will be crucial for the success of any proposed control strategy.

8.2 M. bovis: Zoonotic Aspects

i) The risk posed to the human population in developing countries by animal tuberculosis was recognized. There is an urgent need to establish the extent of the risk posed. This situation is made more critical by the impact of the HIV/AIDS epidemic with the possibility of multi-drug resistant strains of M. bovis.

ii) Basic procedures for the differentiation between members of the M. tuberculosis complex should be adopted by all mycobacteria laboratories. WHO/VPH will issue guidelines for the methodology required to achieve this objective (b).

iii) The use of molecular techniques such as the RFLP will play a key epidemiological role in elucidating the zoonotic aspects of M. bovis. VPH will act as a focal point for information dissemination and coordination for those laboratories and institutions employing or intending to employ molecular techniques for investigating mycobacteriosis in animals and humans.

iv) The group emphasized the need to establish communication between veterinary (VPO) and medical (PHO) public health officers. Ideally each region should have a VPO and PHO with established lines of interaction. VPOs would report instances of bovine tuberculosis to PHOs who would initiate the examination of human contacts. Likewise PHOs would report on the incidence and location of clusters of extra-pulmonary human tuberculosis cases and/or the isolation of M. bovis from human patients.

v) About 9 supra regional reference laboratories are being nominated to provide an advisory service to networks of national and regional laboratories for human tuberculosis. Hopefully, these reference laboratories will agree to differentiate M. bovis from M. tuberculosis and to cooperate closely with veterinary services.
REFERENCES


* unpublished documents are available on request from the Veterinary Public Health unit, Division of Communicable Diseases, World Health Organization, Avenue Appia 20, CH-1211, Geneva 27, Switzerland
ANNEX 1 - LIST OF PARTICIPANTS

Dr J. Berrada, Département de Microbiologie-Immunologie et Maladies Contagieuses Institut Agronomique et Vétérinaire, Hassan II, B.P. 6202, Rabat, Morocco

Dr J. Barajas-Rojas, Department of Microbiology and Immunology, Faculty of Veterinary Medicine, National Autonomous University of Mexico, Mexico City, 04510 Mexico

Dr D. M. Collins, Australian Reference Laboratory for Bovine Tuberculosis, Department of Agriculture, 3 Baron-Hay Court, South Perth, WA 6151, Australia

Dr D. V. Cousins, Australian Reference Laboratory for Bovine Tuberculosis, Department of Agriculture, 3 Baron-Hay Court, South Perth, WA 6151 Australia

Dr C. Daborn, Centre for Tropical Veterinary Medicine, Royal (Dick) School of Veterinary Studies, The University of Edinburgh, Easter Bush, GB-Roslin, Midlothian EH25 9RG, United Kingdom

Professor E. A. Fanning, Tuberculosis Services, 8220-114 Street, Edmonton, Alberta T6G 213, Canada

Dr J. M. Grange, Reader in Clinical Microbiology, Royal Brompton National Heart and Lung Institute, University of London, Dovehouse Street, GB-London SW3 6LY, United Kingdom

Dr J. F. T. Griffin, Associate Professor of Microbiology, Director, Deer Research Laboratory, University of Otago, P.O. Box 56, Dunedin, New Zealand

Dr J. Haagsma, Head, Dept of Bacteriology, Central Veterinary Institute, Ministry of Agriculture, Nature, Management and Fisheries, Edelheertweg, 15 (P.O. Box 65), NL-8200 AB Lelystad, Netherlands

Dr S. Hoffner, Swedish Institute for Infectious Disease Control, S-10521 Stockholm, Sweden

Report of the WHO working group on zoonotic tuberculosis (Mycobacterium bovis), with the participation of FAO.
Dr H. Huchzermeyer, Veterinary Research Institute, Department of Agricultural Development, Private Bag X5, Onderstepoort, South Africa

Dr J. Francisco Garcia Marin, Departamento de Patologia Animal-Medicina Animal Facultad de Veterinaria, Universidad de Leon, Campus de vegetana sin, E-24071 Leon, Spain

Dr G. Moda, Assessorato Sanità (regione Piemonte), National Veterinary Services, 10063 Corso Margherita, Italy

Dr D. Morelli, Istituto Zooprofilattico Sperimentale dell’Abruzzo e del Molise “G. Caporale”, Via Campo Boario, Casella Postale 137, I-64100 Teramo, Italy

Dr Ali M. Moussa*, Chairman, General Organization of Veterinary Services (GOVS) Agriculture Reclamation Building, 1 Nadi El-Seid Street, Dokki - Cairo, Egypt

Dr S. Neill, Department of Agriculture for Northern Ireland, Veterinary Sciences Division, Stoney Rd, Stormont, Belfast BT4 3SD, Northern Ireland

Dr M. Scacchia, Istituto Zooprofilattico Sperimentale dell’Abruzzo e del Molise “G. Caporale”, Via Campo Boario, Casella Postale 137, I-64100 Teramo, Italy

Professor T. Schliesser, (Former Director), Institute of Animal Hygiene & Infectious Diseases, Justus-Liebig-Universitat, Frankfurter Str. 89-91 D-35392 Giessen, Germany

Dr J. M. Sharp, Moredun Research Institute, 408 Gilmerton Road, GB-Edinburgh EH17 7JH, United Kingdom

Professor C. O. Thoen, Coordinator of the WHO/VPH Working Group on Zoonotic Tuberculosis, Department of Veterinary Microbiology and Preventive Medicine, Iowa State University of Science & Technology, P.O. Box 124, Ames, IOWA 50011, USA

Professor M. F. Thorel, Centre National d’Etudes Vétérinaires et Alimentaires Lab. central de Recherches Vétérinaires, 22, rue Pierre-Curie (B.P: No 67), F-94703 Maisons-Alfort, France

* unable to attend
Other Organizations

Dr A. Benkirane, Bacteriology, Animal Production and Health Division, Food and Agriculture Organization, I-00100 Roma, Italy

Secretariat

Dr F.-X. Meslin, Chief, Veterinary Public Health, Division of Communicable Diseases, World Health Organization, Geneva, Switzerland

Dr O. Cosivi, Veterinary Public Health, Division of Communicable Diseases, World Health Organization, Geneva, Switzerland

Dr I. de Kantor, Pan American Institute for Food Protection and Zoonoses (INPPAZ), Veterinary Public Health Program PAHO/WHO, Calle Talcahuano 1660, Casillo de Correo 44, 1640 Martinez, Provincia de Buenos Aires, Argentina