

Full Length Research Paper

Prevalence of *Brucella* antibodies in marketed cow milk in Benue State, Nigeria

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Brucellosis is an endemic disease in the animal population in Nigeria and of great public health importance, particularly among livestock owners and workers who are ignorant of the risk of *Brucella* infection. A total of 102 milk samples comprising 62 from cattle herds and 40 from milk vendors in Benue State, Nigeria were screened for *Brucella* antibodies by the milk ring test (MRT). The herds for the study were randomly selected within randomly selected local government areas from the three geopolitical zones of the state. All herds sampled had no history of vaccination and cows with mastitis were excluded. 17.7% of milk samples from herds and 12.5% samples from milk vendors were positive for *Brucella* antibodies. The overall prevalence was 15.7%. The result shows significantly ($P < 0.05$) higher prevalence in herds where fresh bulk herd milk was collected than those from milk vendors who sold fermented milk. However, prevalence within the geopolitical zones was not significantly different ($P > 0.05$). These findings are of public health significance as about 90% of the milk sold to the public within the study area is neither pasteurized nor boiled. Local milk producers and milk vendors should be educated on the importance of pasteurization or boiling of milk. Adequate pasteurization or boiling of milk before sale and consumption must be enforced. These regulations should be mandatory for milk intended for human consumption.

Key words: Prevalence, *Brucella* antibodies, unpasteurized milk, milk ring test, Benue State Nigeria.

INTRODUCTION

Brucellosis is a zoonosis caused by *Brucella* species. The disease in animals causes abortion, infertility, neonatal mortality and hygroma, and is spread by materials contaminated by body fluids including milk. In humans, brucellosis presents as a febrile flu-like illness and is common among pastoralists in Africa (Nicoletti, 1984; Chukwu, 1987a). It is therefore a disease of great

economic importance and public health significance.

Humans usually acquire brucellosis by consumption of raw milk and milk products such as cheese. Brucellosis is also recognized as an occupational hazard for livestock farmers, veterinarians, and workers in the meat industry within areas with enzootic *B. abortus*. Farmers and workers in the meat industry may contract brucellosis

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percutaneous, conjunctiva, or by nasal mucous membrane infection. Veterinarians may become infected when handling aborted fetuses or apparently healthy calves born to infected cows and by performing gynaecological and obstetric manipulations, or rectal examination of infected cattle (Schnurrenberger et al., 1975; Alausa, 1980). The most important means of humans acquiring brucellosis is through the consumption of raw milk. The prevention of brucellosis infection in humans is a major reason for the advocacy of milk pasteurization worldwide. The risk of infection by milk borne brucellosis is the reason for public health regulations which discourages the informal milk markets that sell unpasteurized milk. This is because of the risk it poses to human health. However, these regulations are not implemented in many developing countries including Nigeria. Over 90% of milk sales in Nigeria is in the hands of pastoral farmers who do not believe that milk could be a potential source of infection to humans and are not prepared to subject their milk to any form of treatment (Bertu et al., 2010). Despite the existence of regulations that require milk pasteurization, over 75% of the milk marketed in many developing countries is sold raw through local informal pathways (Staal et al., 2000). This is the scenario in Benue State, Nigeria as most of those involved in milk marketing are the Fulani milk maids and the milk is consumed raw or fermented as *fura* and *nono*. These informal milk markets thrive because they are immediate source of money and are of financial benefit to these pastoralists and milk agents.

Previously, only the Milk Ring Test (MRT), with a sensitivity of about 80% (Hunter and Allen, 1972) was available for detection of *Brucella* antibodies in fresh milk. A more accurate indirect Enzyme Linked Immunosorbent Assay (ELISA) with sensitivity = 95% and specificity = 99% for testing *Brucella* antibodies in milk has since been improved and validated (Kerkhofs et al., 1990; Nielsen et al., 1996). The milk ELISA is more sensitive than Rose Bengal test (RBT), MRT, and Complement Fixation Test (CFT) (Sutherland et al., 1986; Kerkhofs et al., 1990; Nielsen et al., 1996; Kerby et al., 1997) and reportedly able to detect antibodies in dilutions of up to 1:100. However, the MRT with a sensitivity of 87.5% and specificity of 98.6% (Bertu et al., 2010) was used in this study to determine the prevalence of *Brucella* antibodies in milk samples as the indirect milk ELISA kits were not readily available.

The MRT is cheap, easy, simple and quick to perform. It detects lacteal anti-*Brucella* immunoglobulin M (IgM) and IgA bound to milk fat globules. However, it tests false positive when milk that contains colostrums, milk at the end of the lactation period, milk from cows with mastitis are tested (Alton et al., 1988).

The risk to human health from these informal marketing of milk is the main objective of this study. This paper presents results of a study on the occurrence of *Brucella* antibodies in informally marketed milk in Benue State,

Nigeria and examines the health risks from consumption of raw or untreated milk.

MATERIALS AND METHODS

The study area

Benue State is one of the 36 States of the Federal Republic of Nigeria and it is referred to as the Food Basket of the Nation. It was created on February 3, 1976 with Makurdi as the capital city. The State has 23 Local Government Areas (LGAs) with a population of 4,219,244 (Daily Trust Newspaper, Wednesday, 10th January 2007). The state occupies a landmass of 30,955 square kilometres. The major ethnic groups in the state include Tiv, Idoma and Igede in that order (Benue State Diary, 2007). Benue State is located between latitudes 6°30'N and 8°10'N and between longitudes 7°30'E and 9°50'E. It is located in the middle belt of Nigeria and derives its name from River Benue which is the second largest river in Nigeria. The state lies in the transition belt between the tropical rain forest of Southern Nigeria and the open grassland savannah vegetation of Northern Nigeria. It is divided into three geopolitical zones A, B and C (Benue State Diary, 2007). Benue State experiences a typical tropical climate with two distinct seasons, the rainy season and the dry season. The rainy season lasts from April to October with annual rainfall in the range of 150-180 mm. The dry season begins in November and ends in March while mean monthly temperatures fluctuate between 23 and 30°C in the year.

Benue State is endowed with abundant agricultural resources. About 80% of the population depends on agriculture for their sustenance and livelihood. The state has favourable climatic conditions and fertile soils conducive for the rearing of animals and cultivation of virtually all crops grown in Nigeria. Most prominent among the animals reared are pigs, goats, poultry and cattle. Major crops cultivated include: yam, cassava, rice, soya beans, sesame, maize, citrus, mangoes, vegetables and sugarcane. The State has high migrant populations of nomadic Fulani pastoralists whose main occupation is raising cattle.

Sample collection

About 10 ml of bulk herd milk was collected immediately after milking from 62 randomly selected herds in nine randomly selected Local Government Areas in the three geopolitical zones of Benue State, Nigeria (3 LGAs from each geopolitical zone). None of the herds had record of vaccination against brucellosis. Also another 10 ml of milk from each milk seller (40 in total) at marketing points in the Local Government areas were collected (Table 1). Before samples were collected, the herdsman and milk vendors were asked whether they were aware of the need to boil or pasteurize fresh milk before marketing. The samples were collected into labelled 10 ml sterile plastic tubes with screw caps. The samples were then taken to the laboratory where they were held under refrigeration for 48-72 h and tested thereafter. The duration of sample collection was 12 months (April 2010 to March 2011).

Laboratory testing

The MRT works on the principles that lacteal antibodies essentially agglutinins to *Brucella* attach themselves to fat globules which rise to the surface of the milk and cluster at the cream layer. When haematoxylin stained *Brucella* antigen is added to the milk (Hunter and Allen, 1972), *Brucella* antibodies in the milk if present, form a complex. The complex attaches to the fat globules in the milk forming a blue ring at the cream layer leaving the normal white

Table 1. Summary of milk samples collected from herds and milk vendors for milk ring test.

Zone	LGA	No. of herds	No. of milk vendors
A	K/Ala	9	6
	Ukum	6	4
	V-ikya	4	3
Subtotal		19	13
B	Gboko	11	6
	Gwer East	5	4
	Makurdi	10	6
Subtotal		26	16
C	Ogbadibo	6	3
	Oju	3	2
	Otukpo	8	6
Subtotal		17	11
Grand Total		62	40

skimmed milk below, indicating a positive result. If *Brucella* antibodies are absent, the whole mixture remains homogeneously bluish-white, indicating a negative result.

Prior to testing of milk samples by the milk ring test, the pH for each sample was determined using a pH meter (Hanna Instruments USA, www.clarksonlab.com). The test was done using *Brucella abortus/melitensis/suis* Milk Ring Test (MRT) antigen from the Veterinary Laboratories Agency (VLA) – Weybridge, Surrey, UK as described by Alton et al. (1988). The test was carried out by pipetting 1 ml of milk sample to be tested (thoroughly mixed to disperse the cream evenly) into a 5 ml sterile glass tube. One drop (0.03 ml) of the ring test antigen was added and while closing the top of the tube with the index finger of gloved hands, mixed gently by shaking and inverting the tube several times. The index finger was rinsed and dried between samples. The mixture was allowed to stand for about a minute and examined to make sure that the antigen is thoroughly mixed with the milk. The mixture was then incubated at 37°C for 1 h. The test result was the read using uniform light source. If the intensity of the blue colour in the cream layer is deeper than the skim portion (forming a blue ring), the test was considered positive. If the intensity of the blue colour in the cream layer was less than in the skim portion, the test was considered negative.

Statistical analysis

Data were analyzed using SPSS version 19. Statistical analysis to test for associations between variables was done using the chi-square test and the test level of statistical significance was set at 5% (95% level of confidence, $P < 0.05$).

RESULTS

Of the 62 fresh bulk milk samples collected from herds and tested using MRT, 11 (17.74%) were positive for *Brucella* antibodies while 5(12.50%) of the 40 samples from milk vendors were positive to the MRT. This gives an overall prevalence of 15.69% (16/102) (Table 2).

There was significant difference ($\chi^2 = 0.51$; $P < 0.05$) in prevalence of *Brucella* antibodies in fresh bulk herd milk and milk from vendors. However, prevalence of *Brucella* antibodies in milk was not significantly different between the geopolitical zones of the state ($\chi^2 = 0.38$; $P > 0.05$).

The pH of fresh bulk herd milk samples ranged from 6.5 to 6.8 while that of sour (fermented) milk sold by vendors ranged from 4.5 to 4.8. Of the total 102 herdsmen and vendors interviewed, 72 (70.59%) agreed that they were aware of the need to boil or pasteurize milk before sale and consumption but emphasized that they do not do so to avoid additional costs and time. Only 30 (29.41%) respondents agreed that they usually boil fresh milk collected before sale, but revealed that boiling of a consignment of milk do not usually prevent them from adding fresh milk to it.

DISCUSSION

Of the 62 fresh milk samples from herds tested, 17.74% were positive for *Brucella* antibodies while 12.5% of the 40 milk samples collected from milk sellers (hawkers) tested positive to *Brucella* antibodies. This gave an overall prevalence of 15.69%. Since the herds sampled in the study were not vaccinated, the result is indicative of response to *Brucella* infection and not due to vaccination. Although the serological diagnosis of brucellosis requires the use of more than one test, other tests such as RBPT, SAT and CFT are used only for testing serum samples. The indirect milk ELISA (Kerkhofs et al., 1990; Nielsen et al., 1996) recommended by OIE (2011), could not be carried out due to non-availability of the test kits.

The result showed a significantly higher prevalence of *Brucella* antibodies in fresh milk samples (17.74%) than in milk from vendors (12.5%), which are mainly fermented milk. The overall prevalence of 15.69% agrees with 13.5% reported by Bertu et al. (2010) for Jos and environs and 18.61% reported by Cadmus et al. (2008) for trade cows at Bodija abattoir, Ibadan, South-western Nigeria. However, the result obtained was lower than the 25.5% milk seropositivity reported by Junaidu et al. (2011) from individual lactating cows in Sokoto State, Nigeria. Alton et al. (1988) stated that ring tests carried out on undiluted milk samples from individual cows may give false-positive results shortly after parturition, near the end of lactation, and when mastitis is present.

All the samples collected were from bulk herd milk from various herds and milk sellers each representing a herd. The prevalence obtained is therefore herd prevalence rather than individual animal prevalence. The infected animals serve as sources of infection to healthy animals within the herds as well as other neighbouring herds. This is because grazing of animals is unrestricted and contact between different herds is usual. The herdsmen and their families are also at risk of infection as they handle and milk these animals on daily basis. They can easily get

Table 2. Milk ring test results for *Brucella* antibodies in cattle in different areas of Benue State, Nigeria.

Zone	Area	No. of herds tested	MRT positive (%)	No. of vendor milk tested	MRT positive (%)	Zone Total MRT positive (%)
A	K-Ala	9	2(22.22)	6	1(16.67)	4(12.5)
	Ukum	6	0(00.00)	4	0(00.00)	
	V-ikya	4	1(33.33)	3	0(00.00)	
Subtotal		19	3	13	1	
B	Gboko	11	2(18.18)	6	1(16.67)	7(16.7)
	Gwer East	5	1(20.00)	4	0(00.00)	
	Makurdi	10	2(20.00)	6	1(16.67)	
Subtotal		26	5	16	2	
C	Ogbadibo	6	1(16.67)	3	1(33.33)	5(17.9)
	Oju	3	0(00.00)	2	0(00.00)	
	Otukpo	8	2(25.00)	6	1(16.67)	
Subtotal		17	3	11	2	
Grand Total		62	11(17.74)	40	5(12.5)	16(15.7)

Type of milk. $\chi^2=0.51$; $P<0.05$ Geopolitical zones (A, B and C). $\chi^2=0.38$; $P>0.05$.

infected because of close contact with the animals (Chukwu, 1987b). Megersa et al. (2011) stated that brucellosis in traditional livestock husbandry practices certainly poses a zoonotic risk to the public, in consequence of raw milk consumption, close contact with animals and provision of assistance during parturition. Adherence to traditional farming practices, preference for fresh dairy products and contact with animals have been reported to be risk factors for human exposure (Kassahun et al., 2006; Meko et al., 2007). Adesokan et al. (2013) in a survey for knowledge and practices related to bovine brucellosis transmission among livestock workers in South-western Nigeria revealed that consumption of unpasteurized milk, uncooked meat and its products, co-habitation with animals, and poor hygiene were significant risk practices in the transfer of *Brucella* infection from animals to humans among these workers. Previous reports indicated that about 90% of milk consumed in sub-Saharan Africa is raw or soured (Walshe et al., 1991; Mfinanga et al., 2003). The consumption of unpasteurized dairy products has been identified as a brucellosis risk factor for humans (Kang'ethe et al., 2000; Cadmus et al., 2008). Regassa et al. (2009) reported that 85.7% of cases of human brucellosis in Ethiopia resulted from the consumption of raw milk emphasizing the gravity of the problem.

Most of the milk sold to the public in major settlement areas of Benue State does not undergo any form of treatment such as pasteurization or boiling. The Fulani believe that milk is wholesome and could even be taken directly from the cow. Milk being sold by Fulani milk maids is either fresh or soured (fermented). This means that milk being consumed by the public in the study area is mostly not boiled and therefore a potential health risk

to the consumers. It is a point of public health concern that this work uncovered the unusual practice of adding fresh milk to one already boiled. This practice nullifies the importance and usefulness of boiling milk in the first instance and all efforts must be put in place through education to stop this practice.

Concern about human health risks from these market pathways needs to be addressed in the context of consumer practices such as boiling to reduce or eliminate potential infection by milk-borne pathogens without discouraging the markets through which the smallholders sell their milk (Kang'ethe et al., 2000). Boiling of raw milk achieves higher temperatures and duration than those attained through pasteurization (Kang'ethe et al., 2000). This means that if these milk sellers ensure regular boiling of milk before it is sold to the public, such milk could be said to be safe, as most infectious agents in the milk might have been destroyed during boiling.

In this work, the pH of fresh bulk herd milk ranged from 6.5 to 6.8 while that of fermented milk ranged from 4.5 to 4.8. There is the belief that traditionally fermented milk known as *nono* is safe since it has undergone fermentation. Fermentation (souring) only lowers the pH of milk from about 6.8 to about 4.5. Under this acidic pH, *Brucella* organisms are only mildly affected (Farrell, 1996). Minja (1999) found that the low pH level in sour milk only destroyed *Mycobacterium bovis* after 66 hours. This implies that homemade fermented milk could be a possible source of infection with brucellosis to humans. Although the herdsman, milk sellers and consumers do not believe that they could get infected by drinking raw milk or non boiled fermented milk, it is a major source of infection with brucellosis.

Despite the existence of pasteurization, most of the

milk marketed by traditional milk maids is raw and sold through informal channels. Boiling is not also considered a beneficial means of treating milk to make it safe for human consumption. Milk is a nutritious food for animals and humans and must be free of pathogenic organisms. The risk of infection by milk borne brucellosis is the reason for public health regulations. Adequate pasteurization or boiling of milk before consumption must be enforced. This is the only means by which milk can be made safe for human consumption. A prevalence of 25% in lactating cows was earlier reported in Nigeria by Junaidu et al. (2011) and 80.7% in Pakistan by Soomro (2006). This is of public health importance particularly for those Fulani observed to be drinking raw milk directly from the udder of the cow, since *B. abortus* has been isolated from raw and sour milk of Fulani cattle in Nigeria (Bale and Kumi-Diaka, 1981 and Eze, 1978). Brucellosis remains one of the most common zoonotic diseases worldwide with more than 500,000 human cases reported annually (Seleem et al., 2010). In this area, milk is usually preserved by souring, which does not destroy brucellae as they are preserved in milk fat (Eze, 1978). Unfortunately, many farmers do not take measures to protect them against brucellosis and are quite willing to drink unpasteurized milk. Infected farmers with symptoms of undulating fever and joint pains rarely seek medical help and when they do, the fever is usually ascribed to malaria or typhoid, therefore human brucellosis is likely to be greatly under diagnosed (Njoku, 1995; Mai et al., 2012). Although human brucellosis is essentially an occupational hazard, many people can become infected through the consumption of raw milk or milk products. Milk producers and the general public need to be educated on the danger of drinking raw milk. Adequate pasteurization or boiling of milk before sale and consumption must be enforced. Further work on the isolation and characterization of *Brucella* from marketed milk is suggested.

Conflict of interests

The authors did not declare any conflict of interest.

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