SEROLOGICAL DETECTION OF BORRELIA BURGDORFERI ANTIBODIES IN THE BOVINE AND OVINE SERUM

By

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Summmary

A total of 255 blood samples (114 from cattle and 141 from sheep) were collected from farms with history of Borrelia burgdorferi-digital dermatitis infection. Among these animals 24 (21.1%) cattle and 21 (14.9%) sheep had typical symptoms of the disease, while the rest of samples were collected from contact and non-contact animal groups. B. burgdorferi flagellar protein (41kDa) was used as a coating antigen in indirect ELISA, as well as in Western blot technique to detect its specific antibodies in the serum of clinical cases as well as in contact and non contact animals.

In cattle ELISA could detect 79.2% of the clinical cases as positive to the infection with B. burgdorferi, whereas Western blott could detect 75% of these cases. In sheep, ELISA as well as Western blott could equally detect 85.7% infection among the symptomatic animals. In contact and non contact animal groups, ELISA showed relative superiority over Western blott in detecting the B. burgdorferi infection. In cattle ELISA could detect 10.8% and 4% in contact and non contact animals respectively against 9.2% and 4% detected by Western blot technique. In sheep relatively similar results to those of cattle were obtained. ELISA could detect 6.2% and 4.3% in contact and non contact animals respectively, against 5.2% and 0% by Western blot.

INTRODUCTION

Lyme disease 'Borreliosis' caused by Borrelia burgdorferi is a tick borne disease of most domestic animals and man. The disease is of a world wide distribution, especially in temperate countries where ticks play a pivotal role in the transmission of infection. Rodents and pet animals (mainly dogs) has proved to be highly potential reservoir of infection, which recently has increased the awareness to this zoonotic infection.

In cows the infection was first described in Italy by Cheli and Mortellaro (1974) who described it as 'Mortellaro disease' or 'digital dermatitis'. Later other authors manifested and confirmed the infection in cattle, sheep, goat, horses and other animals (Hovmark et al. 1986; Burgess et al 1887; Burgess, 1988; Burgess et al. 1993; Wells et al 1993; Ji and Collins 1994; Mc Kenna et al. 1995 and Tuomi et al 1998). The infection was mainly manifested as epidemic lameness among the animals. In dairy cattle herds, the lesions were characterised by diffuse or circumscribed inflammation or erosions of the superficial layer of the epidermis at the coronary margin on the planter aspect of the inter digital space just above the coronet between the heel bulbs. Hyperplasia, hyperaemia, pain, foul odour and swelling of the affected site accompanied by severe pain which might force the cow to walk on its toes. Other lesions might be involved due to the infection with B. burgdorferi as, myocardial, renal, lymphadenopthy, and neurologic abnormalities (Blowey and Sharp 1988; Aiello and Mays 1998; Mumba et al. 1999).

Serological techniques as Western blot and Enzyme Linked Immunosorbant Assay (ELISA) has been performed succsessfully to determine the antibodies against B. burgdorferi in the serum of different animals and man. Different protein antigens were used as whole cell sonicate, outer membrane associated protein (OspA-31kDa or OspB-34kDa) and the flagellar protein 41kDa. Moreover immunological differences between these antigens as well as the cross reactivity has been also manifested (Wilske et al. 1989; Karlsson 1991; Assous et al. 1993; Lovrich et al. 1993; Wienecke et al. 1993; Dressler et al.1994; Magnarelli et al. 1994; Wilske et al. 1994; Bunikis et al. 1995; Lovrich et al. 1995; Norman et al. 1996).

Due to the lack of data on B. burgdorferi-digital dermatitis infection in Egypt, this study was considered the first aimed to use an enzyme linked immunosorbant assay (ELISA), as well as Western blot to detect the bovine and ovine B. burgdorferi antibodies in the sera of clinical cases as well as contact and non contact control cattle and sheep.

MATERIALS AND METHODS

Blood samples

A total of 255 blood samples were collected from 114 cattle (buffaloes were not included in this study, only local and mixed breeds cows) and 141 sheep from governmental and private farms in Giza and Kafr-EL-Sheikh governorates. From these animals 24 cattle and 21 sheep had symptoms of digital dermatitis at the time of sampling.The rest of samples were collected from contact and non-contact animal groups.Whole blood was collcted from vein puncture from the coccygeal vein of cows and from the left jagular vein of sheep. Blood was allowed to clott at room temperature and centrifuged at 700xg for 15min. sera were collected and stored in frozen aliquotes untill used.

B. burgdorferri Flagellar protein and control antisera

B. burgdorferi flagellar antigen protein 41kDa as well as positive and negative control antisera were kindly provided by Dr. Petra Winter at the large animal clinic, Faculty of veterinary medicine, Vienna Univerisity.

Sodium Dodecyl sulphate-Polyacryamide Gel Electrophoresis (SDS-PAGE)

The flagellar protein antigen 41kDa was electrophoesed by the SDS-PAGE to investigate its purity and to detect its recognition by the sera from clinical cases, contact as well as non-contact cattle and sheep. The SDS-PAGE was performed in a vertical gel slap by the Laemmli discontineous buffer system (Laemmli 1970). The gel slabs were 140 x 80 x 1.5mm in a vertical electrophoresis unit (Pharmacia). The stacking gel was 4% and the resolving gel was 12%. Equal volumes (20µl) of the flagellar protein (41kDa protein) and sampling buffer were vortexed for 15 seconds and heated in thermomixer for 5 min then loaded in the gel along with a molecular protein marker range from 31kDa to 200kDa. The electophoresis unit was adjusted at 60V for about 6h till the marker blue stain (bromothimol blue) in the sampling buffer reaches the end of the gel. The protein bands were fixed in 50% methanol, 7% acetic acid and 43% dist. Water, then visualised by Coommassie blue stain (Ceceroni et al. 1997 and Demirkan et al. 1999).

Western blot

The electrophoresed protein was blotted on a 0.2µm nitrocellulose membrane in a Bio-Rad transfer unit containing transfer buffer,25mM Tris; 192mM glycine and 20% methanol (pH8.3). The unit was run at 70 V for 5h at 10°C. Protein bands were checked on the nitrocellulose membranes by amido black stain. The unoccupied sites on the unstained nitrocellulose membrane were blocked by 5% gelatin in PBST or for 1 h at room temperature with agitation. The membranes were then washed three times for 10min in PBSTand finally cut into strips. These strips were incubated with 1:10 diluted sera from cows and sheep at room temperature for 1h. The strips were then washed three times with PBST, then incubated with rabbit antibovine IgG or rabbit antiovine IgG conjugated with the horse radish peroxidase for 1h at room temperature in dilution 1:4000 in PBS pH 7.4. The conjugate was then washed and the colour was developed by adding the substrate(30mg 4-chloro-1-naphtholdissolved, 10ml cold methanol, 30µl hydrogen peroxide in 50ml PBS pH7.4) (Ceceroni et al. 1997 and Demirkan et al. 1999).

ELISA

The ELISA was performed in polystyrene 96 well microtiter plates (Dyatech) and coated with 5ug/ml B. burgderferi antigen (flagellar protein 41kDa) in carbonate bicarbonate buffer pH9.6 and incubad for 1h. at 37°C then overnight at 4°C. Unbound antigen was removed by washing three times with PBS pH 7.2-Tween 20 (0.05%) (PBST). Unoccupied sites on the plates were blocked by adding 100µl of 0.1% gelatine solution and allowed to incubate for 1h. at 37°C.The plates were washed three times as before. Cattle and sheep sera were diluted in 1:100 and in 1:50 respectively in PBST and 100µl were pipetted from each sera in the ELISA plates in duplicates including the positive clinical cases and the negative control samples. Plates were incubated at 37°C for 1h, then washed again three times with PBST. Then 100µl rabbit anti-bovine IgG and rabbit anti-sheep IgG conjugated to horse radish peroxidase was added in dilution 1:50000 and 1.2000 (in PBS containing 0.05%Tween 20) respectively. The colour was developed by adding 3,3,5,5,tetramethylbenzidine (TMB) as substrate and the blue developed colour was then converted to yellow by addition of 100µl of 1M H_2SO_4 and the

plates were read at 450nm. Each sample was tested as a duplicate, and on each plate a positive controls as well as negative control were included. The mean OD values greater than 0.32 in cattle serum and greater than 0.6 in sheep serum were graded as positive (Ciceroni et al.1997; Tuomi et al. 1998).

RESULTS

In this study a total number of 255 blood samples were collected from 114 cattle (only cows from local and mixed breeds) and 141 sheep from farms had infection with digital dermatitis. Among these animals 24 (21.1%) cows and 21 (14.9%) sheep had typical symptoms of digital dermatitis with severe lameness, accompanied by hyperplasia, hyperaemia of the skin, pain, and swelling of the affected site which in some cases oozed foul odour pus.

SDS-PAGE electrophoresis and Western blot technique were also carried out to confirm the purity of the flagellar protein and its specificity in reaction with B. burgdorferi antibodies. One protein band of 41kDa was the outcome of the SDS-PAGE of the flagellar protein, which reacted specifically in Western blot with the positive control sera as well as sera collected from clinical and non-clinical cases of cattle and sheep (Figure1 and 2).

All blood samples were tested for the presence of B. burgdorferi antibodies by the ELISA test using the B. burgdorferi flagellar protein (41kDa) as a coating antigen and compared with its corresponding Western blot results. In cattle, ELISA could detect 79.2% of the clinical cases, positive to the infection with B. burgdorferi, whereas Western blott could detect 75% of these cases (table 2). In sheep, which had clinical symptoms, ELISA as well as Western blott could detect equally 85.7% infection rate.

Among contact and non contact animal groups ELISA showed superiority over Western blott in detecting the B. burgdorferi infection.(Table 2 and 3) In cattle ELISA could detect 10.8% and 4% in contact and non contact animals respectively against 9.2% and 4% detected by Western blot. In sheep relatively similar results to those in cattle were obtained as ELISA could detect 6.2% and 4.3% in contact and non contact animals respectively against 5.2% and 0% detected by Western blot.

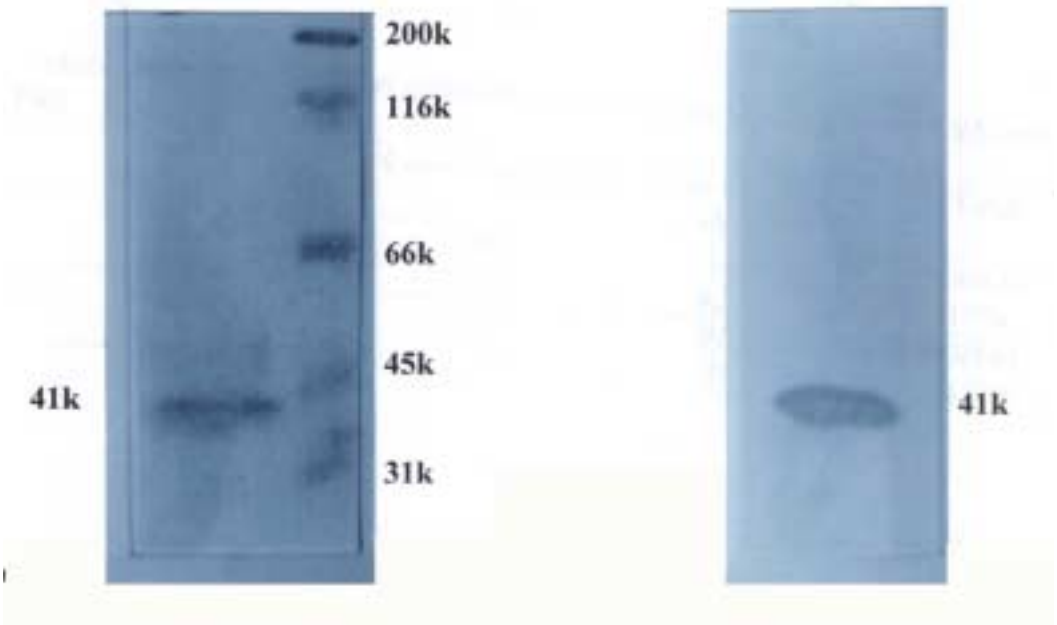

Figure:1) SDS-PAGE electrophoresis of B.burgdorferi flagellin (41kDa) and estimated molecular weights in kilodaltons. Proteins in the gels were stained with Coammassie blue. Figure:2) Western blotting of this flagellin and reaction of antibodies in serum of cattle infected with digital dermatitis. Nitrocellulose membranes were stained with amido black.

TABLE 1: THE PATTERN OF BLOOD SAMPLES COLLECTED FROM COWS AND SHEEP

Animal and clinical symptoms	Blood from clinical cases (with symptoms of digital dermatitis)	Blood from contact apparently healthy animals.	Blood from animals not in contact with infected animals	Total
CATTLE	24/114 21.1%	65/114 57%	25/114 21.9%	114/114 100%
SHEEP	21/141 14.9%	97/141 68.8%	23/141 16.3%	141/141 100%

TABLE 2: DETECTION OF B. BURGDORFERI ANTIBODIES IN THE SERUM OF CATTLE BY ELISA AND WESTERN BLOT

Serological test	Clinical cases among cattle	Contact cattle	Non contact cattle	Total
ELISA	19/24 79.2%	7/65 10.8%	1/25 4%	27/114 23.7%
Western blot	18/24 75%	6/65 9.2%	1/25 4%	25/114 21.9%

TABLE 3: DETECTION OF B. BURGDORFERI ANTIBODIES IN THE SERUM OF SHEEP BY ELISA AND WESTERN BLOT

Serological test	Clinical cases among sheep	Contact animals	Non contact sheep	Total
ELISA	18/21 85.7%	6/97 6.2%	1/23 4.3%	25/141 17.7%
Western blot	18/21 85.7%	5/97 5.2%	0/23 0%	23/141 16.3%

DISCUSSION

Digital dermatitis is one of the most common infectious diseases of claws in cattle and sheep. B. burgdorferi has been proved to be the primary and the most dominant cause of infection as it was isolated and detected microscopically and histopathologically from many clinical cases infected with digital dermatitis. Other bacteria as bacteroides and campylobacter species were found to participate and aggravate the infection but their role in the pathogenesis of digital dermatitis was not as potential as B. burgdorferi. In many cases virus isolation from the affected tissues proved to be negative (Blowey and Sharp 1988; Bassett et al. 1990; Mumba et al. 1999).

Our results revealed, that in closed confined cohort of 114 cattle and 141 sheep, digital dermatitis was clinically manifested in 21.1% of the cattle and 14.9% of the sheep (Table 1). This incidense was considered low when compared by some authors who reported the severity of infection to reach 70% in some dairy farms. The symptoms were severe lameness, accompanied by hyperplasia, hyperaemia of the skin, that might be accompanied by fever in advanced cases, pain when touching or approaching the affected limb with swelling of the affected site, that might show erosions of the superfacial layer of the epidermis and oozed foul odour pus. Which agreed with other authors (Burgess et al. 1993; Wells et al 1993; Ji and Collins 1994; Tuomi et al 1998).

Clinical symptoms supported by serological evidences of bovine and ovine B. burgdorferi infection revealed the relationship between infection with digital dermatitis and the seropositive response to B. burgdorferi (Blowey et al. 1994). Different B. burgdorferi antigens as whole cell sonicate, outer membrane associated protein (OspA-31kDa or OspB-34kDa) and the flagellar protein 41kDa were used in different serological tests. Moreover immunological differences between these antigens as well as the cross reactivity has been also manifested (Wilske et al. 1989; Karlsson 1991; Assous et al. 1993; Lovrich et al. 1993; Wienecke et al. 1993; Dressler et al.1994; Magnarelli et al. 1994; Wilske et al. 1994; Bunikis et al. 1995; Lovrich et al. 1995; Norman et al. 1996). Bunikis et al. (1995) used outer membrane protein as antigen in the detection of infection and suggested a relatively high risk of false negative results. The flagellin protein (41kDa) which most specific protein was found to be the first protein that antibodies directed against after infection with B. burgdorferi (Zoller et al. 1991; Ma et al. 1992; Demirkan et al. 1999)

This flagellar protein was One band of 41kDa (Figur 1) when electrophoresed with SDS-PAGE and reacted specifically in Western blot with the positive control sera as well as sera collected from clinical and non-clinical cases of cattle and sheep (Figure 2).

All blood samples were tested for the presence of B. burgdorferi antibodies by the ELISA test using the flagellar protein (41kDa) as a coating antigen and compared with its corresponding Western blot results. In cattle, ELISA could detect 79.2% of the clinical cases, positive to the infection with B. burgdorferi, whereas Western blott could detect 75% of these cases (table 2). In sheep with clinical symptoms, ELISA as well as Western blot detected 85.7% infection This high rate of seropositive cases among clinically recognised cases confirmed the infection with B. burgdorferri, whereas the seronegative cases from the clinically affected group could be attributed to other causes.

In contact and non contact animal groups ELISA showed superiority over Western blot in detecting the B. burgdorferi infection in cattle and sheep(Table 2 and 3), which agreed with the findings of previous authors. The Relatively low incidenc rate of seropositve cases among contact and non contact animals when compared with clinically infected animals was possibly due to control of infection by isolation of infected animals, application of disinfectants, adminstration of local and general antibiotcs as well as control of ticks and vector animals. It was noticed over the last two decades, that digital dermatitis caused by B. burgdorferi has remained one of the major skin lesion causes lameness in dairy cattle and sheep and may be

responsible for great economic losses to farmers, which might motivate more research to be conducted on the pathogenesis as well as the posibility of vaccine production against B. burgdorferi infection (Demirkan et al. 1999).

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