THE PREVALENCE OF ANTIBODIES TO *BRUCELLA ABORTUS* IN MARKETED MILK IN KENYA AND ITS PUBLIC HEALTH IMPLICATIONS.

Kang'ethe, E.K.[1]. Arimi, S.M.[1], Omore, A.O[2,3], McDermott, J.J.[3], Nduhiu, J.G.[1], Macharia J.K.[1] and Githua, A[1].

[1]Department of Public Health, Pharmacology and Toxicology, University of Nairobi, P.O. Box 29053, Nairobi, Kenya; [2]Kenya Agricultural Research Institute (KARI), P.O. Box 57811, Nairobi, Kenya; [3]International Livestock research Institute (KARI), P.O. Box 30709, Nairobi, Kenya.

Paper prepared for ORAL PRESENTATION **at the 3rd All Africa Conference on Animal Agriculture. 6 – 9 November 2000.**

Summary

The risk of infection by milk-borne brucellosis is one reason for public health regulations which discourage informal milk markets that sell unpasteurized milk. However, these regulations are not generally implemented in many developing countries. Kenya is a typical example, with over 85% of milk sales passing through informal channels. Consumer practices to reduce or eliminate potential infection by milk-borne health hazards under these circumstances have rarely been studied.

Seasonal survey data were collected between January 1999 and January 2000 from informal milk market agents of various cadres and from households consuming unpasteurized milk in rural and urban locations in central Kenya. Respondents were randomly selected within production system (extensive and intensive) and human population density (urban, peri-urban and rural) strata. In addition, pasteurized and packaged milk samples from five processors were collected. Samples were screened for antibodies to *Brucella abortus* using the milk ring test (MRT) (unpasteurized milk) and indirect antibody ELISA (both unpasteurized and pasteurized milk).

Milk samples originating from farms in the extensive production system and those containing milk from many sources were associated with higher antibody detection proportions. Five percent of all raw milk samples collected from consumer households and 4% of samples collected from various levels of bulking of market samples were positive to the ELISA. There was poor to no agreement between the two antibody detection tests. All urban consumers and 96% of rural consumers of unpasteurized milk indicated that they boil the milk (in tea or otherwise) before consumption. The implications of these results on milk marketing in Kenya are discussed.

Key words: *Brucella abortus, unpasteurised milk, milk marketing, Kenya.*

Introduction

Bovine brucellosis is a zoonosis commonly caused by *Brucella abortus*. The disease in cattle causes abortions and is mainly spread by material contaminated by body fluids. In humans, brucellosis presents as a febrile flu-like illness and is common among pastoralists in Africa (Berman, 1981; Chukwu, 1987; Nicoletti, 1984; Seifert, 1996) and Kenya (Muriuki *et al*., 1994). It is less prevalent in intensive smallholder production systems (Kadohira *et al.,* 1997). The prevention of brucellosis infection in humans is a major reason for the advocacy of milk pasteurization worldwide. Despite the existence of regulations that require milk pasteuriuzation, over 75% of milk marketed in many developing regions is sold raw through informal channels (Staal, 2000). The informal milk markets thrive because they provide social and economic benefits to smallholder producers, small market agents and consumers in terms of higher farm-gate prices, creation of employment and competitive consumer prices. In Kenya, over 85% of marketed milk is not pasteurized and is sold through informal market pathways (Omore *et al*., 1999). Concerns about human health risks from these market pathways need to be addressed in the context of consumer practices, such as boiling, to reduce or eliminate potential infection by milk-borne health hazards, without

discouraging the markets through which the majority of smallholders sell their milk.

Application of serological diagnostic tests for bovine brucellosis has been achieved in diverse areas using the Rose Bengal Plate Test (RBPT) (Kagumba and Nandokha, 1978; Turkson and Boau, 1992) and complement fixation test (CFT) (Cargill *et al.,* 1982 and Sutherland *et al*., 1986). Until recently, only the Milk Ring Test (MRT), with a sensitivity of about 89% (Nicoletti, 1969; Hunter and Allen, 1972) was available for detection of brucella antibodies in fresh milk. A more accurate indirect ELISA (sensitivity = 95% and specificity = 99%) for testing brucella antibodies in milk has also been recently improved and validated (Kerkhofs *et al*., 1990; Nielsen *et al*., 1996). The milk ELISA is more sensitive than MRT, CFT and RBPT (Sutherland *et al*., 1986; Kerkholfs *et al*., 1990 Neilsen *et al*., 1996; Kerby *et al* 1997) and reportedly is able to detect antibodies in dilutions of up to 1:100 (Forschner and Buegner,1986). This paper presents results of a study on the occurrence of *Br. abortus* antibodies in informally and formally marketed milk in Kenya using the MRT and milk ELISA tests and it evaluates consumer practices to reduce potential milk-borne health risks from consumption of raw milk.

Materials and methods

As part of a large study to assess public health hazards associated with marketed milk, samples were collected between January 1999 and January 2000 from 212 and 222 raw (unpasteurized) milk consuming households in the dry and wet season, respectively. At the marker-level, 262 and 246 informal market agents were interviewed and sampled during two seasons. Informal market agents sampled included dairy co-operatives, milk bars, milk shops and kiosks and mobile traders on foot, bicycle or motorised transport. A total of 110 formally (pasteurized) marketed milk samples from retail outlets with and without refrigeration facilities in Nairobi and Nakuru were also tested.

Respondents were randomly selected within production system (extensive and intensive) and human population density (urban, peri-urban and rural) strata. Nakuru and Narok districts represented extensive production systems and low population density (also medium market access). Nairobi and Kiambu Districts represented intensive production systems and high population density (also high market access). Informal market agents sampled included dairy co-operatives, milk bars, milk shops and mobile traders on foot, bicycle or motorised transport. Attempts were made during the wet season to interview and sample the same respondent as in the dry season. Where this was not possible, substitution was made within the same locality. Samples were screened using the milk ring test (MRT) (unpasteurized milk) and indirect antibody ELISA (both unpasteurized and pasteurized milk).

Brucella Milk Ring Test (MRT)

The MRT works on the principle that antibodies to *Br. abortus* attach themselves to fat globule agglutinins in milk which rise to the surface of the milk and cluster in the cream layer. When haematoxylin stained *Br. abortus* antigen combines with brucella antibody (if present), a complex which adheres to the fat globules in the cream layer of milk is formed. The test often detects a high proportion of false positives (low sensitivity) due to positive reactions from samples taken shortly after parturition, near the end of lactation period, or from mastitic quarters (MacMillan, 1990). MRT was conducted by pipetting 1 ml of milk into a 1.2ml Skatron tubes (Skatronas, Lier, Norway), adding and mixing one drop of stained *Br. abortus* antigen. The tubes were thereafter incubated at 37^0C for 1hr and results read. A positive control was included with each set of tests.

Indirect milk ELISA

The method described by Neilsen *et al*., (1996) was adopted with slight modification. Briefly, polystyrene 96-well flat bottomed plates were coated with 100µl of 0.5mg/well of *Br. abortus* smooth lipopolysacccharide antigen in coating buffer (0.06 M carbonate buffer pH 9.6) and kept overnight in a humid box. The plates were thereafter washed five times with phosphate buffer (0.01M phosphate buffer of pH 7.2 containing 0.05% Tween-20 and 0.15M NaCl), dried and blocked using 200ul/well of 0.1% gelatin and incubated at 25^0C for 30 mins. The plates were washed again, dried and milk samples added at 100 µl/well diluted 1:2 in milk diluent (0.01M phosphate buffer, pH 6.3, containing 0.15M NaCl, 0.05% Tween-20, 15 mM EDTA and 15 mM EGTA). The plates were shaken for 2 minutes in an orbital shaker and incubated for 28 mins at 25^0C. The plates were then washed and 100µl/well of monoclonal antibody

conjugated (dilution 1:1600) to horse radish peroxidase added and incubated for 1hr at 25^0C. The plates were washed again, dried and the substrate (0.05M Citrate buffer pH 4.5 containing 1mM hydrogen peroxide and 4mM ABTS) added at 100 µl/well. The plates were incubated for a maximum of 15 mins and the absorbance read at 414 nm. Brucella positive and negative serum and milk controls were included. The control serum samples were diluted 1:50, while milk samples were diluted 1:2 in the milk diluent. Each milk sample was tested in duplicate. The modification in this procedure was that the cut-off value was determined by using twice the mean of the negative control samples (Savingy and Voller,1980) and not by the targeted reading described by Wright *et al*., (1985).

Results and Discussion

Indirect ELISA classified more consumer- and market-level samples as *Br. abortus* positive than MRT (Tables 1). Overall prevalence of brucellosis at consumer-level as determined by both ELISA and MRT were 4.9% and 3.9%, respectively. At the informal market level, ELISA and MRT classified 2.4% and 3.4%, respectively, as positive. Informally traded bulked raw milk from dairy co-operatives and milk bars had the highest proportion of ELISA and MRT positive samples. Nearly all these samples were from Narok District where extensively grazed pastoralist zebu herds predominate. The ELISA test classified nine (8.2%) of pasteurised milk samples as positive. Six of the nine positive samples were from one milk processor in Nakuru. Agreement between the test results were poor (*Kappa* = 0.32, 95%, confidence interval = 0.07-0.56) to moderate (*Kappa* = 0.40, 95% confidence interval = 0.19-0.60) for the market- and consumer-level samples, respectively, with the ELISA test classifying more samples as positive. Two consumer households in Nakuru reported having had a member diagnosed with brucellosis in the previous one year.

Table 1. Numbers and proportions of milk samples from consumer households and various market agents (two seasons) in rural and urban areas in Kenya testing positive for *Br. abortus* using MRT and ELISA antibody tests

Source of milk samples	Antibody Prevalence			
	MRT		ELISA	
	n	%	n	%
Consumer households				
Urban consumers (Nairobi and Nakuru)	10	4.7	11	5.1
Rural consumers (Nakuru)	7	3.2	10	4.6
Informal market agents in high market access and intensive production area (Nairobi/Kiambu)				
Coops/collection centers/Self help groups	3	4.8	2	3.1
Milk Bars	1	0.8	2	1.6
Milk Shops/kiosks	2	2.1	1	1.0
Small mobile traders	1	1.7	0	0
Informal market agents in medium market access and extensive production area (Nakuru/Narok)				
Coops/collection centers/Self help groups	0	0	0	0
Milk Bars	9	15.0	5	12.2
Milk Shops/Kiosks	4	4.4	0	0
Small mobile traders	0	0	2	3.4
Pasteurised milk in Nairobi & Nakuru	-	-	9	8.2

The test results generally reflect previous findings from serological studies (e.g., Kagumba and Nandokha, 1978; Kadohira *et al*., 1997) indicating higher farm-level prevalence of brucellosis in extensive and/or communal grazing areas than in smaller stall-fed herds. Kagumba and Nandokha (1978) reported a prevalence of 10% bovine brucellosis in extensive production systems in Nakuru, and Kadohira *et al*., (1997) reported a 2% apparent prevalence of bovine brucellosis in the smallholder system in Kiambu. Human brucellosis is also more common where extensive cattle production systems predominate. Muriuki *et al*., (1997) found that as high as 21% of human flu-like cases reported in health facilities in Narok were diagnosed as brucellosis (tests were done using Rose Bengal Plate test).

Boiling of raw milk (alone or in tea) achieves higher temperatures and duration than those attained during pasteurisation. These conditions, like pasteurisation, destroy all zoonotic health hazards. Given the very high proportion of households that boil milk, the health risks from bacterial pathogens were determined to be very low. One area that requires attention is the consumption of traditionally fermented milk (*maziwa lala*), by 6% of households (mainly in rural areas) in this survey. This milk is often not boiled before fermentation, which lowers the pH of milk from about 6.8 to about 4.5. *Br. abortus* are only mildly affected by acidity at this level (Farrel, 1996). In a related investigation, Minja (1998) found that the low pH level in sour milk only destroyed *Mycobacterium bovis* after 66 hours. This would imply that home-made fermented milk could be a possible source of milk-borne infection to humans. It is note-worthy that the number of consumer households reporting a member having been affected by brucellosis was generally low. These households were in Nakuru rural sample area where more unboiled and/or home-made fermented milk is consumed. It is apparent that bulking of raw milk by large-scale raw milk market agents or failures in large-scale pasteurisation can increase risks of infection with brucellosis.

Acknowledgements

1. This research was funded by the Smallholder Dairy (R&D) Project (SDP) of the Kenya Ministry of Agriculture and Rural Development, KARI and ILRI. SDP is funded by the UK Department for International Development (DFID). The views expressed are those of the authors and not necessarily those of DFID or SDP.
2. Animal Diseases Research Institute, Ontario, Canada, (through Drs K. Neilsen and S. Tan) for kindly donating the indirect milk ELISA reagents.

References

1. Berman, D.T. (1981). Diseases of cattle in the tropics, economic and zoonotic relevance. In: Ristic, M., Mclntyre, I. (eds). Current topics in veterinary medicine and animal science Vol. 6. The Hague, Martinus Nijhoff. 271-286.
2. Blythman, I. G . and Forman, A. J. (1977). The use of preserved milk samples in the Brucella milk ring Test. *Aust. Vet. J.* 53 184 - 186
3. Cargill, C., Lee, K. and Clarke, I. 1985. Use of enzyme linked immnunosorbent assay in a bovine brucellosis eradication program. *Aust. Vet. J.* 62 49 –52
4. Chukwu, C.C. (1987). Brucellosis in Africa II. The importance. *Bull. Anim. Hlth Prod. Afr*. 35 92-98.
5. Farrell, I. D. (1996). Brucella. In Mackey and Carter, Practical Medical Microbiology, 14th edition, Eds; J.G. Coller, A.G. Fraser, B.P. Marimion and A. Simmons. Churchill Livingstone Publishers. pp 473 - 478.
6. Forchner, V. E. and Buenger, I. (1986). Detection of IBR/IPV, EBI and brucellosis antibodies in samples of bulk milk with ELISA using a single method for concentration of antibodies. *Dtsch. Tieraertzl. Wochensch*. 93: 112.
7. Hunter, D. and Allen, J. (1972). An evaluation of milk and blood tests used to diagnose brucellosis. *Vet. Rec*. 91: 310.
8. Kagumba, M. and Nandokha, E. (1978). Survey of the prevalence of bovine brucellosis in East Africa. *Bull. Anim. Hlth Prod. Afr*. 26: 224-229
9. Kadohira, M., McDermott J. J., Shoukri, M. M. and Kyule M. N. (1997). Variations in the prevalence of antibody to brucella infection in cattle by farm, area and district in Kenya. *Epidemiology and Infection* 118: 35-41.
10. Kerby, P. J., Quiroga, J. L., McGrane, J.J. and Stagg, D.D. (1997). Field evaluation of an indirect ELISA for detection of brucellosis in lowland Bolivia. *Trop. Anim. Hlth Prod.* 29: 65-72
11. Kerkhofs, P., Botton, Y., Thiange, P., Dekeyser, P., Limet, J. N. (1990). Diagnosis of bovine brucellosis by Enzyme immunoassay of milk. *Vet. Microbio.l* 24: 73-80.

12. MacMillan, A. (1990). Conventional serological tests. In: Animal Brucellosis. Eds Neilsen, K.H. and Duncan, J.R. CRC Press, Boca Raton, Florida

13. Minja, (1999). Occurrence and survival of Mycobacterium species in fermented milk from traditional cattle herds: A case study of Usangu Plains, Southern Highlands, Tanzania. *M.Sc. Thesis*. University of Agriculture, Sokoine. 91pp.

14. Muriuki, S. M., McDermott, J. J., Arimi, S. M., Mugambi, J. T. and Wamola, I. A. (1997). Criteria for better detection of brucellosis in the Narok District of Kenya. *East Africa Med. J*. 74: 317 – 320.

15. Neilsen, K., Smith, P., Gall, D., Perez, B., Cosma, C., Muller, P., Trottier, J., Cote, G., Boag, L. and Bosse, J. (1996). Development and validation of an indirect enzyme immunoassay for detection of antibody to *Brucela arbotus* in milk. *Vet. Microbiol*. 52:165-173.

16. Nicoletti, P. (1984). The control of brucellosis in tropical Africa and subtropical regions. *Prev. Vet. Med*. 2: 193-196.

17. Omore, A.O., Muriuki, H., Kenyanjui, M., Owango, M. and Staal, S. (1999). The Kenyan Dairy Sub-Sector: A Rapid Appraisal: Research Report of the MoA/KARI/ILRI Smallholder Dairy (R&D) Project. International Livestock Research Institute. Nairobi (Kenya).51pp

18. Savingy de, D. and Voller A. J. (1980). Communication of ELISA data from laboratory to clinicial use. *J. of Immunoassay* I 105-128.

19. Seifert, H.S.N, (1996). Brucellosis. Tropical Animal Health. Kluwer Academic Publishers. pp356-368.

20. Sutherland, S.S., Evans, R. J. and Bathgate, J. (1986). Application of an enzyme linked immunosorbent assay in the final stages of a bovine brucellosis eradication program. *Aust. Vet. J*. 63: 412-415

21. Turkson, P. K. and Boadu, D.Q. (1992). Epidemiology of bovine brucellosis in the Coastal Savanna zone of Ghana. *Acta.. Trop*. 52: 39-43

22. Wright, P. F., Kelly, W. A. and Gall, D.G. (1985). Application of a timing protocol to the reduction of interplate variability in the indirect enzyme immunoassay for detection of anti brucella antibody. *J. Immunoassay* 6: 189.