Serologic detection of antibodies to Brucella spp. using a commercial ELISA in cattle in Grenada, West Indies

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Abstract. Bovine brucellosis, caused mainly by Brucella abortus, a zoonotic bacterium, has been reported from many areas of the world, including Central and South America, and the Caribbean island state of Trinidad and Tobago. Although brucellosis has been eradicated from domestic cattle in Canada it still exists in one or two herds in the United States. Serological tests are important in estimating prevalence of Brucella exposure in order to target eradication programmes. In this study, serum samples from 150 cattle were tested using a commercial competitive enzyme linked immunosorbent assay (SVANOVIR®Brucella-Ab C-ELISA) which detects antibodies to both B. abortus and Brucella melitensis. All cattle tested were greater than 6 months old and were unvaccinated. Sampled cattle were from 35 herds representing animals from all 6 parishes of Grenada. Nine of the 150 animals (6%) were positive for antibodies to B. abortus and/or melitensis by the C-ELISA. Of the 35 herds, 7 (20%) had C-ELISA-positive animals. Three of the 6 parishes contained positive herds. Based on the high sensitivity (98%) and specificity (99.7%) of the C-ELISA, these results strongly indicate the presence of cattle exposed to B. abortus and/or melitensis in Grenada.

INTRODUCTION

Brucellosis in cattle is mainly caused by Brucella abortus, and less commonly by Brucella melitensis (Verger, 1985; Jimenez de Bagues et al., 1991). Occasionally, Brucella suis may cause infection in cattle, but it has not been known to cause abortion or to spread among cattle (Ewalt et al., 1997). In the last decade bovine brucellosis was reported to be endemic in several countries in Central and South America with individual cow prevalence ranging from 3% to 11% and herd prevalence as high as 15% (Radostitis et al., 2007). Information on brucellosis from Caribbean islands is limited. Serological evidence for Brucella exposure in goats and sheep was reported from Saint Croix (Ahl et al., 1993). A 1997 review of the literature indicated that brucellosis was not detected in Barbados, Surinam, or St. Kitts/Nevis (Corbel, 1997). However, more recently serologic detection of Brucella exposure and culture-confirmed infection in cattle has been reported in Grenada’s neighboring island of Trinidad (Fosgate et al., 2002), and seropositive sheep have been detected in Grenada itself (Stone et al., 2012). In Grenada the estimated cattle population is around 4000 and typical herd size is from 2 to 10 animals. Cattle are frequently pastured along with sheep and goats. Thus, the recent detection of sheep positive for antibodies against Brucella spp. is an important rationale for testing cattle on this island. The objective of this study was to serologically test cattle from all 6 parishes in Grenada using a competitive ELISA (C-ELISA), a test with a high performance index compared to other serologic tests (Gall & Nielsen, 2004).
C-ELISA used in this study detects serum antibodies to both *B. abortus* and *B. melitensis*. Our results will facilitate implementation of control programmes for this important zoonotic disease.

**MATERIALS AND METHODS**

A total of 150 cattle were randomly selected from 35 herds representing all 6 parishes of the island of Grenada. Herd size ranged from 2 to 10 animals and only animals greater than 6 months of age were selected. Both males and females were tested. The cattle were of Holstein/Friesian-local mixed breed and clinically normal. None of the animals tested had received any vaccinations, nor was there any herd history of abortion. Cattle in Grenada are reared in a free-range pasture environment often together with sheep and goats. Ten mls of venous blood was collected from each animal in a sterile, red-top tube and allowed to clot. The samples were transported on ice to the diagnostic laboratory of the St. George's University School of Veterinary Medicine. Serum samples were collected after centrifugation (at 3000 rpm for 10 minutes) and stored at -80°C until tested for antibodies to *Brucella* spp. The SVANOVI®Brucella-Ab C-ELISA antibody test kit (SVANOVA Biotech AB, Uppsala, Sweden) was used to test the serum samples for antibodies to *B. abortus* and *B. melitensis* according to the manufacturer’s instructions. The optical density (OD) values for each of the controls provided in the kit and serum samples in the wells were read at 450 nm using a microplate photometer (Universal Microplate Reader, Bio-Tek Instruments, Inc.). The percent inhibition (PI) values were calculated according to the manufacturer’s instructions. The results were expressed as negative for *Brucella* antibodies (PI <30%) or positive for *Brucella* antibodies (PI ≥30%).

**RESULTS**

Nine of the 150 cattle sampled were positive by the C-ELISA test, thus giving a seroprevalence of 6% for *Brucella* exposure in cattle in this study. The seroprevalence was highest in St. David parish at 16.3% (5 of 31 animals positive) followed by a 5.26% seroprevalence for cattle from St. Andrew parish (3 of 57 animals positive). Of 33 cattle sampled from St. John parish, only one was positive (3.03% seropositive). None of the cattle sampled from the remaining 3 parishes tested positive (Table 1).

**DISCUSSION**

The SVANOVI®Brucella-Ab C-ELISA detects serum antibodies to both *B. abortus* and *B. melitensis*. In this assay, serum samples are exposed to *B. abortus* smooth lipopolysaccharide (S-LPS) coated wells on microtiter plates together with a mouse monoclonal antibody (mAb) specific for an epitope on the o-polysaccharide side chain of the S-LPS antigen and labeled with horseradish peroxidase. If the test serum contains *B. abortus-* and/or *B. melitensis-* specific antibodies (positive) they compete

<table>
<thead>
<tr>
<th>Parish</th>
<th>No. of herds</th>
<th>No. of samples</th>
<th>No. positive</th>
<th>% positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>St. Andrew</td>
<td>15</td>
<td>57</td>
<td>3</td>
<td>5.26</td>
</tr>
<tr>
<td>St. John</td>
<td>8</td>
<td>33</td>
<td>1</td>
<td>3.03</td>
</tr>
<tr>
<td>St. David</td>
<td>7</td>
<td>31</td>
<td>5</td>
<td>16.13</td>
</tr>
<tr>
<td>St. Patrick</td>
<td>3</td>
<td>11</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>St. George’s</td>
<td>1</td>
<td>10</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>St. Mark</td>
<td>1</td>
<td>8</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>35</td>
<td>150</td>
<td>9</td>
<td>6</td>
</tr>
</tbody>
</table>

Table 1. Seroprevalence of brucellosis in cattle in Grenada, based on C-ELISA
with the mouse mAb binding to the o-polysaccharide portion of S-LPS antigen resulting in a decrease or lack of colour change after addition of substrate. C-ELISAs using the Brucella LPS antigen significantly increased the specificity of antibody tests and allowed for the differentiation between Strain-19-vaccinated animals (low affinity antibody) and naturally exposed animals. (Nielsen et al., 1996). Prior vaccination was not an issue in the present study as cattle in Grenada are not vaccinated against Brucella. The sensitivity and specificity of the C-ELISA as reported by the manufacture are 98% and 99.7% respectively (SVNOVIR®Brucella C-ELISA (Svanova, 2012), which is higher than most other serological tests used in screening cattle for exposure to Brucella spp. The sensitivity was based on 331 samples confirmed positive by culture. The specificity was based on 1144 samples from negative herds in Canada. The competitive Brucella ELISA is a marked improvement over conventional ELISAs where false-positive reactions were common (Hariharan et al., 1986). Importantly, the C-ELISA has been demonstrated to be an effective tool for detecting exposure to Brucella spp. in both cattle and buffaloes (Fosgate et al., 2011). In this study, 6% of cattle tested were serologically positive for Brucella antibodies. This reflects natural exposure and not current infection or disease. The actual prevalence of Brucella exposure, however, may be higher because animals can lose their antibody titers over a period of time and there may be a few false negative due to the C-ELISA having a sensitivity of less than 100% (Godfroid et al., 2010). However, C-ELISA is an excellent confirmatory assay for detecting exposure to Brucella in most mammalian species (Nielsen & Yu, 2010). Although the seroprevalence of bovine brucellosis in regions of the world varies considerably, our results are in agreement with other seroprevalence reports from the Americas (3-11%) reflecting data from Argentina, Brazil, Guatemala, Paraguay, and Venezuela (Radostits et al., 2007). Importantly, brucellosis has been confirmed in cattle and water buffaloes in Trinidad (Fosgate et al., 2002).

The C-ELISA kit used in this study is designed to detect serum antibodies to B. melitensis as well as B. abortus. In Latin America, of the 1377 strains isolated in 1968-1991, nearly 32% were from cattle, most of them being B. abortus, but a few B. melitensis as well (Lucero et al., 2008). A recent study conducted in Grenada showed a seroprevalence of 3.6% for brucellosis in sheep, which used an indirect ELISA with the smooth LPS antigens of B. abortus strain S-99 (Stone et al., 2012). In Trinidad, B. abortus has been cultured from the lymph nodes of both cattle and buffaloes (Fosgate et al., 2011). The ‘gold standard’ diagnostic test for brucellosis continues to be based on isolation of the organism from the organs and lymph nodes of the fetus, the placenta, milk, vaginal mucus or uterine exudate (Radostits et al., 2007). Further studies are required to confirm brucellosis in cattle in Grenada by culture or PCR, and to determine the species of Brucella associated with positive antibody titers.

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REFERENCES


