

**Risk associated with *Mycobacterium bovis* infections  
detected in selected study herds and slaughter cattle  
in 4 countries of West Africa**

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## SUMMARY

During October 2000 to March 2003 several pilot studies to assess the prevalence of tuberculosis in cattle (BTB) were carried out in The Gambia, Senegal, Guinea and Guinea Bissau in form of abattoir and on-farm screenings using different diagnostic approaches.

In The Gambia 1,595 cattle in two abattoirs located in the Greater Banjul Area were examined using classical meat inspection to detect tubercle-like lesions. In Guinea (Kankan) and Guinea Bissau (Bissau), where one abattoir per country was selected, Comparative Intradermal Tuberculin Test (CIDT) prior to slaughter and followed by meat inspection of positive reactors was applied on 933 and 450 cattle respectively. Suspected tubercle-like lesions found in meat inspection and also positive in the Ziehl-Neelsen stain were sent to the National Reference Laboratories for specific culturing for *Mycobacterium bovis*. On-farm screenings using CIDT were carried out on 20 farms (465 cattle) in the Central River Division (CRD) of The Gambia and on 30 farms (479 cattle) in the Bassin Arachidier of Senegal. Doubtful positive reactors for *Mycobacterium bovis* in the skin test were retested after a period of 3 months for confirmation.

To assess the importance of reported positive cases in cattle, investigation in risk groups (herders, farm members etc.) was also envisaged. A questionnaire was therefore applied at abattoirs to identify the places of origin of individual slaughter animals. In addition, information was collected from the national public health authorities on their diagnostic capacity and recorded cases of bovine tuberculosis in man.

Results indicate an absence of any clinical and immunological infection due to *M. bovis* in cattle. All doubtful reactions in the skin test (Senegal n= 5, The Gambia n= 5; Guinea Bissau n= 2) or suspected lesions in meat inspection (The Gambia n= 1) were not confirmed by further testing.

Except for Guinea (16%) very high proportions (> 50%) of cattle reacted positive to PPD *M. avium* indicating a high exposure to environmental mycobacteria, which might have epidemiological and diagnostic implications. Recent findings have shown that environmental infections with other mycobacteria influence the immune response to *M. bovis* to an unknown extent. Further research based on more sensitive test methods is essential for interpretation of the results and the understanding of the mechanism involved.

From the investigations in public health centres it can be concluded, that mainly due to low capacity and high costs involved, specific *M. bovis* diagnosis is not routinely applied. Therefore information on the prevalence of *M. bovis* infection in man is insufficient and not representative.

# 1 INTRODUCTION

Human and animal tuberculosis is wide spread in Africa with very close genetic similarity between the causative organisms, *Mycobacterium tuberculosis* (*M. tuberculosis*) and *Mycobacterium bovis* (*M. bovis*). The presence of *M. bovis* in cattle can pose a serious health risk for man since close contact between people and animal, and consumption of raw milk form part of the society characteristics. In sub-Saharan Africa nearly 2 million tuberculosis (TB) cases per year occur in man and the genus *Mycobacteria* has pathogenetic and zoonotic importance (DABORN et al., 1996). It is estimated that in countries where pasteurisation of milk is rare and bovine tuberculosis (BTB) in cattle is common 10% to 15 % of human cases of TB are caused by *M. bovis* (ASHFORD et al., 2001).

In West Africa, BTB has been reported in domestic ruminants in Senegal, Burkina Faso, Mauritania, Ghana and Nigeria (BENKIRANE, 1998, BONSU et al., 2000, DUSAI. et al., 1995, DELAFOSSE, 1995) and has been suspected in all other countries of the region. Except for these few available publications there is a substantial lack of knowledge of the distribution, epidemiological pattern and zoonotic implication of BTB in livestock and man in West-African countries. The latest information for The Gambia and Guinea Bissau is more than 30 years old. Cases of BTB in man are reported for Guinea (GOUMOU, 2001), Nigeria (IDIGBE et al., 1986) and Guinea Bissau (HOFFNER et al., 1993). No information is available on the origin of the infections in man in Guinea and Guinea Bissau.

Man can acquire tuberculosis of bovine origin directly by the aerogenous route and indirectly by consumption of milk and meat. As the main route of entry is the oral route, BTB in man is mainly extra pulmonary, resulting in abdominal, bone and joint tuberculosis as well as infection of the cervical and mesenteric lymph nodes (DABRON and GRANGE 1993, EDELSTEIN, 1995, ASHFORD et al., 2001).

BTB as a predominantly milk-borne zoonosis may have a significant impact on private and social costs in milk consuming societies in urban and rural areas of Africa, where poor economies can not afford culling and refund practices, a strategy which led to successful elimination of bovine tuberculosis in many countries in Europe. Zoonotic transmission of *M. bovis* to man could be prevented by either eradicating the disease in animals or educating people about the risk factors (consumption of raw milk). There is an urgent need to investigate veterinary and human health aspects of *M. bovis* infections jointly.

In order to generate the necessary information to develop strategies to minimise the risk of transmission of BTB, several pilot studies to assess the prevalence of BTB in cattle were carried out between October 2000 and April 2003 in Senegal, The Gambia, Guinea and Guinea Bissau in the form of abattoir and on-farm surveys.

To determine the potential importance of infections in cattle for humans, positive cattle were traced back to the place of origin where investigation in people at risk of contact with infected animals were envisaged. This part of the study as well as further confirmation tests were proposed to be conducted by the National Reference Laboratories for Tuberculosis or International Research Organisations (e.g. Medical Research Council located in The Gambia).

## **2 MATERIALS AND METHODS**

### **2.1 Investigations in cattle**

#### **2.1.1 Approaches, study population and sampling strategy**

For the diagnosis of BTB in cattle three different approaches were applied as shown in table 1.

*Classical meat inspection* to detect tubercle like lesions due to *M. bovis* was applied in two main abattoirs located in the Greater Banjul Area (GBA), Abuko and Banjul of The Gambia. The sample size consisted of all animals present at the 3 times weekly visits during the period November 2000 to March 2001. A total of 1595 cattle was investigated in the 5 months sampling period. Cattle slaughtered were mainly trypanotolerant N'Dama. Few cattle were of Zebu type and originated from Senegal.

*Tuberculinisation* prior to slaughter *followed by meat inspection of positive reactors* was carried out at the city abattoir in Kankan (Guinea) and Bissau (Guinea Bissau). These diagnostic procedures lead to a higher sensitivity compared to simple meat inspection, which is known to be low in developing countries (BARWINEK and TAYLER, 1977; DeKANTOR et al., 1987). In Kankan 933 cattle were tuberculinised during the period March to November 2002. Similarly, 450 cattle were tested at Bissau abattoir during March 2002 to March 2003. The calculation of sample sizes was based on an assumed prevalence of 1 % and a 95 % confidence interval (THRUSFIELD, 1995). The predominant breed of slaughter cattle was the trypanotolerant N'Dama.

*On-farm screening* using *tuberculinisation* was carried out on 30 randomly selected herds chosen from a bigger sampling frame in the Bassin Arachidier of Senegal and in the Central River Division (CRD) of The Gambia where 20 herds were chosen in a similar way. On each farm up to 45 animals were chosen randomly and stratified by age assuming a within-herd prevalence of at least 5% and a 95 % confidence interval (THRUSFIELD, 1995). The Bassin Arachidier of Senegal is characterised by its present transition from the low input to the market orientated farming system. The common cattle breed is the Zebu. The CRD of The Gambia is known for its high cattle density in a traditional farming system. The predominant breed is the N'Dama.

Table 1: Population and approaches for testing

	The Gambia	Guinea	Guinea Bissau	Senegal
Single meat inspection (abattoir)	1595 slaughter cattle at Banjul abattoirs (Oct 00 - Mar 01)	-	-	-
CIDT*/meat inspection** (abattoir)	-	933 cattle at Kankan abattoir (Mar–Nov 02)	450 cattle at Bissau abattoir (Mar 02-Mar 03)	-
CIDT on-farm screening*	465 N'Dama cattle from 20 farms in the CRD (Jun 01 – Jan 02)	-	-	479 F1 and Zebu cattle from 30 farms in the Bassin Arachidier (Jun 01 – Jan 02)

\* Suspected positive reactors in CIDT were retested after 3 months

\*\* Meat inspection of suspected positive reactors for *M. bovis* in the CIDT

### 2.1.2 Diagnostic techniques and interpretation

Tuberculinisation was carried out in form of CIDT using PPD *M. avium* and *M. bovis* and applied in cattle older than six months. The tuberculin used for the test was purchased from WDT® Germany. All selected cattle were inoculated with 50,000 IU bovine PPD and 25,000 IU avian PPD. For inoculation two sites on the middle of the neck were shaved about 12 cm apart from each other, the skin thickness measured (HAUPTNER® cutimeter) and injected intradermally with 0.1 ml tuberculin. After a period of 72 hours the results were noted. Test results were interpreted according to the German Tuberculosis Prescription (Version from 13.3.1997). When differences in skin thickness were greater at the site for avian PPD than for bovine PPD, then the animal was considered as positive for *M. avium* or other atypical mycobacteria. Reactions for *M. bovis* were interpreted as shown below.

Interpretation for *M. bovis* (German Tuberculosis Prescription from 13.3.1997):

$$av2 - av1 = avd \quad \text{and} \quad bv2 - bv1 = bvd$$

Then,  $bvd - avd < 2 \text{ mm}^*$  = negative reaction

$bvd - avd$  between  $2 \text{ mm}^*$  and  $4 \text{ mm}$  = doubtful\*

$bvd - avd > 4 \text{ mm}$  = positive reaction

Key:  $av1$  = injection site for avian PPD (skin thickness before injection)

$av2$  = injection site for avian PPD (skin thickness 72 h after injection)

$bv1$  = injection site for bovine PPD (skin thickness before injection)

$bv2$  = injection site for bovine PPD (skin thickness 72 h after injection)

$avd$  = skin thickness difference of avian PPD

$bvd$  = skin thickness difference of bovine PPD

\*To further increase the sensitivity for *M. bovis* and in modification of the German standard, a reaction was interpreted as doubtful between 1.5 mm and 4 mm. Following this German standard, all cattle with doubtful reaction detected on-farm (The Gambia and Senegal) were retested after a period of 3 months for confirmation.

For meat inspection all carcasses of selected cattle (The Gambia) or only carcasses of cattle with doubtful or positive response to PPD *M. bovis* in the skin test (Guinea and Guinea Bissau) were examined macroscopically. Bronchial, mediastinal, submandibular, retro pharyngeal, mesenteric lymph nodes and the lungs were examined for tuberculosis-like lesions (i.e. nodular and/or caseating lesions). Such suspected lesions were trimmed from the carcass, packed in plastic bags, stored on ice packs, transported to the laboratory and screened using acid-fast Ziehl Neelsen stain for the presence of tubercle like bacilli. In case of positive results in Ziehl Neelsen, samples would have been sent to the National Reference Laboratory for TB in Guinea or Medical Research Council (The Gambia) for specific cultivation for *M. tuberculosis* and *M. bovis*.

## **2.2 Investigations in man**

To assess the importance of positive cases in cattle in relation to their potential risk for humans, investigation in people at risk, living in close contact to their cattle (herders and butchers), were also envisaged. A questionnaire was therefore applied at abattoirs to identify the places of origin of individual slaughter animals. In addition, the National Reference Laboratories for TB were visited in each country to gather information on clinical cases of BTB in man as well as to assess the diagnostic capacity for *M. bovis* infections.

## **2.3 Data analysis**

For data analysis, graphical presentations and statistical calculations Microsoft EXCEL 7.0, STATGRAPHICS PLUS (Version 2.1 for Windows) and EPIINFO (Version 6.01b) was used.

# **3 RESULTS**

## **3.1 Investigations in cattle**

### **3.1.1 Results for skin tests and meat inspection**

Findings in CIDT and/or meat inspection carried out in Senegal, The Gambia, Guinea and Guinea Bissau are shown in table 2. Skin reaction due to *M. avium* infections are presented in table 3.

Table 2: *M. bovis* infections in cattle for The Gambia, Guinea, Guinea Bissau and Senegal

<b>Results for <i>M. bovis</i></b>	<b>The Gambia</b>	<b>Guinea</b>	<b>Guinea Bissau</b>	<b>Senegal</b>
<b>Single meat inspection</b>	1595 cattle	Not applied	Not applied	Not applied
Suspected positive cases in meat inspection	1	Not applied	Not applied	Not applied
Confirmed cases of infection*	0	0	0	Not applied
<b>CIDT/meat inspection*</b>	Not applied	933 cattle	450 cattle	Not applied
Total suspected positive cattle in CIDT**	Not applied	0	2	Not applied
Confirmed in meat inspection	Not applied	0	0	Not applied
<b>CIDT on-farm screening</b>	465 cattle from 20 farms	Not applied	Not applied	479 F1 and local cattle from 30 farms
Herds with suspected positive cattle in CIDT***	3	Not applied	Not applied	3
Total suspected positive cattle in CIDT***	5	Not applied	Not applied	5
Confirmed positive reactor in CIDT (after retest)	0	Not applied	Not applied	0

\* Positive in Ziehl Neelsen and specific culturing for *M. bovis*

\*\* Meat inspection of suspected positive reactors to PPD-*M. bovis* in the CIDT

\*\*\* Doubtful reaction in skin test

No confirmed case of *M. bovis* infection was found. The few cases that showed a doubtful reaction in the skin test (n= 12) could not be confirmed; neither during meat inspection (Guinea and Guinea Bissau) nor in repeated skin test after a period of 3 months (Senegal and The Gambia) and were therefore considered negative.

Table 3: *M. avium* infections in cattle for The Gambia, Guinea, Guinea Bissau and Senegal

Results for <i>M. avium</i>	The Gambia	Guinea	Guinea Bissau	Senegal
<b>CIDT (abattoir)</b>	Not applied	933 cattle	450 cattle	Not applied
Total suspected positive cattle in CIDT	Not applied	127 (14%)	238 (53%)	Not applied
<b>CIDT on-farm screening</b>	465 cattle form 20 farms	Not applied	Not applied	479 F1 and local cattle form 30 farms
Total suspected positive cattle in CIDT	234 (50 %)	Not applied	Not applied	298 (62%)

Very high responses due to *M. avium* infection in the skin test (> 50%) were found in The Gambia, Guinea Bissau and Senegal and a moderate response in Guinea.

Individual skin test readings to PPD-*M. bovis* and PPD-*M. avium* are presented in form of scatter plots for each country in the ANNEX (Graph 1 - 4). The cut-off points for suspected and confirmed reactions to PPD *M. bovis* infections and for reactions to PPD *M. avium* infections are included.

Results for The Gambia, Senegal and Guinea Bissau show a similar pattern with few suspected positive reactions to PPD *M. bovis* (n= 12) and generally high responses to PPD *M. avium*. For Guinea the individual readings for PPD *M. bovis* and PPD *M. avium* are less scattered with significantly less immunological reaction to PPD *M. avium* (p< 0.01).

### 3.1.2 Selected parameters for the study population (age and origin of slaughter cattle)

The age of slaughter cattle was recorded at Kankan and Bissau abattoir. With an average age of 7.7 years slaughter cattle at Kankan abattoir were significantly older than cattle at Bissau abattoir where animals were on average 5.5 years old. At Banjul abattoir the age of slaughter animals was not recorded.

A questionnaire was applied to get information on the origin of slaughter cattle. Results are presented in Table 4.

Table 4: Origin of cattle at selected abattoirs in The Gambia, Guinea and Guinea Bissau

	The Gambia		Guinea		Guinea Bissau	
No,	1595 cattle		933 cattle		450 cattle	
<b>Origin of slaughter cattle</b>	CRD	1180 (74%)	Kankan	746 (80%)	Gabu	225 (50%)
			Mandiana	110 (12%)	Bafata	126 (28%)
	Senegal	399 (25%)	Kouroussa	64 (7%)	Oio	54 (12%)
			Sigiuri	2	Ouinara	5 (1%)
	Unknown	16 (1%)	Kerouaré	10 (1%)	Guinea	13 (3%)
			unknown	1	unknown	27 (6%)

Nearly one quarter of the slaughter cattle in The Gambia originate from Senegal, mainly Casamance. At Kankan abattoir the majority of cattle slaughtered was reared in the same district (80%), no animals of the study population were imported from a neighbouring country. At the city abattoir of Bissau most of the cattle slaughtered are from Gabu and Bafata, the districts with the highest cattle density in Guinea Bissau. Thirteen percent of cattle are from Guinea.

### 3.2 Investigations in man

Information on the diagnostic capacity to detect BTB and on cases reported in man was collected at the National Reference Laboratories or other institutions in each country. The results are presented in table 5.

Table 5: Cases of BTB in man and diagnostic capacity of National Reference Laboratories

	The Gambia	Guinea	Guinea Bissau	Senegal
Capacity to detect <i>M. bovis</i> in human sputum samples*	Yes	Yes	No	Yes
Confirmed cases of <i>M. bovis</i> in man for the year 2002	No**	Yes	No	No
Cases mentioned in literature (last 10 years)	No	Yes	Yes	?

\* Not applied routinely!

\*\* Few cases of infection of the cervical lymph nodes were reported.

The diagnostic capacity to isolate *M. bovis* in human sputum samples is presently available in The Gambia, Senegal and Guinea, where it is restricted to the National Reference Laboratories (Senegal and Guinea) or the Medical Research Council (The Gambia). The application of the diagnostic tests, however, is limited, due to the high cost of 15-20 US \$ per sample. Thus the standard procedure in cases of suspected tuberculosis stops usually after the Ziehl Neelsen stain without further culturing.

Confirmed cases of BTB are only reported for Guinea (GOUMOU, 2001). In The Gambia, few cases of infection of the cervical lymph nodes, which are stated to be pathognomic for *M. bovis* infections were reported. However, none of these cases were confirmed by culturing.

#### 4 DISCUSSION

This survey was an attempt to provide base line information on the occurrence of BTB and the associated public health risk for selected districts of Senegal, Guinea, Guinea Bissau and The Gambia. Information on BTB for the 4 countries is scarce, not up to date and usually not representative for the respective countries.

Our major finding is the clinical and immunological absence of *M. bovis* infections in the cattle population under investigation. This result was not expected since *M. bovis* infections are known to be endemic in cattle in other West African countries, like Ghana (BONSU et al., 2000) and Burkina Faso (DELAFOSSÉ et al., 1995). Moreover, in none of the four countries any regular control measures are applied. However, the number of cattle tested in this study is considerably high and representative for wide regions in the four countries (see table 1).

When comparing the present findings with the few historical data available for the region it appears that *M. bovis* infection in cattle seems to be rare in the 4 countries. Among 100 cattle tested in The Gambia in 1974 no positive reactor was found (CLIFFERT, 1999). Also less than one percent of positive reactors for *M. bovis* in the Simultan Intradermal Tuberculin Test (SIDT) were found in cattle selected for export in the late seventies (DLS, 2000). The reported prevalence might even be overestimated considering the lower specificity of the SIDT compared to the CIDT used in the present survey.

Results from Kankan abattoir (Guinea) are in agreement with a previous survey carried out in a different region of this country. Out of 900 slaughtered cattle at Conakry abattoir no positive case for *M. bovis* was found in meat inspection. However, a direct comparison of the previous and present study is not possible as the study population (origin of cattle) differed clearly.

The absence of *M. bovis* infection in the tested cattle population in Guinea and Guinea Bissau did not shed any light on the present (Guinea) and previous (Guinea Bissau) reported cases of BTB in man in these countries (GOUMOU, 2001, HOFFNER et al., 1993). Possible explanations are: Firstly, there is no direct link between the observed cases in man and the cattle population investigated. Secondly, there is a link, but the chosen study population in cattle was still not sufficient to detect any relation to the observed cases in man because of the very low number of infections (i.e. in Kankan only 2 cases of *M. bovis* were confirmed during 2001 to 2002, countrywide only 33).

When discussing the absence of the infection in cattle in the present survey the phenomenon of anergy and the high number of positive reaction to tuberculinisation using PPD *M. avium* (Senegal, Guinea Bissau and The Gambia) have to be considered and may have epidemiological and diagnostic implications. The significantly lower number of positive reactions to PPD *M. avium* as well as the generally lower individual immunological responses reported for Guinea, however, might also indicate a reduced activity of the used tuberculin as regular, long lasting power failures are common at Kankan.

Anergy is a long recognized phenomenon in the tuberculin test, which results in false negative reactions. The reasons for this are poorly understood but recently infected cattle (< 6 weeks), cattle under stress (malnutrition, gastrointestinal parasitosis and concurrent infections) and cattle with generalised severe tuberculosis are anergic (AMENI and MEDHIN, 2000; LEPPER et al., 1977; MONOGHAN et al., 1994) and fail to react. Stress due to starvation (end of dry season) and concurrent infections like trypanosomosis, tick-borne diseases and high burden of gastro-intestinal parasites are common in the study herds. In addition, about 30% of cattle in the periparturient period can give false negative results returning later to a positive state (BLOOD and RADOSTIS, 1989). The test is more efficient in medium or high-infected herds. In low infected populations it is therefore recommended to repeat the skin test regularly or to use more sensitive test (e.g. BOVIGAM® ELISA) to increase sensitivity.

More than 50 % of tuberculinised cattle in Guinea, Guinea Bissau and Senegal reacted positive for PPD *M. avium*. Recent findings have shown that environmental infections with other mycobacteria can influence the immune response to *M. bovis* to an unknown extent (BUDDLE et al., 2002; VORDERMEIER et al., 2001). It is therefore assumed, that the unexpected absence of *M. bovis* in the study population may not reflect the true epidemiological picture. The phenomenon of interference of environmental infections with the classical infection pathway, explained by cross-immunity mechanism, has been well reported in humans (BLACK et al., 2002). The observed variation in immuno-responses to *M. tuberculosis* in man may reflect geographic variations in the exposure to environmental mycobacteria, as it was

reported recently by BLACK et al. (2002) comparing vaccinated populations in Malawi and UK. When tested with the specific antigen for *M. tuberculosis* (ESAT-6) in comparison with the PDD, a larger proportion of patients reacted positively to the PPD, a clear indication for exposure to environmental mycobacteria. The role of the unspecific, non-pathogenic mycobacterial infections in the epidemiology of *M. bovis* is not known and may be very specific for West Africa. As described for *M. tuberculosis* (BLACK et al., 2002) and in consideration of the very close genetic similarity between *M. bovis* and *M. tuberculosis*, potential candidates for protective cross-immunity for *M. bovis* could be *M. avium*, *M. intracellulare* and *M. marinum*.

## **5 CONCLUSIONS AND RECOMMENDATIONS**

Based on the large number of cattle sampled in the different regions in this study it can be concluded that clinical and immunological infection due to *M. bovis* are very rare or even likely to be absent in the four countries investigated. To confirm these observations further investigations using more sensitive test methods are highly recommended. Future studies should also be directed to the research on the influence of exposure to environmental mycobacteria and their influence on manifestation of *M. bovis* infections. Results might provide more differentiated information on the role of Mycobacteria sp. prevalence in cattle and the role of environmental versus pathogen mycobacteria.

The most effective method of controlling BTB in a cattle population with less than 5 % prevalence is the test and slaughter method based on a nationwide disease surveillance system. However, mainly due to financial constraints such a disease surveillance has not yet been established in any of the study countries. Thoroughly applied meat inspection at all abattoirs, followed by tuberculinisation of identified herds of origin of positive cattle in meat inspection is the most appropriate method. Positive cattle in the skin test should be retested after a 3-months period and, if remaining positive, be slaughtered. Such infected herds should be always tested twice until only negative test results occur. In addition, investigation in people at risk (e.g. herdsman, owner etc.) are recommended.

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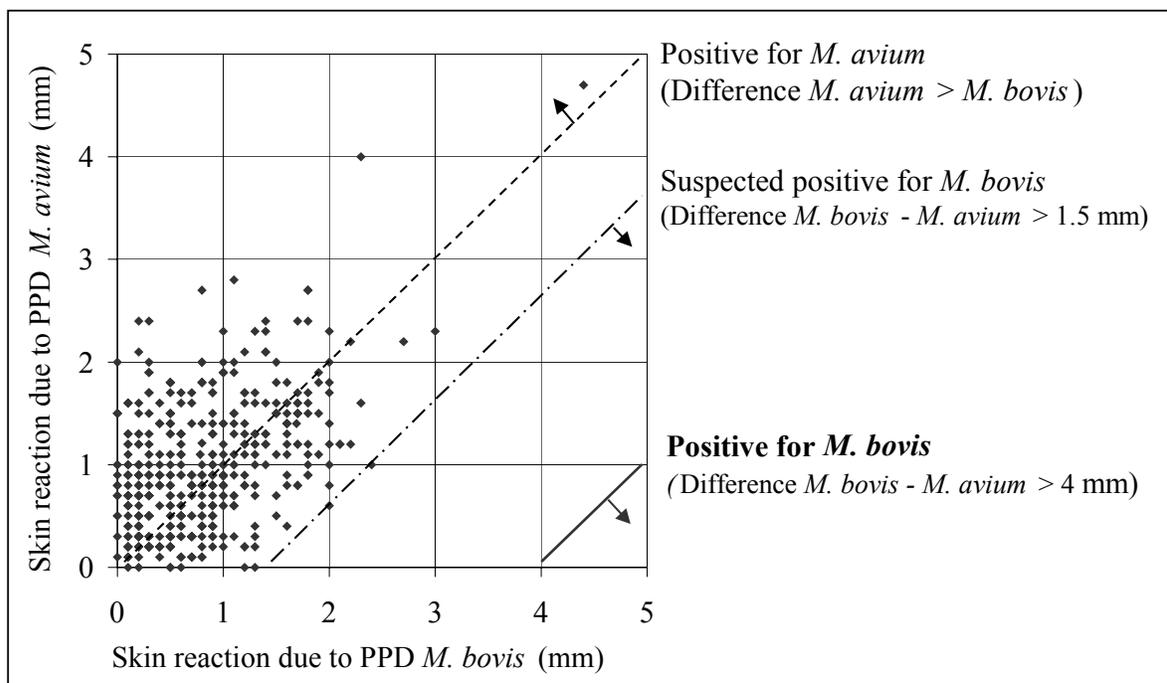
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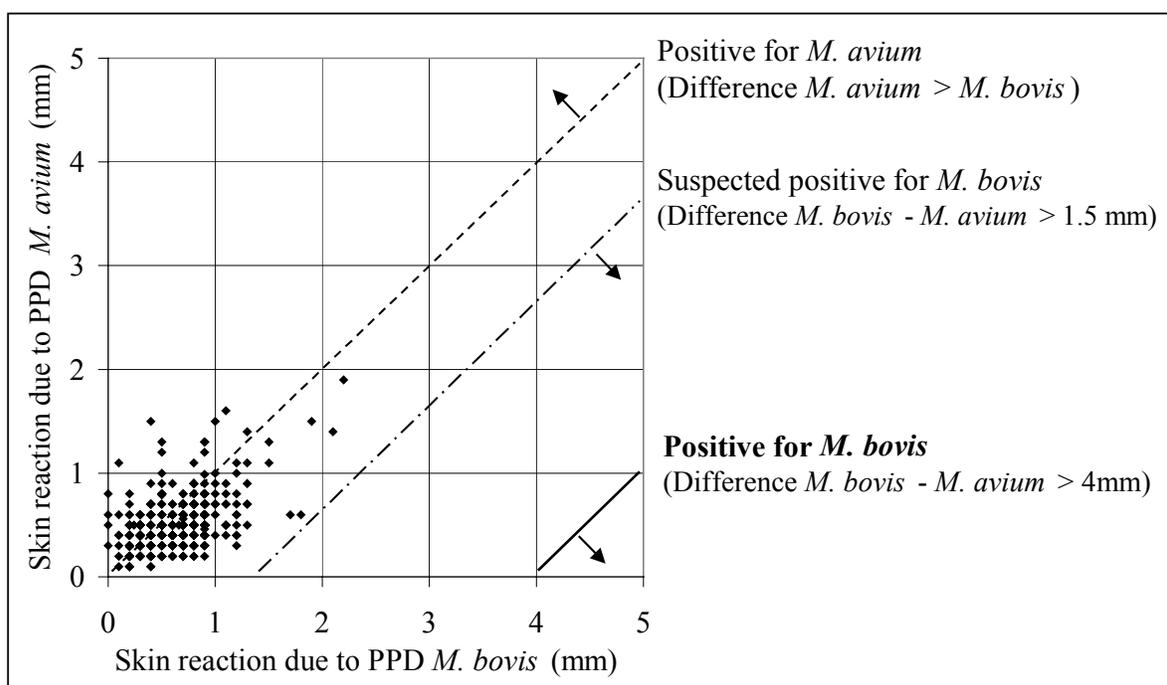
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## 7 ANNEX

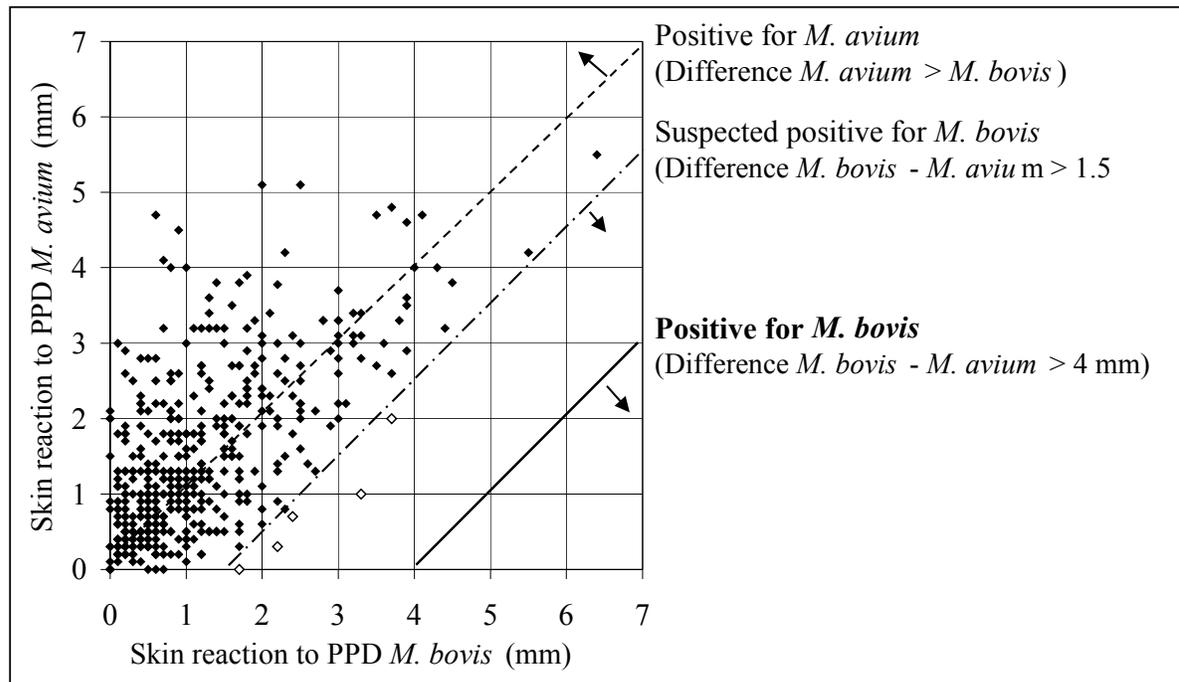
Graph 1: Skin reaction to PPD *M. bovis* and PPD *M. avium* in the skin test in cattle at Bissau abattoir (Guinea Bissau), March 2002 – March 2003



Graph 2: Skin reaction to PPD *M. bovis* and PPD *M. avium* in the skin test in cattle at Kankan abattoir (Guinea), March – November 2002



Graph 3: Skin reaction to PPD *M. bovis* and PPD *M. avium* in the skin test in cattle herds at Bassin Arachidier (Senegal), June 2001 – January 2002



Graph 4: Skin reaction to PPD *M. bovis* and PPD *M. avium* in the skin test in cattle at CRD herds (The Gambia), June 2001 – January 2002

