

## ***Brucella Abortus* Antibodies in Raw Cow Milk Collected from Kraals within the Coastal Savannah Zone of Ghana**

**Gloria Ivy Mensah<sup>1</sup>, Kennedy Kwasi Addo<sup>1\*</sup>, Kwame George Aning<sup>2</sup>, Naomi Nartey<sup>1</sup>, George Kwasi Nipah<sup>3</sup> and Henk Lucas Smits<sup>4</sup>**

<sup>1</sup>Bacteriology Department, Noguchi Memorial Institute for Medical Research, University of Ghana, Legon, Ghana

<sup>2</sup>Microbiology Department, School of Veterinary Medicine, University of Ghana, Legon, Ghana

<sup>3</sup>Epidemiology Department, Veterinary Services Directorate, Ministry of Food and Agriculture, Accra, Ghana

<sup>4</sup>KIT Biomedical Research, Royal Tropical Institute / Koninklijk Instituut voor de Tropen (KIT), Amsterdam, The Netherlands

### **ABSTRACT**

There has been a recent upsurge in the marketing of fresh un-pasteurized milk and yoghurt in some urban areas in Ghana. This has raised concerns about human health risks associated with the consumption of unpasteurized milk especially with respect to *B. abortus* as bovine brucellosis is common in Ghana.

The Milk Ring Test (MRT) and the indirect milk ELISA were used for *Brucella abortus* antibody detection in raw milk samples collected from 224 kraals involved in the production and marketing of fresh cow milk within the coastal savannah zone of Ghana. *Brucella* IgG and IgM lateral flow assay (LFA) was used for the detection of antibodies to *B. abortus* in whole blood from 75 herdsman and family members.

The MRT detected *B. abortus* antibodies in 21.9% of the milk samples while the ELISA detected specific antibodies in 58.9% of the samples. Two individuals (2.7%) tested positive with the LFA; one for specific IgG antibodies and the other with specific IgG and IgM antibodies.

These results indicate that although *B. abortus* antibodies are present in raw cow milk produced and marketed within the coastal savannah zone of Ghana, human brucellosis infection among herdsman and their family members is low.

**KEY WORDS:** Raw milk, *B. abortus*, Kraal, MRT, ELISA

### **INTRODUCTION**

Brucellosis is a chronic zoonotic disease caused by *Brucella species* which are Gram-negative and facultative anaerobic non-motile intracellular bacteria [1] with *Brucella abortus* being the principal cause of brucellosis in cattle. As a result of compulsory pasteurization of milk products and strict control of the disease in dairy cattle, the incidence of brucellosis has steadily declined in most industrialized countries unlike in most developing countries where people still consume unpasteurized milk and milk products [2]. It is estimated by the World Health Organization (WHO) that more than 500,000 new cases of human brucellosis occur globally each year with most of the cases reported from the Mediterranean area, Central Asia and East-Africa [3]. Relatively little information is available on the occurrence of brucellosis in livestock and the transmission to the human population in West-African countries.

In Ghana bovine brucellosis is caused almost exclusively by *B. abortus* [4] and the disease in cattle has been reported from the coastal and northern savannah zones [5, 6] as well as the middle forest belt [7]. The detection of *B. abortus* as well as several other pathogens in raw milk from Ghana [8] in the past did not raise the needed concern possibly because the consumption of raw dairy was not widespread. In recent years however, the marketing of fresh dairy in Ghana, especially in the urban and peri-urban areas has increased considerably in response to a growing demand for such products [7]. A high prevalence of *B. abortus* antibodies was reported in raw cow milk samples taken from retailers and wholesalers in Accra and Kumasi, two major cities in Ghana [9].

To investigate the extent of the problem we have designed a larger study to determine the prevalence of *B. abortus* antibodies in raw cow milk produced and marketed within the entire coastal savannah zone of Ghana; where the major livelihood is raising of cattle. Dairy thus forms a substantial portion of the dietary needs of these communities and marketing of fresh dairy is an important source of income. Also we have investigated the prevalence of human brucellosis infection among a section of herdsman and their family members.

---

**\*Corresponding Author:** Dr. Kennedy Kwasi Addo, Bacteriology Department, Noguchi Memorial Institute for Medical Research, University of Ghana, Legon, Ghana. E-mail: kaddo@noguchi.mimcom.org

## MATERIALS AND METHODS

### Selection of cattle kraals

Ghana is divided into ten regions (subdivided into districts) and portions of three of these; Central, Greater-Accra and Volta Regions lie within the coastal savannah zone. Districts (12) with intensive dairy production were purposively selected for the study. Cattle owners in each district were identified with the help of District Officers of the Ministry of Food and Agriculture. The aim of the study was explained to the owners/caretakers for verbal consent and 224 kraals out of 400 were randomly selected for the study. The selected kraals produced about 50 to 500 litres of milk per day, being classified as small to medium scale producers. The milk was either sold raw or processed into cottage cheese for sale to distributors and/ or processors. In all the kraals, milking was manual.

### Collection of milk samples

From each kraal, approximately 100ml of milk was aseptically withdrawn from the final container (containing the pooled milk for the day) into sterile bottles and sent to the Bacteriology Department of the Noguchi Memorial Institute for Medical Research on ice packs at 4°C. Milk samples were tested for antibodies to *B. abortus* with the *Brucella* Milk Ring test (MRT) immediately upon arrival in the laboratory and the remaining portion of the samples was stored frozen at -70°C until ELISA was performed. Samples were collected between May and July 2007 during the calving season.

### Brucella Milk Ring Test

Using a sterile pipette, 1ml of each milk sample was withdrawn into miniature test tubes after which 50µl of stained *B. abortus* antigen was added. The mixture was homogenized by shaking the tubes and thereafter incubated at 37°C for 1 hour. Results were read after further incubation at 2-8°C for 24 hours. A positive control was included with each set of tests. A blue ring on top of the tube indicated a positive reaction whilst a white layer on top indicated a negative reaction.

### Indirect Milk ELISA

Milk samples previously frozen at -70°C were thawed and screened in bulk using a 96 well *Brucella* milk ELISA screening kit (Institut Pourquier, Montpellier, France) for the detection of *B. abortus* lipopolysaccharide (LPS) specific antibodies. Milk samples were diluted by adding 50µl of milk to 200µl of dilution buffer in the wells of the plate. The contents of the wells were homogenised by gently shaking the plate and then incubated for 1 hour and 30 minutes at 21°C. After incubation the contents of the plate was emptied by a flick off method and washed three times with the wash solution, a peroxidase conjugated anti-ruminant IgG was added and the plates were incubated at 21°C for another 30 minutes. After a final washing, the enzyme substrate 3, 3', 5, 5'-tetramethylbenzidine (TMB) was added to the conjugate and the reaction was stopped with H<sub>2</sub>SO<sub>4</sub> 0.5M solution. With each run a positive (duplicate) and a negative control milk sample were included.

### Interpretation

The reaction was considered valid when; (1) minimal mean OD<sub>450</sub> value of the positive control milk was >0.400 and (2) the ratio between the OD<sub>450</sub> values of the positive and negative control samples was ≥3.0. For each sample the ratio S/P% was calculated ( $S/P\% = 100 \times (\text{OD}_{450} \text{ value of the sample} - \text{OD}_{450} \text{ value of the negative control}) / (\text{mean OD}_{450} \text{ value of the positive control} - \text{OD}_{450} \text{ value of the negative control})$ ). Samples with an S/P % ≤45% were interpreted as negative and coming from a herd which had not been in contact with *B. abortus*, samples with an S/P% >45 % and <55 % were interpreted as doubtful, and samples with an S/P% ≥55% were interpreted as positive and considered coming from a herd in which at least one animal had been in contact with *B. abortus*. The assay was repeated for samples with a doubtful result.

### Recruitment of participants for brucellosis screening

A cross section of kraals in the Central and Greater-Accra Regions where milk samples had already been screened for antibodies to *B. abortus* were revisited and herdsman and their family members, 6 years and above within the kraal household were recruited using informed written/verbal consent. All study participants were interviewed prior to testing using a pre-structured questionnaire to capture personal information as well as habits and practices of relevance to possible infection with brucellosis. Because of logistic reasons kraals in the Volta Region were not included in the human screening.

### Lateral Flow Assay (LFA)

A disposable lancet was used to induce bleeding, blood was collected with a 50µl heparinized glass capillary and 10µl spotted directly onto the sample application pad in the sample well of each of the two devices (IgG and IgM LFA). The test liquid was then added to completely fill the well and the result was read 10 to 15 minutes later by visual inspection for staining of the antigen and control lines in the test window. Tests were considered valid when the control

line was stained, and the assay was scored negative when there was no staining of the antigen line and positive when a distinct staining of the antigen line was observed. The antigen line stains at different intensities hence positive results are subjectively rated 1+ for weak staining, 2+ for moderate staining, 3+ for strong staining, and 4+ for very strong staining. Undetermined staining represented by very weak (+/-) staining is considered negative.

### Data Analysis Framework

Data was entered into Microsoft Office Excel 2003 (Microsoft Excel, Palisade Corp, Newfield, NY, USA) and analyzed using the Statistical Package for Social Sciences (SPSS Inc., version 11 and 16, Chicago, Illinois, USA). Chi-square was used to detect statistical significant difference between the regions ( $P$  value  $< 0.05$ ).

### Ethical issues

Ethical clearance was granted by the Ghana Health Services' ethical review board on research. Consent was sought from individuals/household head before blood samples were taken for brucellosis screening. The objectives of the study were explained to them in their local dialect.

## RESULTS

### Participating kraals

The mean herd size of participating kraals was 108.78 (SD 95.68, range 18-900) and only 13.4% (30/224) had vaccinated the herd against brucellosis. Within the year, 33.5% (75/224) had experienced abortions in the herd (OR 0.992, CI 0.44-2.24), with most (86.7%) of the aborted cases recorded in the non vaccinated herds. The number of abortions in the unvaccinated herds was not significantly higher ( $P=0.99$ ) compared with the vaccinated herds.

Table: I. Detection of *Brucella* positive milk samples

Region (Number of milk samples)	Number of positive (%) samples in the following assay	
	MRT	ELISA
Central (n=38)	6 (15.8%)	24 (63.2%)
Volta (n=95)	27 (28.4%)	60 (63.2 %)
Greater-Accra (n=91)	16 (17.6%)	48 (52.7%)
Total (n=224)	9 (21.9%)	32 (58.9%)

### Screening of bulk milk samples

*B. abortus* specific antibodies were detected in 21.9% of the milk samples by MRT and in 58.9% of the milk samples by ELISA. All the samples which tested positive for the MRT also tested positive for ELISA (Table 1). There was no significant difference in the number of positive milk samples recorded per region ( $P > 0.05$ ). Vaccination status had no significant effect on test outcome whether MRT ( $p=0.1$ ) or ELISA ( $p=0.3$ ). A positive result by MRT was associated more ( $p=0.024$ ) with abortion within the herd than ELISA.

Table: 2. Number of households and participants screened for antibodies to *B. abortus* and relationship with the detection of antibodies in milk

Region	Number of seropositive (%) / participants tested		Number (%) kraals with a seropositive milk samples	
	Households	Household members	MRT	ELISA
Central	2 (22.2)/9	2(11.2)/18	2 (22.2)	8 (88.9)
Greater-Accra	0(0)/12	0(0)/57	1(8.3)	4 (33.3)
Total	2(8.3)/24	1(1.8)/57	3 (12.5)	12 (50)

### Screening of household members

A total of 75 individuals from 21 households participated in the study (Table 2); Nine households in the Central Region and twelve in the Greater-Accra Region. Bulk milk samples from the kraal of twelve households had tested positive for antibodies to *B. abortus*. Blood samples from two (2.7%) individuals tested positive for *Brucella* antibodies with the LFA. One individual tested positive for the presence of specific IgG antibodies and the other tested positive for both specific IgG and IgM antibodies. In both cases the staining intensity was weak (1+). One of the seropositive individuals came from a kraal with a positive milk sample while the other was from a kraal with a negative milk sample. The mean ages of the consenting participants was 26.91 (SD 16.843), range 6 and 67 years with the majority (37%) being within the 20-40 year group. More males (72%) compared to females (28%) were tested. While 59% of the participants were involved with all duties at the kraal, 29% of the respondents were not directly involved in any of the activities at the kraal, while the rest (12%) were involved in specific duties such as milking, grazing and cleaning. All but two participants regularly consumed dairy and of the regular consumers of dairy, 11% always boiled their milk before consumption, 49% consumed the milk raw while 40% consumed their milk either boiled or raw (Table 3). None of the participants had ever been diagnosed with brucellosis but all had experienced fever in the past and only 2 out of 75 did

not visit the hospital as a result of the fever. Those who visited the hospital were diagnosed with malaria. Of the 21 kraals, 11 (55%) had recorded abortions in the herd within the past year while 10 had not had any case of abortion. Raw milk from 8 (72.7%) out of the 11 kraals that had recorded abortions in the herd within the year were positive for *B. abortus* antibodies by one or both tests while 3 (27.3%) were negative by both tests. Of the 10 kraals with no aborted cases in the past year, 5 (50%) recorded the presence of *B. abortus* by one of both tests with the remaining half negative by both tests. The number of aborted cases per kraal ranged from 1 to 10. All kraals disposed of aborted fetuses by burying.

Table: 3. Participants' role in kraal activities

Question	Response	Frequency	Percent
<b>Status at kraal</b>	Herdsmen	24	32.0
	Wife	8	10.7
	Child	33	44.0
	Relative	5	6.7
	Worker	5	6.7
<b>Main duty at kraal</b>	None	22	29.3
	Milking	1	1.3
	Grazing	3	4.0
	Cleaning	5	6.7
	General	44	58.7
<b>Mode of consumption of milk</b>	Nil*	2	2.6
	Boiled	8	10.7
	Raw	36	48.0
	Boiled or raw	29	98.7
<b>Ever been diagnosed with brucellosis?</b>	Yes	0	0
	No	75	100

\* Do not consume raw milk/milk products

## DISCUSSION

For bulk milk samples the sensitivity (98.1%) of the ELISA is greater ( $p=0.0001$ ) than the MRT (72.2%) although the specificity of ELISA (88.1%) is no different ( $p=1.0$ ) from that of the MRT (90.5%), [10] which is also true for our study limited to screening of bulk milk.

The proportion of kraals with positive bulk milk samples indicate that bovine brucellosis is not only an important veterinary problem in the coastal savannah zone of Ghana but presents a potential public health risk as well. Infected animals shed *Brucella* from the udder and infected supra mammary lymph nodes into the milk and concentrations of the pathogen may be as high as 2,000,000 organisms/ml of milk [11]. Fresh dairy forms an important constituent of the daily dietary needs of the local population and the fact that many individuals indicated a preference for the consumption of fresh dairy that has not been boiled suggests that the population is regularly exposed to the pathogen. High levels of contaminated milk samples have also been reported from other countries in West Africa including Nigeria and Mali [12, 13]. Aside the public getting infected through the consumption of contaminated unprocessed milk, milk products and meats, *Brucella* has a low infectious dose making infection a genuine risk to those occupationally exposed such as farmers, veterinarians and butchers [14]. *Brucella* replicates in the placenta to very high concentrations thus is easily transmitted to farmers/veterinarians during their daily work especially when milking, assisting with calving or handling aborted materials without wearing proper protective clothing. The high proportion of farmers reporting abortions in their cattle also presents an economic problem as abortions together with reduced milk production, weak off-springs and infertility weakens the economic position of the farmers. One may wonder whether the small proportion of cattle herds that have been vaccinated against brucellosis helps to protect against the infections. Vaccinated herds may still be in regular contact with unvaccinated infected animals and become infected. Abortions were also reported among vaccinated herds and although abortions may have various other causes this could be an indication of infection with *Brucella*. A more widespread implementation of vaccination and the introduction of other preventive measures may be desirable.

Evidence that brucellosis is transmitted to the human population and presents a serious public health problem is still lacking. Studies performed in Eritrea [15] and Ethiopia [16] have shown that brucellosis is transmitted to humans but in these countries *B. melitensis* is the likely cause of brucellosis. Testing of farmers and their family members living at the kraals indicated that 2 out of 75 individuals tested seropositive for antibodies against *Brucella*. This relatively low seroprevalence notwithstanding suggests that people living at the kraals are indeed exposed to the pathogen. A similar rate was reported for communities' endemic for brucellosis in Turkey [17]. The rapid test used for the testing of the local population is highly specific (99%) and sensitive (96%) and is specifically designed for the field testing for brucellosis [18]. None of the individuals tested was ill and the detection of specific antibodies in healthy individuals may be taken as an indication of previous exposure and perhaps the development of some degree of immunity. Only during outbreak situations will much higher seroprevalence rates be observed [19]. Earlier studies on human brucellosis in Ghana did not record a single positive case [7]. Studies have shown that oligosymptomatic, or even asymptomatic and self-limiting episodes of *Brucella* infection are common in endemic areas [20, 21] and that IgG anti-*Brucella* antibodies can persist for

many months after the completion of treatment [22, 23]. This accounts for the high seroprevalence of anti-*Brucella* antibodies in endemic regions [24, 25] and in individuals who are exposed repeatedly [26].

## CONCLUSIONS

In conclusion, human brucellosis is not regarded as a public health problem in Ghana. It is not a notifiable disease and could be easily overlooked and misdiagnosed without sufficient awareness of medical staff and if laboratory tests are not used. Individuals living in the kraals were always diagnosed with malaria in cases of febrile illness and brucellosis could be easily confused with malaria. The introduction of laboratory testing for malaria and other causes of febrile disease including brucellosis could help to sort out the major causes of disease in these communities. Besides, health education may be needed to reduce the risk of infection.

## ACKNOWLEDGEMENTS

The Ghanaian-Dutch collaboration for health research and development (Project ID number 2005/OD/08) funded this research. The authors are most grateful to The Animal Research Institute, Ministry of Food and Agriculture and the Veterinary Services Directorate for the support of their staff during the sample collection. Gratitude is also extended to all farmers and stakeholders in the small scale diary business whose cooperation contributed to the success of this work.

## REFERENCES

1. Cutler, S. J., Cutler, R. R., 2006. Brucellosis, the most common bacterial zoonosis? The biomedical scientist 50 (4), 336-341.
2. Cadmus, S., Palmer, S., Okker, M., Dale, J., Gover, K., Smith, N., Jahans, K., Hewinson, R. G., Gordon, S. V., 2006. Molecular analysis of human and bovine tubercle bacilli from a local setting in Nigeria. J Clin Microbiol 44(1), 29-34
3. Pappas, G., Papadimitriou, P., Akritidis, N., Christou, L., Tsianos, E. V., 2006. The new global map of human brucellosis. Lancet. Infect. Dis. 6, 91-99.
4. Corbel, M. J., 1997. Brucellosis: an overview ( 1<sup>st</sup> International conference on emerging zoonosis) Emerging Infectious diseases 3(2) URL: <http://www.cdc.gov/ncidod/EID/vol3no2/corbel.htm>
5. Oppong, E. N.W., 1966. Bovine brucellosis in Southern Ghana. Bull. Epizootic Dis. Africa 14, 397-403.
6. Turkson, P. K., Boadu, D. Q., 1992. Epidemiology of bovine brucellosis in the Coastal Savannah zone of Ghana. Acta Tropica 52, 39-43.
7. Kubuafor, D. K., Awumbila, B., Akanmori, B. D., 2000. Seroprevalence of brucellosis in cattle and humans in the Akwapim-South district of Ghana: public health implications. Acta Tropica 76, 45-48.
8. Ayebo, A., Assoku, R. K. G., 1976. A study of bacteriology of raw milk produced on Accra plains of Ghana. Ghana Journal of Science 16, 9-18.
9. Aning, K. G., Sampene-Donkor, E., Omere, A., 2002. Analysis of milk-borne public health risks in milk markets in Ghana. Final report of the peri-urban milk marketing and public health research report. CSIR-ARI, KNUST & International Livestock Research Institute (ILRI). 33pp.
10. Vanzini, V. R., Aguirre, N. P., Valentini, B. S., Torioni de Echaide, S., Lugaesi, C., Marchesino, M.D., Nielsen, K., 2001. Comparison of an indirect ELISA with the *Brucella* milk ring test for detection of antibodies to *Brucella abortus* in bulk milk samples. Vet. Microbiol. 82 (1), 55-60
11. Corbel, M. J., 1988. Brucellosis, In Laing, J.A. (ed.) Fertility and Infertility in Veterinary Practice. p. 189-221 4<sup>th</sup> ed. ELBS, Bailliere Tindall.
12. Bonfoh, B., Fane, A., Traore, A. P., Tounkara, K., Simbe, C. H., Alfarouh, O. I., Schalch, L., Farah, Z., Nicolet, J., and Zinsstag, J., 2002. Use of indirect enzyme immunoassay for detection of antibody to *Brucella abortus* in fermented cow milk. Milk Science International 57(7), 361-420.
13. Junaidu, A. U., Oboegbulem, S. I., Salihu, M. D., 2008. Seroprevalence of brucellosis in prison farm in Sokoto, Nigeria. Asian Journal of Epidemiology 1(1), 24-28.
14. Smits, H. L., Cutler, S. J., 2004. Contributions of biotechnology to the control and prevention of brucellosis in Africa. African Journal of Biotechnology. 3 (12), 631-636.
15. Omer, M. K., Assefaw, T., Skjerve, E., Teklehiorghis, T., Woldehiwet, Z., 2002. Prevalence of antibodies to *Brucella* spp. and risk factors related to high-risk occupational groups in Eritrea. Epidemiol Infect 129, 85-91.

16. Regassa, G., Mekonnen, D., Yamuah, L., Tilahun, H., Guta, T., Gebreyohannes, A., Abraham, A., Abdoel, T. H., and Smits, H. L., 2009. Human Brucellosis in Traditional Pastoral Communities in Ethiopia. *International Journal of Tropical Medicine* 4 (2), 59-64.
17. Kose, S., Smits, H. L., Abdoel, T. H., Ozbel, Y., 2006. Prevalence of Brucella antibodies in rural and suburban communities in three provinces of Turkey: need for improved diagnosis and prevention. *J Infect.* 53(5), 308-14.
18. Smits, H. L., Abdoel, T. H., Solera, J., Clavijo, E., Diaz, R., 2003. Immunochromatographic Brucella-specific immunoglobulin M and G lateral flow assays for the serodiagnosis of human brucellosis. *Clin Diagn Lab Immunol.* 10, 1141–1146.
19. Irmak, H., Buzgan, T., Evirgen, O., Akdeniz, H., Demiroz, A. P., Abdoel, T. H., Smits, H. L., 2004. Use of a new, simple and rapid diagnostic test, the Brucella-IgM and IgG flow assays in the serodiagnosis of human brucellosis in an endemic area in eastern Turkey. *Am. J. Trop. Med. Hyg.* 70, 688–694
20. Coghlan, J. D., Longmore, H. J., 1973. The significance of Brucella antibodies in patients in a rural area. *Practitioner* 211, 645–652.
21. Poole, P.M., 1975. A 6-year survey of human brucellosis in a rural area of north-western England and north Wales. *Postgrad Med J.* 51, 433–440.
22. Young, E. J., 1991. Serologic diagnosis of human brucellosis: analysis of 214 cases by agglutination test and review of the literature. *Rev Infect Dis.* 13, 359–372.
23. Ariza, J., Pellicer, T., Pallare's, R., Foz, A., Gudiol, F., 1992. Specific antibody profile in human brucellosis. *Clin. Infect Dis* 14, 131–140.
24. Tasei, J. P., Ranque, P., Balique, H., Traore, A. M., Quilici, M., 1982. Human brucellosis in Mali; Results of a seroepidemiological study. *Acta Trop.* 39, 253–264.
25. Abo-Shehada, M. N., Odeh, J. S., Abu-Essud, M., Abuharfeil, N., 1996. Seroprevalence of brucellosis among high risk people in northern Jordan. *Int. J Epidemiol.* 25, 450–454.
26. Kumar, P., Singh, D. K., Barbuddhe, S. B., 1997. Sero-prevalence of brucellosis among abattoir personnel of Delhi. *J Commun Dis.* 29, 131–137.