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Comparative assessment of fluorescence polarization and tuberculin skin testing for the diagnosis of bovine tuberculosis in Chadian cattle

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ABSTRACT

Effective surveillance of bovine tuberculosis (BTB) in developing countries where reliable data on disease prevalence is scarce or absent is a precondition for considering potential control options. We conducted a slaughterhouse survey to assess for the first time the burden of BTB in Southern Chad. Altogether, 954 slaughter animals were consecutively sampled and tested using the single intra-dermal comparative cervical tuberculin (SICCT) test, a recently developed fluorescence polarization assay (FPA) and routine abattoir meat inspection after slaughter. Gross visible lesions were detected in 11.3% (CI: 9.4–13.5%) of the animals examined and they were mostly located in the lymph nodes and the lung. Significantly more Mbororo zebus (15.0%) were affected by lesions than Arab zebus (9.9%; OR = 2.20, CI: 1.41–3.41%; $p < 0.001$). Of all animals tested, 7.7% (CI: 6.2–9.6%) reacted positively to SICCT if OIE guidelines were applied. However, receiver operating characteristic (ROC) analysis using *Mycobacterium tuberculosis* complex (MTBC) infected animals as the positive population and lesion negative animals as the negative population, revealed a better SICCT performance if the cut-off value was decreased to >2 mm. SICCT reactor prevalence rose to 15.5% (CI: 13.3–18.0%) and FPA did not perform better than SICCT, when this setting adapted cut-off was applied.

1. Introduction

Bovine tuberculosis (BTB) is a considerable threat in many respects. It causes economic loss by its effects on animal health and productivity and by international trade restrictions (Zinsstag et al., 2006b). BTB has also a large impact on animal wellbeing in wildlife populations and hence entire ecosystems (Renwick et al., 2006). Moreover, infected wildlife serves as an animal reservoir and hampers BTB eradication programs in several countries (Corner, 2006). BTB is also of concern for public health as it can cause zoonotic disease in humans, e.g. through close contact to infected animals or consumption of contaminated raw milk (Cosivi et al., 1998; Ayele et al., 2004). In Africa, the disease is present virtually on the whole continent with only very few countries being able to apply control measures due to the lack of financial resources.

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Moreover, laboratory and technical capacity is very limited in most countries with diagnosis of tuberculosis relying exclusively on microscopy (Cosivi et al., 1998; Ayele et al., 2004; Zinsstag et al., 2006a).

In a representative survey in the Chari-Baguirmi and Kanem region in Western Chad, Schelling et al. found 17% of transhumant nomadic cattle to be positive by single intra-dermal comparative cervical tuberculin (SICCT) testing (Schelling et al., 2000). A subsequent study at the abattoir of N'Djaména in Chad revealed that 7.3% of the animals had gross visible BTB suspect lesions with Mbororo zebu breeds being more affected than Arab zebus. The differential susceptibility was even more significant, when only confirmed *Mycobacterium bovis* infected animals were considered (Diguimbaye-Djaïbe et al., 2006a). Spoligotyping (Kamerbeek et al., 1997) demonstrated a homogeneous population structure of the isolated bacteria with the most predominant strains showing the same patterns as previously identified in studies from Northern Cameroon and Nigeria (Njanpop-Lafourcade et al., 2001; Cadmus et al., 2006; Diguimbaye-Djaïbe et al., 2006a).

Current ante mortem diagnosis of BTB mainly relies on SICCT testing, which although imperfect could not yet be replaced by any other more accurate or satisfactory diagnostic method (de la Rua-Domenech et al., 2006). Also the γ-Interferon (γ-IFN) test (Bovigam®, Prionics) has gained increasing importance for BTB diagnosis in cattle (Gormley et al., 2006).

SICCT and the γ-IFN test are both based on cell mediated immune (CMI) responses against tuberculosis infection. Tuberculosis in cattle is characterized by an early Th1 type CMI response, whilst humoral immune responses develop as disease progresses. CMI responses can wane and animals become anergic, and SICCT as well as γ-IFN tests have been shown to give false negative results in such disease stages (Welsh et al., 2005; Pollock et al., 2005; de la Rua-Domenech et al., 2006). Importantly, anergic animals are thought to be heavily diseased and highly infective (Pollock et al., 2005). In low income countries, where control measures are absent, the predicted higher prevalence of such animals might considerably affect disease spread and persistence (Pollock et al., 2005; de la Rua-Domenech et al., 2006; Palmer and Waters, 2006). Thus, development of a diagnostic test targeting late stage diseased animals is of specific importance for high-incidence countries with restricted resources for BTB surveillance and control.

Anergic animals may be detected by serological tests if the host's immune response shifts from a predominant CMI response to an antibody-based response. In this context, a number of diagnostic tests have been developed, however sensitivity and/or specificity was low compared to SICCT (de la Rua-Domenech et al., 2006). Fluorescence polarization (FP) constitutes an alternative technique for antibody detection with a shown potential for diagnostic purposes (Jolley and Nasir, 2003). An assay for the detection of *M. bovis* antibodies has been described some years ago, utilizing fluorescein-labelled MPB70 protein as antigen (Lin et al., 1996; Surujballi et al., 2002; Waters et al., 2006; Jolley et al., 2007). The assay has recently been modified by employing a polypeptide-based tracer derived from MPB70 protein, named F-733. Jolley et al. previously provided a more detailed description of the test and have evaluated its performance for the assessment of the BTB herd status in various settings (Jolley et al., 2007). However, this study provided no ROC analysis for cut-off selection and only poorly assessed FPA test sensitivity and specificity for the identification of infected animals.

The present study aimed at the comparison of SICCT testing and FPA for the diagnosis of BTB in naturally infected cattle, in an African setting. Moreover, our results revealed first insights into the prevalence of BTB in Southern Chad.

2. Materials and methods

2.1. Animals

In the absence of a sampling frame, a total of 954 slaughter animals were sampled during three intervals of approximately one month between July and November 2005 at abattoirs in Southern Chad. Sample size calculation for diagnostic test comparison was based on a prevalence of 17%, a standard error of the estimate <10% and a difference in sensitivity to be detected of 10% (sensitivity of the SICCT test was assumed to be 82%, level of confidence of 95%, power of 80%; www.openepi.com, 2004). The far majority of animals (n = 944) was sampled at the abattoir of Sarh and a few (n = 10) at the abattoir of Moundou. The study area was located approximately 500 km from N'Djaména, where a previous slaughterhouse survey was conducted (Diguimbaye-Djaïbe et al., 2006a). The animals were raised in a long distance transhumant livestock production system with frequent trans-border movements of herds between the Central African Republic and Chad (Ben Yahmed, 2006). Because of the regional farming system, focusing primarily on milk production (Dicko et al., 2006), relatively small amounts of animals (surplus males and old cows) are usually sold from the same herd to different traders, which in turn, sell on their animals to different butchers. Therefore, we assume that the tested animals can be considered a representative sample from a large number of different herds and an extensive area of Southern Chad. However, because of poor documentation and the multiple selling-on the origin of the animals could not be traced. All available animals were subjected to this study. The reticence of some butchers limited the number of animals that could be sampled to approximately one third of the animals slaughtered during our presence at the abattoir. Different zebu breeds were frequently intermixed in the same cattle herds. Most likely, none of the animals has ever undergone tuberculin skin testing. Four types of phenotypic breeds were encountered: Arab (n = 658), Mbororo (n = 286), Bogolodjé (n = 7) and cross-breeds (n = 3) of which only the former two were used for statistical analysis.

2.2. Physical examination of animals

All 954 animals were physically examined before slaughter. Body condition was categorized by assigning