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Evaluation of different enzyme-linked immunosorbent assays for the diagnosis of brucellosis due to *Brucella melitensis* in sheep

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ABSTRACT

Six serological assays for the diagnosis of ovine brucellosis, due to *Brucella melitensis* were evaluated. Reference serum samples from sheep of known *B. melitensis* infection status ($n = 118$) were assessed using the Rose Bengal test (RBT), complement fixation test (CFT) and four commercial enzyme-linked immunosorbent assays (ELISAs), including two indirect ELISAs (iELISAs), one competitive ELISA (cELISA) and one blocking ELISA (bELISA).

The highest differential positive rates (DPR) were obtained with the cELISA and bELISA, while the lowest DPR was estimated using iELISAs. A latent class analysis was performed to estimate the accuracy of the CFT, RBT and bELISA using 1827 sera from sheep undergoing testing as part of a surveillance and control programme. Lower sensitivity and specificity were obtained for the three serological tests when the field samples were used. A higher DPR was achieved by the CFT, compared to bELISA and RBT. The results suggest that ELISAs, and particularly the bELISA, might be suitable for inclusion in the European Union legislation on intra-community trade for diagnosing *B. melitensis* infection in sheep, as it has a similar test performance compared to the RBT.

Introduction

Brucellosis is a zoonotic infection, caused by bacteria of the genus *Brucella*, which infect a wide range of domestic and wildlife species (Ko and Splitter, 2003). The disease has important health and socioeconomic impact in both humans and animals (Corbell, 1997). Despite control and eradication campaigns, conducted over recent decades in Europe, the incidence of the disease is still increasing in some regions (Seleem et al., 2010).

Sheep and goats are primarily affected with brucellosis caused by *Brucella melitensis* (Corbell, 1997). Brucellosis in small ruminants is an acute disease of pregnant ewes, causing late gestation abortion, reproductive problems and loss of milk and meat production (Alton and Forsyth, 1996). Although bacterial isolation and identification of *Brucella* spp. is defined as the 'gold standard' for diagnosis of brucellosis, serological tests are routinely used in brucellosis control and eradication programmes (EFSA, 2006). Different diagnostic tests have been validated for diagnosing brucellosis in small ruminants, but only the Rose Bengal test (RBT) and the complement fixation test (CFT) are approved for diagnosis of small ruminant brucellosis in the European Union (EU) legislation on intra-Community trade (Council Directive 91/68/EEC).[1] However, there is evidence that both tests are less sensitive and specific for the diagnosis of brucellosis in sheep and goats than in cattle (Blasco et al., 1994; Garin-Bastuji et al., 1998).

Efforts to improve the serological detection of brucellosis in small ruminants have led to development of new assays, including indirect ELISAs (iELISAs), competitive ELISAs (cELISAs), the modified Rose Bengal test (MRBT) and the fluorescence polarisation assay (FPA) (Blasco et al., 1994; Díaz-Aparicio et al., 1994; Marín

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[1] See: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:1991:046:0019:0036:ES:PDF.

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