iMedPub Journals
Our Site: http://www.imedpub.com/

ARCHIVES OF CLINICAL MICROBIOLOGY

2013
Vol. 4 No. 4:2
doi: 10.3823/270

Immunohistochemical detection of Brucella mellitensis and *Coxiella burnetii* antigens in formalin-fixed tissues of West African Dwarf goats

Benjamin O Emikpe[1], Sabri Mohd Yussouf[2], Chukwunonso K Ezeasor[1], Polycarp N Tanko[2]

1 Department of Veterinary Pathology, University of Ibadan, Ibadan, Oyo State, Nigeria.

2 Department of Pathology and Microbiology, Faculty of Veterinary Medicine, University Putra Malaysia, Malaysia.

Correspondence:

banabis2001@yahoo.com

Summary

Background: the information on the evidence of *Brucella mellitensi* and *Coxiella burnetti* infections or co-infection in goats had been scanty; this investigation reports the immuno-histochemical evidence of *Brucella mellitensi* and Coxiella burnetti infections in West African dwarf goats.

Methods and findings: goats presented for post mortem in the Department of Veterinary Pathology, University of Ibadan were used for this investigation. By simple randomisation, lung, kidney, liver and spleen of fifteen goats were used for this immunohistochemical evaluation using standard methods 60% of the goats examined were positive for *Brucella mellitensis*, 33% were positive for *Coxiella burnetti* while 7% were positive for both infection. The spleen liver, kidney and lungs were positive for *Brucella mellitensis* while only the spleen and lungs were positive for *Coxiella burnetti*. *Coxiella burnetti* antigens were located in the cytoplasm of macrophages of alveoli of the lung and in the red splenic pulp while Brucella melitensis antigens were located in the cytoplasm of macrophages in the lung, in the red splenic pulp, Kupffer cells of the liver, macrophages in the glomeruli and epithelial cells of cortical tubules.

Conclusions: this appears to be the first report of immunohistochemical detection of Brucella and Coxiella antigens in tissues of West African dwarf goats. The presence of these antigens in apparently healthy and pneumonic goats showed the level of risk posed by goats' meat and meat products in the spread of these zoonotic diseases hence the need for facilities for rapid detection of these diseases.

Key words: *Brucella mellitensis*, Coxiella burnetii antigens, goats.

This article is available from:
www.acmicrob.com

Introduction

Brucellosis in goats is a major zoonotic disease of economic importance in Africa. In Nigeria, studies have shown that the infection is had been reported in ruminants within the northern and southern savanna zones [1-5] of Nigeria and the human neurobacillosis cases had also been reported [6-7]. *Coxiella burnetti* infection, a similar abortion induced zoonotic pathogen had also been reported in Nigeria with emphasis on cattle [8-10]. The diagnoses of these pathogens are usually by isolation which often does not offer rapid diagnosis and must be correlated with other diagnostic methods [11-12] for it to be diagnostic; this may be due to the nature of the organism which often resides in macrophages. With dearth of information on the evidence of infection or co-infection in Nigerian goats and the problem often faced in the diagnosis of the zoonotic reproductive diseases both on field and in laboratory, there is need to employ the immunohistochemical technique which is rapid, specific and without the need to use tissues containing viable organism to detect the infection. This investigation reports for the first time the immunohistochemical detection of *Brucella mellitensi* and *Coxiella burnetti* infections in formalin-fixed tissues of West African dwarf goats.

Goats presented for post mortem in the Department of Veterinary Pathology, University of Ibadan were used for this investigation. This diagnostic laboratory receives cases and referrals from veterinarians all over Nigeria. The animals were both sexes; their ages varied from six months to five year-old. The gross diagnosis as obtained from the departmental post mortem records showed that majority of these goats died of pneumonia and Peste des Petits Ruminants (PPR) with a few, ruminal impaction. By simple randomisation for the surveillance of these zoonotic infections in goats presented, lung, spleen, kidney and liver samples from fifteen cases were used for this immunohistochemical evaluation as described by Dilbeck and McElwain [13], Ilhan and Yener [14].

The samples were routinely processed, cut at 4-5 µm thickness onto silane-coated glass slides. Two sets of the slides were dried for 15 min at 56-60°C, dewaxed in xylene and rehydrated through a graded alcohol series. The slides were washed with PBST for 10 min. Endogenous peroxidase activity was blocked with freshly prepared 3% hydrogen peroxide for 5 min in room temperature and rinsed and washed with PBST for 2 min. In enhancing the tissue to be immunoreactivity, the heat-mediated antigen retrieval with citrate solution in microwave oven was used. Sections were blocked with blocking buffer 1% normal goat serum and PBST, and then each set of the sections were incubated with anti-*Brucella mellitensis* and *Coxiella burnetti* polyclonal antibody prepared in rabbit with the dilution of 1:100 for at least 1 hour at 37°C in an incubator. Then, the procedure was followed by rinsing and washing with PBST for 5 min. Sections were incubated again at 37°C for 30 min with secondary antibody, biotinylated goat anti-rabbit immunoglobulin Gc with the dilution of 1:1000 for *Brucella* and 1:500 for *Coxiella*. The slides were rinsed and washed with PBST for 5 min before tissue sections were incubated with 3, 39-diaminobenzidine tetrahydrochloride (DAB) which was applied 1 ml diluents to a 1 drop of DAB for the development of the colour change. Once the sections became brown, the slides immediately rinsed with distilled water and the slides were stained using Mayer's haematoxylin solution for the background colour. The specific primary antibody was replaced by non immune rabbit serum in tissue sections used as negative controls. All the slides were analyzed and captured using image analyzer NIS-Elements D 3.2 (Nikon, Japan). The immunostaining was graded into slight, moderate and marked to reveal the intensity of the presence of the antigens [14].

Table 1. The tissues examined and the cells that were immunopositive.

No	Identification no	Tissue	Agents involved	Degree	Cells involved
1.	1023	kidney	Brucella	+	Tubular cells and glomeruli
		liver	Brucella	+	Kupfer cells, in blood vessels
		spleen	Brucella	++	Macrophages in the red pulp.
2	2c2x	spleen	Brucella	++	Same
3	3a2	spleen	Brucella	++	Same
4	3a	spleen	Brucella	++	Same
5	306	spleen	Brucella	++	Same
6	1c23	spleen	Brucella	++	Same
7	2c24	liver	Brucella	+	Kuppfer cells
		spleen	Brucella, coxiella	++	Macrophages in the red pulp.
8	4a23	kidney	Brucella	+	Tubular cells and glomeruli
		lungs	Brucella	+	Alveolar macrophages, in blood vessels
9	3023	liver	Brucella	+	Kupfer cells
10	4224	Liver	Brucella	+	Kupffer cells
		spleen	Brucella	++	Macrophages
11	2c4	Spleen	Coxiella	++	Macrophages in the red pulp.
12	2c23	lung	Coxiella	+	Alveolar macrophages
13	3b23	Spleen	Coxiella	++	Macrophages in the red pulp.
14	11325	Lung	Coxiella	+	Alveolar Macrophages.
		Spleen	Coxiella	++	Macrophages in the red pulp.
15	2a2c	Spleen	Coxiella	++	Macrophages in the red pulp.

Results

The results showed that 60% of the goats examined were positive for *Brucella mellitensis*, 33% were positive for *Coxiella burnetti* while 7% were positive for both infections. The spleen liver, kidney and lung samples were positive for *Brucella mellitensis* while only the spleen and lung samples were positive for *Coxiella burnetti* (**Table 1**). *Coxiella burnetti* antigens were located in the cytoplasm of macrophages in the cellular debris of alveoli of the lung, intracellularly within the cytoplasm of macrophages in the red splenic pulp (**Fig. 1A–1B**). *Brucella melitensis* antigens were located in the cytoplasm of macrophages in the cellular debris of alveoli of the lung, intracellularly within the cytoplasm of macrophages and Kupffer cells of the liver, in the cytoplasm of macrophages in the red splenic pulp; cytoplasm of epithelial cells of cortical tubules, and macrophages in the glomeruli (**Fig. 2A–2F**).

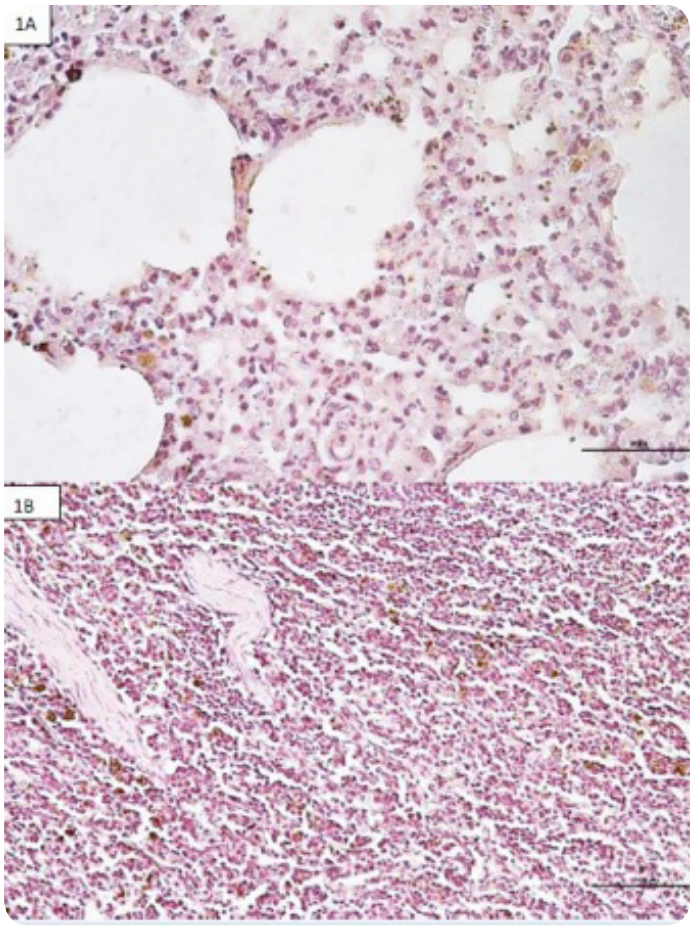

Figure 1. Goat; **A)** Lung. Immunoreactivity to the anti- Coxiella burnetti polyclonal antibody in the cytoplasm of macrophage within alveoli. **B)** Spleen. Immunoreactivity to the anti- Coxiella burnetti polyclonal antibody in the cytoplasm of macrophages of the red pulp of the spleen ABC method, Mayer's hematoxylin counterstain.

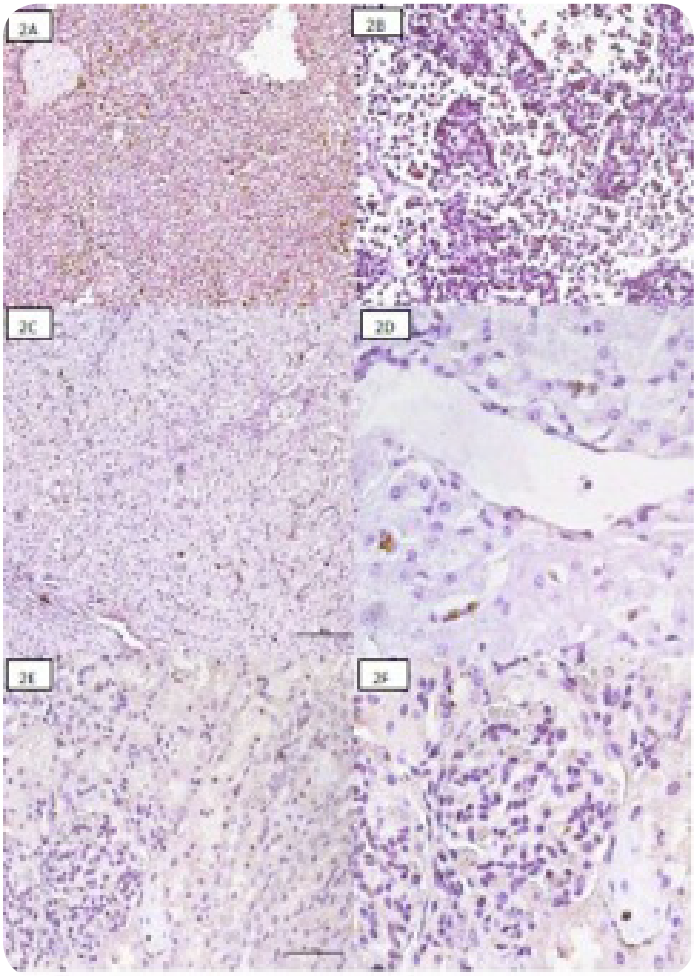

Figure 2A and 2B. Goat; (**2A, B**) Spleen. Immunoreactivity to the anti- Brucella mellitensis polyclonal antibody in the cytoplasm of macrophage the Red pulp. 2C and 2D **B)** Liver. Immunoreactivity to the anti- Brucella mellitensis polyclonal antibody in the cytoplasm of macrophages and Kupffer cells. 2E and 2F, Kidney. Immunoreactivity to the anti- Brucella mellitensis polyclonal antibody in cytoplasm of interstitial macrophages and tubular epithelial cells of the kidney. ABC method, Mayer's hematoxylin counterstain.

Discussions

Although there had been reports describing the serological evidence and prevalence of brucellosis and *Coxiella* infection in Nigerian breeds of ruminants [3, 15-16] so also cattle in Ghana [17], this appears to be the first report of immunohistochemical detection of *Brucella* and *Coxiella* antigens in tissues of West African dwarf goats. The detection of *Brucella* antigen in the lung, spleen, kidney and liver of goats further reveals the predilection sites of these organisms especially in chronic infections [14] and the immunolocalisation

in macrophages further showed the role of macrophages in the two infections [11-13]. The presence of these antigens in blood and in the kidney further leads credence to the fact that they can easily be transmitted through blood and urine. The presence of these antigens in randomly selected goats brought for necropsy showed the threat of the disease in Nigeria with no facilities for rapid and specific method of the disease diagnosis, especially with the possible reports of human cases. The use of immunohistochemical technique is fast and can adapt for us in a developing country with difficulty in stable electricity and where fresh sample submission is difficult. With the use of formalin-fixed sample, retrospective studies using this technique with stored specimens are possible [13]. Definitive and rapid diagnosis of *Brucella* and *Coxiella*-induced abortion will facilitate identification of infected dams thereby contributing significantly to prevention of zoonotic transmission. Efforts are presently on the detection of constant and intermittent shedders in these diseases using some quicker diagnostic procedures.

Conclusions

The presence of these antigens in randomly selected goats brought for necropsy showed the threat of the disease in Nigeria. The use of immunohistochemical technique can be adapted for use in the definitive and rapid diagnosis of Brucella and *Coxiella*-induced abortion especially in resource poor settings.

Acknowledgements

The author acknowledge the support technical staff of the department of microbiology and Pathology, Universiti Putra Malaysia for the research.

References

1. Okoh, AE. Abortion in sheep near Kano, Nigeria. Trop Anim Health Prod. 1980; 12 (1): 11-14.
2. Falade, S. Brucellae isolated from goats. Zentralbl Veterinarmed B. 1981; 28 (3): 205-209.
3. Ocholi, RA., Ezeokoli, CD., Akerejola, OO., Saror, DI. Use of the enzyme161 linked immunosorbent assay for screening cattle for Brucella antibodies in Nigeria. Vet Q. 1996; 18 (1): 22-24.
4. Ocholi, RA., Kwaga, JK., Ajogi, I., Bale, JO. Abortion due to Brucella abortus in sheep in Nigeria. Rev Sci Tech. 2005; 24 (3): 973-979.
5. Cadmus, SI., Adesokan, HK., Adedokun, BO., Stack, JA. Seroprevalence of bovine brucellosis in trade cattle slaughtered in Ibadan, Nigeria, from 2004-2006. J S Afr Vet Assoc. 2010; 81 (1): 50-53.
6. Alausa, OK. Incidence and seasonal prevalence among an occupationally 169 exposed population to brucellosis. Trop Geogr Med. 1980; 32 (1): 12-15.
7. Obiako, OR., Ogoina, D., Danbauchi, SS., Kwaifa, SI., Chom, ND., Nwokorie, E. Neurobrucellosis--a case report and review of literature. Niger J Clin Pract. 2010; 13 (3): 347-350.
8. Adesiyun, AA., Jagun, AG., Tekdek, LB. Coxiella burnetii antibodies in some Nigerian dairy cows and their suckling calves. Int J Zoonoses. 1984; 11 (2): 155-160.
9. Adesiyun, AA., Jagun, AG., Kwaga, JK., Tekdek, LB. Shedding of *Coxiella burnetii* in milk by Nigerian dairy and dual purposes cows. Int J Zoonoses. 1985; 12 (1): 1-5.
10. Blondeau, J., Yates, L., Martin, R., Marrie, T., Ukoli, P., Thomas, A. Q fever in Sokoto, Nigeria. Ann N Y Acad Sci. 1990; 590: 281-282.
11. Neta, AV., Mol, JPS., Xavier, MN., Paixao, TA. Review: Pathogenesis of bovine brucellosis. Vet. J. 2010; 184: 146-155.
12. Xavier, MN., Paixao, TA., den Hartigh, A.B., Tsolis, RM., Santos, RL. Pathogenesis of *Brucella* spp. Open Vet Sci J. 2010; 4: 109-118.
13. Dilbeck, PM., McElwain, TF. Immunohistochemical detection of *Coxiella burnetii* in formalin-fixed placenta. J Vet Diagn Invest. 1994; 6: 125-7.
14. Ilhan, F., Yener, Z. Immunohistochemical detection of *Brucella melitensis* antigens in cases of naturally occurring abortions in sheep. J Vet Diagn Invest. 2008; 20: 803-806.
15. Sixl, W., Rosegger, H., Schneeweiss, H., Withalm, H., Schuhmann, G. Serological investigations in Nigeria for anthropozoonoses in human sera: brucellosis, echinococcosis, toxoplasmosis, chlamydial diseases, listeriosis, rickettsiosis (*Coxiella burnetii* and *Rickettsia conori*). J Hyg Epidemiol Microbiol Immunol. 1987; 31 (4 Suppl.): 493-495.
16. Ocholi, RA., Kwaga, JK., Ajogi, I., Bale, JO. Phenotypic characterization of Brucella strains isolated from livestock in Nigeria. Vet Microbiol. 104 (3-4): 229-230.
17. Kubuafor, DK., Awumbila, B., Akanmori, BD. Seroprevalence of brucellosis in cattle and humans in Akwapim-south distict of Ghana: Public health implications. Acta Trop. 2000; 76: 45-48.