Bacteriological investigation of sheep and goats milk for brucellosis in government farms in Northern Nigeria

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Abstract

*Bacteriological investigations of sheep and goats brucellosis were carried out in Northern Nigeria. Seven government or quasi-government farms were investigated. A total of 418 sheep and goats milk samples were examined culturally. Investigation revealed a 34.75 per cent and 15.88 per cent rate of infection in goats and sheep by milk ring test. Of 277 sheep and 141 goats milk samples examined culturally, brucellae were isolated from four sheep and six goats giving a total of ten isolates. Biochemical and serological studies of the isolates revealed that they were *Br. melitensis* indicating *Br. melitensis* as probably the common cause of brucellosis in sheep and goats in the areas surveyed. The results show that there was greater chance of isolating *Brucella* from milk which were strongly positive than from those which were weakly positive. The economic importance and public health significance of brucellosis in sheep and goats are discussed.*

Keywords: Brucellosis, sheep, goats, Nigeria

Introduction

Brucellosis is widespread in all domestic animals. The clinical manifestations however are mostly frequent and economically important in cattle, sheep, goats and swine. In these animals it is a disease causing abortion, infertility and orchitis (Bruce, 1930; Boyd, 1950; Allsup, 1969). *Brucella melitensis* and *Br. abortus* are the etiological agents of brucellosis in sheep and goats (Nilakantan and Pande, 1948; Stoenner, 1951; Bruce, 1930; Meurou and Pinca, 1937). *Br. ovis* was also described as a specific species of *Brucella* whose host is sheep (Buddle, 1956; Philpott and Auko, 1971). The route of infection of brucellosis include the ingestion of contaminated feed or water; inhalation of dust contaminated with droplets or sometimes

through unbroken skin. The spread of the organism could occur by movement of infected animals or their products (FAO, 1964). The route of excretion of *Brucella* from sheep and goats include: milk (Nilakantan and Pande, 1948; Stoenner, 1951), semen (Anon, 1961; Cameron *et al.*, 1971), vaginal discharge (Polydorou, 1974) urine and other body fluids (FAO, 1964). Specimens from which *Brucella* can be isolated include: milk, urine, faeces, vaginal discharge, from lymph nodes such as - supramammary, submaxillary or retropharyngeal and internal iliac. Other organs include: uterus, spleen, aborted foetus and aborted foetal membranes (Alton *et al.*, 1975). Abortions, together with contagious caprine pleuropneumonia (CCPP), pneumoenteritis complex (CEP), helminthiasis and

*Deceased

nutritional problems, accounts for serious economic loss in sheep and goats population in Nigeria (Beaton, 1928; Beaton, 1931; Ojo, 1971; Schillhorn Van Veen, 1973; Akerejola *et al.*, 1979).

The presence of brucellosis in sheep and goats have been recognized since 1934 (Eze, 1977). Focal serological surveys showed that the incidence in sheep in Eastern Nigeria was 0.9% and in goats was 0.6 per cent (Kramer *et al.*, 1967). In Ibarapa division of Western State of Nigeria Falade *et al.*, (1974) reported that the incidence of brucellosis in goats was 8.9 per cent. Also in parts of Northern Nigeria Morrison *et al.*, (1975) showed that 1.8 per cent of sheep and 1.5 per cent goats surveyed had brucellosis. Northern Nigeria has long been the scene of animal movements between seasons of the year and as a source of animals to the southern parts of the country. Cultural study of *Brucella* organisms from sheep has not been undertaken. However, in a study of caprine brucellosis in southern part of Nigeria *Br. abortus* was isolated from one of 590 milk samples examined (Falade, 1978). It has not been possible so far to find out the distribution of brucellosis in sheep and goats since screening for the disease among the private farms, villages and the Fulanis covering the whole country is a gigantic task to perform.

There is paucity of literature on small ruminant brucellosis in Northern Nigeria where over 80 per cent of the country's sheep and goats reside. There are no reports of cultural studies to determine species and biotypes of *Brucella* in these animals. In view of this lack of reports in the literature regarding the isolation and biotypes, it becomes necessary to investigate in order to fill these existing gap. The investigations reported here are on the isolation and biotyping of *Brucella* spp in sheep and goats in the government farms in some parts of Northern Nigeria.

Materials and methods

Background of the farms

Flock No. I belongs to National Animal Production Research Institute (NAPRI), Shika, Zaria. The

breeds of sheep on the farm are Yankasa, Udah, Balami and crosses of the three. The breeds of goats are Sokoto red, Kano brown and their crosses.

The sheep and goats are for research purposes and they graze together.

Flock No. II is Goat Improvement Centre (GIC), Kukar Aljana. Sokoto red goats are predominant though there are few exotic breeds. History showed that there were no cases of abortions in this flock nor was any sheep grazing with the goats. Breeding is throughout the year. Crossbreeding with exotic breeds is also practiced.

Flock No. III is a Mutton Improvement Centre (MIC), Katsina (Gidan Kwakwa). It was established with a breeding policy of cross-breeding local sheep that is Balami, Udah and Yankasa with imported Merino and Wensleydale rams in order to produce dual-purpose breed for mutton and wool as secondary product for local cottage industries. There were cases of abortion on the farm.

Flock No. IV is a Goat Improvement Sub-centre (GIS), Rimi which is an extension of flock No. II. The flock consists of 130 goats which were made up of Sokoto red and Anglo-Nubian goats. There were crossbreeding between local and the Anglo-Nubian goats.

Flock No. V is a Federal Government Sheep Production Project Centre (FGSPPC), Tuma. The objective of the farm was to improve domestic production of mutton for meat and export purposes.

Improvement was to be accomplished through line breeding and cross-breeding among the three main indigenous sheep breeds - Yankasa, Udah, and Balami. The flock consists of 950 sheep consisting of Yankasa, Udah, Balami and crosses of the three different breeds.

Flock No. VI: This Rano farm stocks (RFS) exotic breed of sheep - fat tailed Sudanese sheep from Sudan. There were previous cases of abortion on the farm.

Flock No. VII. This is another Goat Improvement Centre (GIC) in Dangora. There are two breeds of goats stock in the farm namely: Kano red and Anglo-Nubian goats. There are crosses of the two

breeds also. Breeding is throughout the year.

Sources of specimens

The milk samples used for cultural examination were obtained from sheep and goats in the following government farms: NAPRI, Shika; GIC Kukar Aljana; MIC, Katsina; FGSPPC, Tuma; RFS Rano; GIS Rimi and Dangora. In each Government farm, milk samples were collected from lactating animals at various stages of lactation

Sampling technique

Milk samples were taken from an individual female sheep or goats on survey farms. Before taking a milk sample from an individual sheep or goat, the hands of the milker and the teats of the lactating animal were cleansed with clean warm water and dried with paper towel. The first few jets of milk from the animals were discarded, and a sample from both two halves was collected into a sterile 15-ml universal bottle, 0.5 ml of 1% w/v boric acid solution was added to each milk sample and samples packed inside ice-box and brought to the laboratory on the same day. All milk samples were identified, marked and stored overnight at 4°C when the tests were carried out.

Laboratory examination of samples

Milk ring test

The milk ring test (MRT) was carried out on each sample as follows: A milk sample was gently mixed by inverting the universal bottle used for collection.

One milliliter of milk was placed in a narrow Kahn test tubes to give a column of milk of about two centimeters high and 0.03ml of haematoxylin-stained brucella antigen batch 149/50 (Supplied by the Courtesy of the Director, CVL, Weybridge, U.K). The contents of the tubes were gently mixed within one minute of adding the antigen and read after incubating for one hour at 37°C. Positive reaction was indicated by a blue-coloured cream layer or button formation at the bottom of the tube. Reading of the milk ring test was according to standard technique of Morgan *et al.*, (1978).

Isolation and identification

Five millilitres of each milk samples were centrifuged in a screw capped centrifuge tubes at

3000 r.p.m for 15 minutes. The milk under the cream layer was poured off using a sterile applicator stick to pierce the cream layer. The cream was mixed with the sediment thoroughly and approximately 0.2 ml of the cream sediment mixture was inoculated into Brodie and Sinton's liquid medium (BS)(1975). All samples in BS liquid medium were numbered and incubated at 37°C in an atmosphere of 5 to 10 per cent added CO₂ for 5 days. About 0.2 ml from the broth was then plated onto Farrell's solid medium (FSM) (1974) and serum dextrose agar (SDA) and incubated under the same conditions.

Isolated bacteria on FSM and SDA were studied for typical colonies of *Brucella*. Colonies selected for further investigation include (a) the typical smooth colonies, convex in shape and often glistening surface; (b) opaque, off-white and granular colonies typical of the rough strains. Suspected brucellae were restreaked on fresh FSM and SDA for isolation in pure culture and maximum growth. Isolates were stained by Gram's and modified Ziehl-Neelsen stain and examined under microscope x40 and x100 (oil immersion) for staining reaction and cell morphology. Biochemical identification were carried out according to standard techniques (FAO/WHO, 1975; Corbel *et al.*, (1978). Tests included: catalase, oxidase, indole, nitrate, citrate, Voges-Proskauer, H₂S production, dye sensitivity and CO₂ requirements.

Phage typing: Phage typing procedure was done in accordance to Corbel *et al.*, (1978).

Results

A total of 418 samples of milk consisting of 277 from sheep and 141 from goats were tested with milk ring test (MRT) antigen for brucella agglutinin. The same samples were cultured for brucellae. The data on Table 1 show the results of the MRT. Forty four (15.88%) of the sheep and 49 (34.75%) of the goats were positive by the MRT

Table 1 Results of milk ring test on sheep and goats milk obtained from seven government farms

Source of milk	Total No examined	Number positive	Per cent positive	Milk	Ring	Test	Reading**	
				3+	2+	1+	±	-
Sheep	277	44	15.9	24	14	6	0	233
Goat	141	49	34.8	31	6	6	6	92

*Positive test from ± to +++

**Milk ring test reading according to standard technique (Morgan *et al.*, 1978).

Table 2 shows the relationship between the degree of MRT reaction and the presence of brucellae in the milk. Four brucellae were isolated from the sheep samples, three of these were from 24 samples which were strongly positive, and one from 14 moderately positive by MRT. No isolates were

obtained from weakly positive, suspicious or negative samples. In goats, six *Brucella* isolates were obtained, five from strongly positive and one from moderately positive samples. No isolates were obtained from weakly positive, suspicious or negative samples.

Table 2 The relationship between the degree of milk ring test reaction and cultural isolation of brucellae

Source of milk	Total No. examined	MRT positive	Milk		Ring	Test	Reading		and	Culture	Isolation	negative	Culture positive
			3+	Culture positive	2+	Culture positive	1+	Culture positive	±	Culture positive	Culture positive		
Sheep	277	44	24	3	14	1	6	0	0	0	0	233	0
Goats	141	49	31	5	6	1	6	0	6	0	0	92	0

Table 3 shows the flock strength and type of animals in the flock and the results of the MRT and cultural examination in the livestock improvement and breeding centres.

Table 3 *Survey of brucellosis in sheep and goats milk from various establishments using the milk ring test screening method*

No. of flock	L.I.B.C. location	Type of animal	Flock* strength	Cases of abortion	No. of goats sampled	No. of sheep sampled	MRT positive	% MRT positive	No. of milk culture positive
I	NAPRI Shika Zaria	Sheep	275	None	NA	94	14	14.9	0
		Goats	75	None	60	NA	24	40.9	0
II	Kukan Aljanan	Goats	321	None	59	NA	17	28.8	3
III	MIC Katsina	Sheep	391	30	NA	48	6	12.5	2
IV	GISC Rimi	Goats	130	None	10	NA	1	10.0	0
V	Tima	Sheep	950	None	NA	76	4	5.3	2
VI	Rano	Sheep	208	5	NA	59	20	33.9	0
VII	Dangora	Goats	116	None	12	NA	4	33.3	3
Total		Sheep/Goats	2,466	35	141	277	90	21.5	10

* The flock strength refers to the total animals on the farms which includes: rams, ewes, kids, lambs, nannies and doe.

NA = Not applicable.

The MRT results show that all the flocks surveyed were infected with brucellae. However, 10 isolates of the brucella were obtained from four of the seven centres and only two of these four centres had reported abortions in their animals previously.

The brucellae were typed, confirmed by biochemical and serological tests according to standard techniques (Alton *et al.*, 1975; Morgan *et al.*, 1978). The isolates obtained were similar to

those described by Henry (1933). In oblique light they appeared dry, granular and reddish-yellow to yellowish-white. They agglutinated in 0.1 per cent acriflavine in 0.15M NaCl at 80-100°C and by anti-rough monospccific antiserum. They were thus described as rough strain of *Br. melitensis*. Their biotypes could not be determined by phage typing because they were rough strains of *Brucella* spp. (Table 4).

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Table 4 *Biochemical reactions and typing of brucellae isolated from milk*

Strain Laboratory Number	Milk Source	Cata- lase	Oxid- ase	Indole	Methyl red	Voges Proskauer	Nitrate	Modified Ziehl- Neelsen Stain	CO ₂ reagent	H ₂ S reagent	Aggluti- nation in 0.1% acridavine antiserum	Agglutination with mono- specific antiserum	Initial phage lysis	Lipase activity	Growth on 1% w/v		
															Basic nutrient	Threonine	
A M R RTD 10 ⁴ RTD 1:25000 1:50000 1:100000 1:200000 1:400000																	
Dangara 5	Goat	+	-	-	-	-	Gm -ve cocci/bacilli	-	trace	-	-	+	NL	NL	-	-	+
Dangara 6	Goat	+	+	-	-	-	Gm -ve cocci/bacilli	-	trace	-	-	+	NL	NL	-	-	+
Dangara 9	Goat	+	-	-	-	-	Gm -ve cocci/bacilli	-	trace	-	-	+	NL	NL	+	+	+
MFC 18	Sheep	-	-	-	-	-	Gm -ve cocci/bacilli	-	-	+	-	+	NL	NL	-	+	+
Tuma 18	Sheep	-	+	-	-	-	Gm -ve cocci/bacilli	-	-	-	-	+	NL	NL	-	+	+
Tuma 39	Sheep	-	+	-	-	-	Gm -ve cocci/bacilli	-	-	+	-	+	NL	NL	-	+	+
Kukan Ajman 24	Goat	+	+	-	-	-	Gm -ve cocci/bacilli	-	-	-	-	-	NL	NL	trace	+	+
Kukan Ajman 48	Goat	+	+	-	-	-	Gm -ve cocci/bacilli	-	-	-	-	-	NL	NL	-	+	+

Positive +; Strongly positive ++; Negative -; No lysis NL; (from previous tests) -

Discussion

All the isolates obtained in this study were serologically, biochemically proved to be *Br. melitensis* but were the rough strains. Previous work by Alton (1960) on goats milk showed that there was a frequent occurrence of dissociated strains of *Br. melitensis* in the milk of goats in Malta. It was further observed that the crystal violet method of staining colonies does not reveal any differences in the degree of dissociation, and it is likely that many of the dissociated strains, were, in fact, intermediate forms. Polding (1939) also recorded some information about dissociated strains of *Br. melitensis* encountered in Malta. Milk from lactating animals is generally easier to obtain than blood specimens and in addition it can be used for cultural examinations from which *Brucella* can be obtained more easily than blood because of tissue predilection of the organism (Ebadi, 1971). The MRT is particularly valuable as a screening test on a herd or flock basis and in individual animals. Isolation of brucellae are more probable in strongly MRT-positive specimens as shown by this work.

Mathur (1967) showed that abortions and a positive MRT test were two very important indicators of brucellosis in a herd or flock. The "false-positive" reaction of MRT, that is, where MRT- positive failed to yield *Brucella* was most likely due to non-specific gammaglobulin but in which the antibody titre is usually low. Another situation where an animal could be MRT positive but culture negative is where there could be infection but without excretion of the organism in the milk at the time of examination, as *Brucella* is known to be excreted intermittently (Alton, 1960). The stage of lactation of many of the sheep and goats under study could not be determined precisely and this could have contributed to the high incidence of MRT-positive but culture negative samples in this study.

The figure obtained here however, still appear much higher than previous report in which 590 goat milk were examined and 288(48.9%) were positive by

MRT but only one isolate of *Br. abortus* was obtained from among the MRT positive milk samples (Falade, 1978).

This study has shown that brucellosis is present in our sheep and goats. It has not been possible so far to find out the actual incidence of brucellosis and the role of the disease in abortion and infertility in sheep and goats in Nigeria since screening for the disease among the private farms, pastoralist and among the households covering the whole country is rather difficult to perform. In addition, cases of abortion in sheep and goats are not reported by private farms, households generally and the pastoralists.

Infected goats are particularly dangerous source of brucellosis to man and other animals as the disease in these animals often assumes a chronic form without obvious symptoms. Infected sheep on the other hand do not constitute much threat as disease in sheep is often self-limiting. Brucellosis among goats and sheep in Nigeria has not received the attention that it deserves. They are important not from the point of human infection with *Br. melitensis* by drinking unpasteurised infected animal milk and milk by-products but also from the point of view of a possible danger of infection to other livestock and as an occupational hazard to man.

Conclusions

Control of the infection is definitely desirable in any infected area where practicable, but unfortunately in many area such as among the private farms, households and the pastoralists, if present control will be extremely difficult to carry out. Sanitary measures to prevent spread of infection from animal to animal and animal to man are of the greatest importance but, not easy to put into practice. Ideally all sheep and goats which are reactors should be slaughtered, but this is impossible with local people and the Fulanis with poor resource bases. Where possible such as Governmental and institutional farms, control of spread of infection should aim at

high level of environmental sanitation, separate room facilities for kids/lambs and early weaning of kids/lambs to a *Brucella*-free area. All government farms where they breed sheep and goats should screen all the animals against brucellosis twice every year with six monthly intervals. Sero-survey and milk culture with one month intervals except where there are no positive and no suspicious cases or from the animals of already infected farms. All reactor animals should be slaughtered and consumed after cooking except for the internal and genital organs which should be incinerated in cases of strong reactors. There should be proper disinfection of the infected premises and proper disposal of the aborted fetuses and placentas in cases of abortion.

All livestock farmers must be enlightened about disease reporting especially cases of abortions in order for investigation of abortions due to *Brucella* and other infectious agents.

They must be encouraged to utilize the diagnostic services provided at the government veterinary diagnostic centres and the Universities or Institutes. Area for future investigation should include cultural and serological studies in sheep and goats of brucellosis on a wider coverage of the country. The role of sheep and goats as a source of infection of human either through contact or their by-products should be investigated. This area will require a team work consisting of medical and veterinary personnel.

Mode of transmission of infection between large and small ruminants need to be study. We should also investigate brucellosis serologically and bacteriologically in other animals with particular emphasis on pigs, dogs, horses, donkeys and camels.

One of the obstacles to livestock improvement is the lack of exact knowledge of Nigerian livestock population. The official figures in the past are usually arrived at as a result of calculation based on the payment of cattle tax, which the bulk of herd owners tend to dodge. Information is obtained mainly from restricted sample surveys, which also

suffers from large margins of error. The direct collection of population data is often difficult: the nomadic nature of the large proportion of livestock owners, the widespread illiteracy among them, poor communications, the serious shortage of finance and of qualified staff all add to the difficulties in the way of collecting accurate population data of cattle, sheep and goats. All these must be addressed for us to move on to develop livestock sub-sector of agriculture.

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