ANIMAL BRUCELLOSIS IN THE NORTH EASTERN PROVINCE OF KENYA

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D E C L A R A T I O N

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SUMMARY

Brucellosis is an important worldwide zoonosis which causes abortions and infertility in animals and a debilitating disease in humans. The disease has a wide host range comprising humans, domestic and wild animals. The animal world acts as the reservoir for human infection. Brucella abortus, Brucella melitensis, Brucella suis, Brucella ovis and Brucella canis are important pathogens while Brucella neotomae has not been associated with any specific disease.

Animal brucellosis is widely distributed in Kenya with the highest incidence occuring in areas where extensive animal husbandry is practised and animal populations are high such as North Eastern Province, Masailand, Nyanza Province and Eastern Province. Reports of Brucella isolations are few with only three reports from North Eastern Province. The few serological surveys carried out in North Eastern Province have been limited utilizing one test at a time and one or two animal species. None of these investigations included camels which are an important source of meat and milk besides being an important means of transport in the province. Recently, clinical cases of brucellosis in

both humans and animals have been reported by the veterinary and medical authorities in the province (Fazil, personal communication, 1975).

A serological survey of cattle, sheep, goats and camels has been done to get an indication of the prevalence of brucellosis in the area. Seven hundred serum samples comprising of 174 camel sera, 220 bovine sera, 145 caprine sera and 161 ovine sera were analysed for Brucella antibodies using Rose bengal plate test (RBPT), serum agglutination test (SAT) and the complement fixation test (CFT). A high reactor rate was found in all the species tested. SAT and CFT reactors occured in approximately equal proportions in RBPT positive and RBPT negative sera. SAT and CFT were found to pick more positives than RBPT in camels. There was no significant difference between SAT and CFT. RBPT was found to pick more positives than SAT and CFT in bovine, and caprine sera. In ovine sera, RBPT and SAT were found to pick more positives than CFT with no significant difference between RBPT and SAT. This is the first brucellosis investigation to include camels in Kenya and a close relationship has been found between

the reactor rate in camels and that found in cattle, sheep, and goats.

In this study a high reactor rate was found. A study of the literature has shown the epidemiological significance of wild animals especially those of the two families - Bovidae and Carnivora in brucellosis. Interspecies transmission has been reported (FAO/WHO Brucellosis Committee Report, 1971; Salem, 1975). Due to the above reasons it is suggested that any meaningful control and/or eradication programme must involve all the four domestic species and the main wildlife species. It is recommended that a study should be undertaken to establish whether Brucella abortus S19 vaccine might be useful in camels in view of the importance of these animals for food and transport and epidemiology of brucellosis in North Eastern Province.

1. INTRODUCTION

Brucellosis is a worldwide zoonosis which causes abortions and infertility in animals and a debilitating disease in humans. The disease is of public health significance because it can directly or indirectly be transmitted from infected animals to man with consequent illness and prolonged incapacity. It is also important in animal production because it causes serious losses which arise from abortion, premature birth, infertility and decreased milk yield.

Brucella abortus, *Brucella melitensis*, *Brucella suis*, *Brucella ovis* and *Brucella canis* are the important pathogens while *Brucella neotomae* has not been associated with any specific disease. To date the only *Brucella* species isolated from animals in Kenya have been *Br. abortus* biotypes 1 and 3 from cattle (Vet. Dept. Annual Report, 1934), *Br. ovis* from rams (Vet. Dept. Annual Report, 1961; Cameron *et al.*, 1971), *Br. suis* type 3 from rodents (Heisch *et al.*, 1963) and recently *Br. melitensis* from goats (Philpott and Auko, 1972). According to Annual Reports, Veterinary Research Laboratory, Kabete, in Kenya since 1968, Br. abortus biotype 3 has been the one most commonly isolated followed by *Br. abortus* biotype 1, though *Br. abortus* biotype 9 has also been isolated. *Br. melitensis* biotype 1 has been the one most commonly isolated from sheep and goats (Table 1).

A serological survey by Wegener (1968 - 1969) using SAT indicated that Kenya can be roughly divided into an area of low prevalence covering areas along both sides of the Mombasa to Kisumu Road and an area of high prevalence along both sides of the remaining parts of Kenya (Tables 2 and 3). Average positive rates of 6.8% in cattle, 0.9% in sheep, 0.57% in goats and about 4% in wildlife were found. Nagy and Sorheim (1969) in a survey on cattle sera from the Kenya Meat Commission abbatoir at Athi River used the CFT and found that the highest reactor rate was in cattle from Nyanza Province (15.78%) whereas the lowest occured in cattle from Central Province (1.67%). From field reports, the highest percentage of reactors in sheep and goats come from Meru District and Athi River in Eastern Province (Table 4). Of 1091 farms of problem herds tested for brucellosis in the period 1969 - 1973 at the Veterinary Research Laboratory, Kabete, 25% of the farms were found positive (Table 5). Waghela and Gathuma (1975) found a low prevalence of swine brucellosis in Kenya.

Table 1: Brucella Species and Biotypes Isolated in Kenya Since 1968

Place of Isolation	Province	Isolated from	Species and Biotypes	
Njoro	Rift Valley	Bovine fetus	Br.abortus biotype	1
"	"	"	"	3
Eldoret	"	"	"	1
"	"	"	"	3
Kitale	"	"	"	9
Ol Kalou	Central	"	"	1
T. Falls	Rift Valley	"	"	3
Kiboko	Eastern	"	"	3
"	"	"	"	3
Wajir	North Eastern	Bovine Semen	"	3
Nanyuki	Rift Valley	Bovine fetus	Br.melitensis biotype	1
Marsabit	Eastern	Caprine Cervical Swab.	"	1
"	"	"	"	3
Narok	Rift Valley	"	"	?
"	"	"	"	1
Mandera	North Eastern	Caprine Semen	"	2+
Wajir	"	Caprine Milk	"	1

+ Not confirmed.

Table 2: Results of Serum Agglutination Test in Kenya Cattle Examined at Random for Brucellosis (Wegener, 1968 - 1969).

Area	Number	Positive	Percentage
Kiambu, Thika	208	5	2.4
Ngong, Kajiado	557	22	3.9
Nairobi	667	74	11.1
Meru, Embu, Machakos	665	32	4.9
Taita, Kwale, Tana River, Lamu, Kilifi, Mombasa	690	74	10.7
Thomson Falls, Nyeri	43	9	21
Marsabit, Isiolo	496	23	4.6
Mandera, Garissa, Wajir	962	86	8.9
Turkana, Samburu	682	28	4.1
Kisumu	185	0	0
Overall	5158	353	6.8

Source: Waghela (1975a)

Table 3: Results of SAT in other animal species from Kenya examined for Brucellosis (Wegener 1968 - 1969).

Animal Species	Area	Number	Positive	Percentage
Goats	Narok Machakos Baringo Marsabit	705	4	0.57
Sheep	Narok Machakos Baringo Marsabit	655	6	0.9
Game	Kenya	381	9	2.3
Baboons	Aberdare	112	7	6.3

Source: Waghela (1975a)

Table 4: Field Reports Br. melitensis infection in Sheep and Goats

Location of Farm, i.e. Province	No. of Sera Tested	Percent Reactors	Other Details
Thika (Central)	215	19.0	Br. melitensis biotype 2 Isolated from Semen of a billy goat with Epididymitis Abortions
Athi River (Eastern)	127	49.6	Abortion outbreak Br. melitensis biotype 1 from milk - One human affected.
Machakos (Eastern)	822	4.7	A few abortions
Kiboko (Eastern)	644	3.0	Occasional abortions
Meru (Eastern)	568	40.0	Abortions, mastitis and infertility.

Source: Waghela (1975a)

Table 5: Percentage of Positive Farms on Routine Serological Testing of Problem Herds from 1969 - 1973.

Year	No. of farms tested	Percentage Positive
1969	299	23.4
1970	226	24.3
1971	153	28.7
1972	216	20.8
1973	197	29.9
Total	1091	Average 25.0

Source: Waghela (1975a)

The North Eastern Province is comprised of Garissa, Wajir and Mandera districts, but before the Boundaries Review Commission of 1968 the province used to be called the Northern Frontier Province and comprised of Garissa, Wajir, Mandera, Marsabit, Samburu, and Isiolo districts (National Atlas of Kenya, 1970). The province has a very large livestock population. Spinks (1956) quoted in Morgan and Shaffer (1966) estimated the livestock population of the province as consisting of 433,000 cattle, 775,000 camels, 738,000 goats, and 325,000 sheep. The East African Livestock Survey (1967) gave an estimate of 470,000 head of cattle while the most elaborate and recent survey by Watson (1972) estimated the province has 597,000 cattle with a standard error of $\pm$ 45,500, 176,000 camels with a standard error of $\pm$ 9,000, 316,704 goats and 74,601 sheep.

From the review of literature there are only a few reports of brucellosis investigations in North Eastern Province. A reactor rate of 5.75% was found in a serological survey of 2336 cattle from Northern Frontier Province (Dept. Vet. Serv. Annual Report, 1947). A high positive rate of 70% was found by Cooke (1956) who tested 632 serum samples of sheep and goats from Northern Frontier Province. Wegener (1968 - 1969) using SAT found 8.9% positives out of 962 cattle from the three districts of North Eastern Province. Philpott and Auko (1972) using SAT found

two goats out of 89 goats from Garissa to give positive reactions. Oomen and Wegener (1974) reported that a high frequency of brucellosis is found in semi-arid areas such as North Eastern Province and Masailand where extensive cattle husbandry is practised and the cattle population is large. In North Eastern Province since 1968 (Table 1), Br. melitensis biotype 2 has been isolated from caprine semen from Mandera, Br. melitensis biotype 1 from caprine milk from Wajir and Br. abortus biotype 3 from bovine semen from Wajir.

Therefore, very little has been done on animal brucellosis in North Eastern Province. Wegener's survey (1968-1969) is limited in that it deals with only one species and applies only one test. With the known limitations of the various serological tests for brucellosis, information obtained by use of only one test especially SAT, whose relative value as a diagnostic test compared to the other tests is only 61%, is of limited accuracy. The survey quoted in (Dept. Vet. Serv. Annual Report, 1947) again deals with only one species. The percentage positives found by Cooke (1956) who surveyed goats and sheep only seems to be very high. The method of expressing infection rate by the percentage positives is not a true indication of the infection rate since according to the recommendation of FAO/WHO Brucellosis Committee (1971), animals showing suspicious

titres should also be regarded as infected. In each case whether the animal is showing a positive titre or suspicious titre, the ultimate indicator of infection is isolation of Brucella organisms. Finally, the investigation quoted in (Dept. Vet. Serv. Annual Report, 1947) and Cooke (1956) covered the Northern Frontier Province which is a much larger area than the North Eastern Province. Wegener (1968-1969), Philpott and Auko (1972), Oomen and Wegener (1974) and Veterinary Research Laboratory, Kabete, are the only ones who have reported on brucellosis in North Eastern Province.

Because of the large domestic animal population in the North Eastern Province (Spinks, 1956 quoted in Morgan and Shaffer, 1966; East African Livestock Survey, 1967; Watson, 1972), no one survey that has been done so far can be said to be extensive or comprehensive. In view of this, and also due to reports of clinical cases of brucellosis in humans and animals by veterinary and medical authorities in the province (Fazil, personal communication, 1975) it has been deemed necessary to carry out further serological survey work and to include camels which have not been studied in any previous serological and/or bacteriological survey.

2. REVIEW OF LITERATURE

2:1. History:

The first report on brucellosis in man in East Africa was in 1910 when a disease called "Muhinyo" in Uganda was found to be caused by a *Brucella* orgnism (Abstract, 1910). Human *Brucella* infections in Kenya were reported in 1953 by Wright *et al.*, who reviewed 70 cases of patients seen over a period of 10 years. Manson-Bahr (1956) called attention to a specific clinical entity while Cox (1966, 1968) observed many cases in the northern areas of Kenya.

Brucellosis in cattle was first reported in Kenya in 1914 (Dept. of Agric. Annual Report, 1914). An increasing number of cases were recorded after 1922 when six outbreaks of the disease involving 25 animals were reported (Dept. of Agric. Annual Report, 1922). A limited stock survey carried out in 1923 showed a widespread incidence of brucellosis in the country (Dept. of Agric. Annual Report, 1923). The disease was assumed to be endemic in Boran cattle from Northern Frontier Province after a serological survey showed a reactor rate of 5.75% in 2336 animals (Dept. Vet. Serv. Annual Report, 1947). A milk ring test (MRT) survey carried out at Creamery Branches of Kenya Co-operative Creameries (KCC) revealed on overall herd infection

rate of 19% (Dept. Vet. Serv. Annual Report, 1955). Four serological tests were performed by Nagy and Sorheim (1969) on sera obtained from cattle at the Kenya Meat Commission, Athi River. The highest reactor rate using the complement fixation test was found in cattle from Nyanza Province (15.78%) whereas the lowest occured in cattle from Central Province (1.67%). A serological survey by Wegener (1968-1969) indicated that Kenya can be roughly divided into an area of low prevalence, covering areas both sides of the Mombasa-Kisumu Road and an area of high prevalence along both sides of the remaining parts of Kenya. Oomen and Wegener (1974) reported that a high frequency of brucellosis is found in semi-arid areas such as the North Eastern Province and Masailand where extensive cattle husbandry is practised and the cattle population is large.

In Kenya, Br. melitensis has been isolated more frequently than Br. abortus as the etiological agent of human brucellosis (Wright et al., 1953; Manson-Bahr, 1956; Cox, 1966). It was not till 1972 when Philpott and Auko reported abortion in goats in East Africa caused by Br. melitensis. A limited survey and other field evidence suggested that caprine brucellosis is common in certain areas of Kenya. The incidence of Br. melitensis infection in goats has been increasingly

reported by the Veterinary Research Laboratory, Annual Reports, 1971 - 1974. The only other Brucella species isolated from animals in Kenya have been Br. abortus biotypes 1 and 3 (Vet. Dept. Annual Report, 1934), Br. ovis from rams (Vet. Dept. Annual Report, 1961; Cameron et al., 1971) and Br. suis type 3 from rodents (Heisch et al., 1963).

2:2. Microbiology of the Genus Brucella:

2:2.1.: Definition.

Brucellae are Gram-negative coccobacilli or short rods, 0.5-0.7 u by 0.6 - 1.5 u, arranged singly and rarely in short chains. They have no capsules, are non-motile, non-spore forming and do not show bipolar staining. They are strict aerobes, but some require 5-10% added carbon dioxide (CO_2) for growth especially on initial isolation; temperature range for growth is 20 to 40°C. and pH range is 6.6 to 7.4. Though they are strictly aerobic, they can also grow anaerobically when nitrate is present as an electron acceptor (Bergey's Manual, 8th Edition, 1974).

2:2.2. Classification.

The Brucella Subcommittee of the International Committee on Bacterial Nomenclature (Jones, 1967) recommends that 3 species be recognised, namely Br. abortus, Br. melitensis and Br. suis. Within these species are 3 biotypes of Br. melitensis, 9 of Br. abortus and 5 of Br. suis. The differential tests are based on cultural, biochemical, serological and bacteriophage typing properties.

Br. abortus, Br. melitensis, and Br. suis form the so-called "classical" species of Brucella. Recently three new species have been added to the "classical" ones, namely, Br. neotomae (Stoenner and Lackmann, 1957), Br. canis (Carmichael and Brunner, 1968) and Br. ovis (Buddle, 1956; Buddle and Boyes, 1953). Br. ovis differs from other species in its utilization of amino acid substrates and oxidation of carbohydrates (Meyer, 1969b). Its possession of much the same polynucleotide sequences as Brucella (Hoyer and McCullough, 1968b) justify its being placed in the Brucella group.

Br. neotomae conforms so closely to the general characteristics of the Brucella group that the Sub-committee agreed to regard it as a separate and valid species. Br. canis differs from the rest in more particulars. It uses neither glucose nor erythritol,

it does not reduce nitrates to nitrites (Meyer, 1969b) and it is antigenically rough. On the other hand, its water soluble antigens show a close similarity to those of Br. melitensis (Diaz et al., 1967) and it has the same polynucleotide sequences as other members of the genus Brucella (Hoyer and McCullough, 1968a). On the whole it seems best to include it in the Brucella group and recognize it as a distinct species.

Paramelitensis, parasuis and paraabortus once used to be regarded as distinct species but are now known to be rough variants of the original smooth forms. They are therefore called rough Br. melitensis, rough Br. suis and rough Br. abortus strains, respectively.

Isolations of previously undescribed Brucella organisms from new hosts led to speculations on the mutability of the Brucella species (Roux and Bouvier, 1946; Jacotot and Vallee, 1951; Bouvier et al., 1954; Nyiredy, 1954; Niznasky et al., 1956; Tudoriu et al., 1957; Tudoriu et al., 1958; Karsten, 1959; Karsten, 1960). However it has not been established that Brucella organisms mutate from one biotype to another within the same species, let alone from one species to another (Meyer, 1976).

Tables 6 and 7 show the characteristics of Brucella species and biotypes. The World Health Organisation (WHO) reference strains of the four main species are designated Br. abortus strain 544, Br. suis strain 1330, Br. melitensis

Table 6: General differential characters of Brucella species and biotypes

Species	Biotype	Lysis by Phage RTD	Lysis by Phage 10^4xRTD	CO_2 Required	H_2S Produced	Growth on dyes (a*) Basic Fuchsin II	Basic Fuchsin III	Thionin I	Thionin II	Thionin III	Agglutination by monospecific sera (b*) A	Agglutination by monospecific sera (b*) M	Anti-rough serum	Most common host reservoir
Br. melitensis	1	-	-	-	-	+	+	-	+	+	-	+	-	sheep, goats
	2	-	-	-	-	+	+	-	+	+	+	-	-	" "
	3	-	-	-	-	+	+	-	+	+	+	+	-	" "
Br. abortus	1			±	+	+	+	-	-	-	+	-	-	cattle
	2	+	+	+	+	-	-	-	-	-	+	-	-	"
	3	+	+	±	+	+	+	+	+	+	+	-	-	"
	4	+	+	±	+	+	+	-	-	-	-	+	-	"
	5	+	+	-	-	+	+	-	+	+	-	+	-	"
	6	+	+	-	±	+	+	-	+	+	+	-	-	"

Table 6 (Contd)

Species	Biotype	Lysis by Phage RTD	Lysis by Phage 10^4xRTD	CO_2 Required	H_2S Produced	Growth on dyes (a*) Basic Fuchsin II	Basic Fuchsin III	Thionin I	Thionin II	Thionin III	Agglutination by monospecific sera (b*) A	Agglutination by monospecific sera (b*) M	Anti-rough serum	Most common host reservoir
Br. abortus	7	+	+	-	±	+	+	-	+	+	+	+	-	cattle
	8	+	+	+	-	+	+	-	+	+	-	+	-	"
	9	+	+	±	+	+	+	-	+	+	-	+	-	"
Br.suis	1	-	+	-	+	-	-	+	+	+	+	-	-	Pigs
	2	-	+	-	-	-	-	-	+	+	+	-	-	Pigs, hares
	3	-	+	-	-	+	+	+	+	+	+	-	-	Pigs
	4	-	+	-	-	+	+	+	+	+	+	+	-	Reindeer

Table 6 (Contd)

Species	Biotype	Lysis by Phage RTD	Lysis by Phage 10^4xRTD	CO_2 Required	H_2S Produced	Growth on dyes (a*) Basic Fuchsin II	Basic Fuchsin III	Thionin I	Thionin II	Thionin III	Agglutination by monospecific sera (b*) A	Agglutination by monospecific sera (b*) M	Anti-rough serum	Most common host reservoir
Br. neotomae		-	+	-	+	-	-	-	-	+	+	-	-	Wood rat
Br. ovis		-	-	+	-	+	+	+	+	+	-	-	+	Sheep (rams)
Br. canis		-	-	-	-	-	-	+	+	+	-	-	+	Dogs

a* = Species differentiation is obtained on Albimi or tryptose agar with the following concentrations of dyes: 1:25,000 (I), 1:50,000 (II), 1:100,000 (III). Other concentrations may be preferrable with other growth media. Interpretation of results should be controlled with the reference strains of each species.

b* = A = abortus, M = melitensis.

Source: Joint FAO/WHO Report - Brucellosis - 5th Report (1971).

Table 7: Utilization pattern of 12 recommended a* substrates by species and biotypes of the Genus Brucella.

	Amino acids								Carbohydrates			
	D-alanine	L-alanine	L-asparagine	L-glutamate	DL-ornithine	DL-citrulline	L-arginine	L-lysine	L-arabinose	D-galactose	D-ribose	D-xylose
Br. abortus types 1-9	+	+	+	+	-	-	-	-	+	+	+	+-
Br. melitensis types 1-3	+	+	+	+	-	-	-	-	-	-	-	-
Br. suis type 1	+-	+-	-	-	+	+	+	+	+	+	+	+
Br. suis type 2	-	-	+-	+	+	+	+	-	+	+-	+	+
Br. suis type 3	+	+-	-	+-	+	+	+	+	-	-	+	+-
Br. suis type 4 type 4 b*	+	-	-	+-	+	+	+	+	-	-	+	-
Br. neotomae	+	+-	+	+	-	-	-	+	+	+	+-	+-
Br. ovis	+	+-	+	+	-	-	-	-	-	-	-	-
Br. canis	+	+-	-	+	+	+	+	+	+-	+-	+	-
Br. suis type 5 b*	+		+	+	+	+	+	+	-	+-	+	+

Table 7: (Contd.)

Key: + = Oxidised (QO_2N value greater than 50) by all strains.
- = Not oxidised by any strain.
± = Oxidised by some strains.
a* = Meyer, M.E. (1969), Amer. J. Vet. Res., 30, 1751.
b* = Br. suis type 5 described by Renoux, G. and Philippon, A. (1969), Ann. Inst. Pasteur, 117, 5241.

Source: Joint FAO/WHO - Brucellosis - 5th Report, 1971.

2:2.3. Culture of Brucella Organisms.

Solid media are preferred for culturing as they limit establishment of non-smooth strains and facilitate isolation and recognition of growing colonies and variants. Some strains grow better in the presence of serum or Tween 40. Serum dextrose agar or Tween dextrose agar are therefore recommended (Alton and Jones, 1967). Addition of antibiotics or ethyl violet suppresses contamination but delays growth of Brucella on selective media. Castaneda (1947) recommended the use of a combination of a solid and liquid medium. The liquid medium contains sodium citrate and is able to support growth from a considerable amount of blood. Subcultures from liquid to solid media can be simplified and performed merely by turning the bottle so that the solid media is exposed. Incubation should occur within 35 days as the development of the colonies may be delayed. Ten percent CO_2 should be added to the culture atmosphere.

2:2.4. Identification of Brucella Organisms.

This is done from colonial and cellular morphology and confirmed by an agglutination test.

Brucella colonies are usually visible by the 3rd or 7th day of inoculation. They are 2 to 3mm. diameter, convex with round margins. They are yellow in color and translucent. The colonial morphology can

be observed using the following methods:-
Direct observation (Henry, 1933) and Acriflavine test (Braun and Bonestiell, 1947). The latter should always be used to confirm the results of direct observation. Acriflavine should be used at a concentration of 1:1,000. The Crystal Violet staining method of White and Wilson (1951) is especially valuable when applied to plates with discrete colonies.

Microscopically, the Brucella organisms are coccobacilli, 0.6-1.5 u by 0.5-0.7 u, usually arranged singly. They are non-motile, non-sporeforming and do not have a capsule. The cellular morphology is demonstrated by using mainly modified Ziehl Neelsen method as given by Stamp et al. (1950).

Identification of the smooth Brucella organisms is confirmed by agglutination of a suspected colony with monospecific antiserum. Dissociated cultures agglutinate poorly or not at all while rough cultures agglutinate spontaneously.

2:2.5. Additional Biochemical Characteristics.

The Brucella organisms have the following additional biochemical characteristics:- They are catalase positive, oxidase positive but Br. neotomae and Br. ovis are oxidase negative. Urea is hydrolysed to a variable extent; nitrate is reduced to nitrite

(except for Br. ovis). Citrate is not utilised and indole is not produced. Methyl Red and Voges Proskauer tests are negative and there is no change in litmus milk. Br. ovis is unique among brucellae in that it does not oxidize either glucose or i-erythritol. Br. melitensis will not ferment inositol, maltose, mannose, rhamnose, or trehalose; Br. abortus will ferment inositol, rhamnose; Br. suis will ferment maltose, mannose and trehalose (Burnett and Schuster, 1974).

2:2.6. Typing of Brucella Organisms

a) Use of Conventional Tests (Huddleson, 1929; Wilson and Miles, 1932).

The requirement for added CO_2 for growth, production of hydrogen sulphide (H_2S) and differential growth on media containing basic fuchsin and thionin are the main tests used. Appropriate known reference cultures should always be included as controls. The urease test is useful for rapid identification of Br. suis. Almost all Brucella species have urease activity but Br. suis strains are able to split urea at the fastest rate. In combination with agglutination with monospecific sera conventional tests are adequate for differentiating all biotypes of the different species with the exception of Br. melitensis biotype 1 and Br. abortus biotype 5.

b) Lysis by Brucella Bacteriophage

Using the Tb phage (Morgan et al., 1960; Jones, 1960; Meyer, 1961; Ostrovskaya and Kaitmasova, 1966) will differentiate all Br. abortus biotypes from the biotypes of other species. Tb phage is used as a reference phage.

c) Manometric Techniques

These are used to measure the oxidative metabolic utilization of amino acids and carbohydrates by aberrant strains (Pickett and Nelson, 1955; Meyer, 1964; Meyer, 1966; Meyer and Cameron, 1961; Stableforth and Jones, 1963). Tables 6 and 7 show the differential characteristics used for typing Brucella species and biotypes.

2:2.7. Brucella Antigens

Brucella organisms have a complex antigenic structure. There are surface antigens and antigens in deeper structures. There is at least one antigen common to all rough strains of Brucella species (Wilson and Miles, 1932). Antigen M in smooth Br. melitensis and antigen A in smooth Br. abortus are specific antigens differentiating the two species. A non-specific group (G) antigen is present in all Brucella species (Olitzki and Gurevitch, 1953). Wilson and Miles (1932)

concluded that antigens A and M are present in different quantitative ratios in smooth Br. abortus, Br. melitensis and Br. suis. By optimal proportion in agglutination tests, Miles, (1939) found Br. abortus has A:M ratio 20:1 whereas in Br. melitensis it was 1:20.

A more detailed antigenic analysis of the genus Brucella was presented by Renoux and Mahaffey (1955). The smooth cultures of the three species, Br. abortus, Br. suis and Br. melitensis have the antigens A, M, Z and R in different quantitative distributions. Br. ovis contains only R and Z and the rough cultures of all species contain only R with or without the Z antigens. Alton, (1960) assumed that Br. abortus had antigens Am and Br. melitensis antigens Ma where capital letters represent the major antigen and the small letters the minor antigen; similarly the rough cultures had the antigenic structure Rm or Ra depending on the species of Brucella.

The surface antigens of Br. ovis and Br. canis and rough strains of Br. abortus and Br. melitensis are similar but not identical. Little antigenic relationship is seen between the surface antigens of Br. ovis, Br. canis, and smooth Br. abortus or Br. melitensis (Diaz et al., 1968). Cross-absorption of Br. neotomae, Br. abortus and Br. melitensis antisera with the three antigens revealed Br. neotomae and Br. abortus to be identical (Stoenner and Lackmann, 1957).

The above together with the findings of Miles (1939) suggest that the minor antibody component of the antiserum can be absorbed leaving most of the major antibody component. This procedure yields monospecific reagents for identification of Brucella bacteria. The use of quantitative agglutination and absorption tests allow the differentiation between Br. melitensis on one hand and Br. abortus and Br. suis on the other, if they are in smooth phase. Also these procedures help in identifying the rough strains from the smooth ones (Dept. Vet. Serv., 1971).

It was recently established that the antigenic determinants used for serological diagnosis of bacteria belonging to the Brucella and Yersinia species are found in the lipopolysaccharide of the cell envelope (Hurvell and Lindberg, 1973; Burnett and Schuster, 1974). The discovery of a serological cross-reactivity between Br. abortus and Yersinia enterocolitica O-group V (previously designated serotype O-9), (Knapp et al., 1973; Winbladh, 1973) complicated the serological diagnostic work (Hurvell, 1973a; Hurvell, 1973b; Hurvell et al., 1973). This cross-reactivity has resulted in an increased risk of false positives in the diagnosis of brucellosis.

The Brucella organisms also have common antigenic components with Vibrio, Pasteurella and Salmonella. Cross-reactions of high titre levels have been described where infections or vaccination with any of these

organisms have occured and also in cases where sub-clinical infection or previous contacts have been excluded. Incubation of sera at 56°C. for 16 hours inactivates these nonspecific antibodies in some cases (Fernberg and Wright, 1951). Parnas and Dominowska (1966) described the antigenic structure of the Brucella cell wall as a generic P (pasteurella) and a species specific AM antigen. Recently, Calsson et al., (1976) revealed antigenic differences between Br. abortus and Yersinia enterocolitica O-group V, previously undetected in tube agglutination and complement fixation tests. They did this by both direct and indirect inhibition assays using Enzyme Linked Immunosorbent Assay (ELISA).

The agar gel precipitation technique of Ouchterlony (1948) and immunoelectrophoresis which differentiate antigens on the basis of immunochemical differences and electrophoretic mobility have been applied in the research of the antigenic composition of the deeper cell structures. Bauhgn and Freeman (1967) found 15 antigens, 5 of which were situated on the surface. None of these structures were species specific and the differences in the deeper cell structures were considered to be of a quantitative nature only. Only a limited number of antigens stimulate antibody production; others might produce hypersensitivity or relative immunity (Rasooly et al., 1968).

(3) Pathogenesis of Brucella Infection.

When Brucella organisms enter the body, irrespective of route, they encounter the primary cellular defence of the body. They are phagocytosed by the free polymorphonuclear leucocytes and macrophages and carried to regional lymph nodes. Brucellae resist intracellular killing by the phagocytes and multiply and cause disruption of the phagocytes. Once the organisms overcome this line of defence they are carried via the blood stream and lymphatic system preferentially to those organs with a rich reticuloendothelial structure such as liver, spleen, bone marrow, lymph nodes and kidneys where they lodge intracellularly. In cattle, sheep, goats and pigs localization of Brucellae in the placenta and fetal products leading to abortion is a permanent feature of brucellosis. Following the observation that erythritol is a stimulant to the growth of Brucella organisms Smith et al. (1962) demonstrated the presence of erythritol in the bovine placenta and postulated that it played a role in this selective localization. In an extended study, Keppie et al. (1965) found that erythritol was also present in the placenta of sheep, goats and swine which are susceptible to Brucella placentitis, and absent in the placenta of humans, rabbits, guinea pigs and rats which rarely develop this complication. In addition, erythritol was found in the seminal vesicles and testicles of bulls, rams, goats and boars. These tissues are similarly prone to the localization of Brucella infection.

Castaneda (1947) discovered in guinea pigs that Brucella organisms prefer an intracellular position in phagocytic cells. Only a few organisms were found extracellularly. He further noticed that intracellular multiplication finally caused disruption of phagocytic cells after which the liberated organisms were re-exposed to phagocytosis. The intracellular location of Brucella organisms is advantageous in two ways:- they are inaccessible by humoral antibodies, antibiotics and complement while their spread to different organs is enhanced.

In spite of phagocytosis Brucella organisms remain viable and even multiply. Holland and Pickett (1958) observed in in vitro experiments that smooth brucellae remained viable for several days in the monocytes of non-immune animals and multiplied abundantly till the host cell was filled to capacity and burst. Virulent strains showed the capacity for intracellular multiplication while avirulent ones seemed to have lost it. Intracellular growth was inhibited in monocytes derived from immune animals. Jeunet et al. (1968) studied the phagocytic activity of different organs of the reticuloendothelial system for the three classical species of Brucella. They concluded that the reticuloendothelial system organs have different degrees and capacity for phagocytosis and that the phagocytic activity of the liver fails in cases of Br. abortus infection.

(4) Immunological response to Brucella Infection.

The immunological response to Brucella infection is humoral as well as cell-mediated.

4:1. Humoral Immunity.

Brucella organisms and other intracellular organisms persist inside the phagocytes. Little is known about the factors which influence the immunogenicity of macrophage associated antigen. It has been suggested that antigen which is highly degraded during catabolism within the macrophage favours delayed hypersensitive (DHS) response (Pearson and Raffel, 1971) and that small amounts of membrane bound antigens are highly immunogenic for the induction of the humoral antibody response (Cruchaud and Unanue, 1971).

In brucellosis and other infections caused by intracellular bacteria, specific antibody appears to promote the engulfment of foreign material by phagocytes, enhances lysosomal killing of organisms (Li and Mudd, 1967) and may play a role in phagocytosis through chemotaxis. Antigen-antibody complexes have a strong chemotactic effect mediated by activation of complement (Boyden, 1962). The antibodies involved are directed against the somatic antigens of the organisms (Muschel, 1965) and only small amounts of antibody are needed (Glynn and Milne, 1967). Therefore, antibodies though

not protective on their own, nevertheless enhance the effectiveness of cellular immunity.

Morgan (1969) showed that the antibody response in cattle to either infection or vaccination with strain 19 consists of an initial IgM production followed by IgG synthesis. IgM was produced as early as the 4th day with a maximum titre on the 13th day post-vaccination. IgG normally appears between the 7th and 42nd day post-vaccination. IgG disappears after 6 months while IgM persists much longer. In case of infection, IgG will persist as long as the disease is active whilst IgM may disappear completely or decrease to a low level. Morgan's finding that in vaccination with S19 vaccine, IgM persists much longer than IgG is an exception to the general rule of immunoglobulin synthesis whereby IgG persists much longer than IgM.

The duration and seriousness of the disease determines the type and quantity of the produced immunoglobulins (Reddin et al., 1965). The presence of IgG reflects the activity of the infection (MacDonald and Elsmie, 1967; Coghlan and Weir, 1967). The presence of IgM in low titres does not necessarily indicate infection since in endemic areas low titres are common.

Presence of non-agglutinating (blocking) antibodies was first observed by Olitzki (1928). The non-agglutinating antibodies belong to the IgG and IgA classes. Experiments have shown that non-agglutinating

antibodies are only induced by a large amount of antigen and will disappear with the cessation of antigenic stimulation. They persist after the titre of agglutinating antibodies has subsided (Glenchur *et al.*, 1961).

Rose *et al.*, (1964) isolated non-specific agglutinins from cattle which might cause false positive reactions on plate agglutination. Non-specific agglutinins are produced by infection with *Yersinia* organisms and through anamnestic reaction in other febrile illnesses.

Antibodies are of diagnostic significance and this is very important since diagnosis is the main basis of control and/or eradication programmes.

The properties of the various immunoglobulins in brucellosis are summarised in Table 8.

Table 8: Physicochemical and Biological Properties of the Gamma Globulins Synthesized in a Brucella infection

	IgG	IgA	IgM
Sedimentation coefficient	7S	7-15S	19S
Molecular Weight	160,000	400,000	900,000
Concentration in Plasma	1.2gm%	0.4gm%	0.1gm%
Complement Fixing Activity	+++	-	+
Agglutinating Activity	+	+	+++
Non-Agglutinating Antibodies	++	++	-
Production	Strong prolonged stimulation		First stimulus

Source: Oomen (1975)

4:2. Cell-mediated Immunity.

Cellular immunity is an acquired form of antimicrobial resistance that depends upon "the perfecting of the phagocytic and digestive powers of the leucocytes" (Metchnikoff, 1905). It resides in activated phagocytes which become able to kill phagocytosed bacteria of strains that would resist intracellular killing in a non-sensitised host.

When an animal is infected with an organism which resists intracellular killing and multiplies within macrophages, for example, Mycobacterium tuberculosis, Brucella abortus, Listeria monocytogenes or Salmonella typhimurium the animal's macrophages become activated and multiply and are able to kill homologous as well as heterologous intracellular organisms with greater efficiency than that observed with normal macrophages (Suter and Ramseier, 1964). There is a latent period of approximately 4 days or more between infection and activation. The details of the events leading to the activation of macrophages are not well understood but it appears that:-

(a) One or more antigens of the infecting organism sensitize(s) lymphocytes.

(b) Once lymphocytes have been sensitised they can interact specifically with antigens of the parasite and bring about the activation of macrophages. From

data obtained mostly in *in vitro* studies, it has been suggested (Mackaness, 1971) that activation may be effected by factors released by sensitized lymphocytes interacting specifically with antigens of the parasite. The specific interaction of sensitized lymphocytes with antigens is believed to be an *in vitro* correlate of DHS (Bloom, 1971).

(c) Activated macrophages:-

(i) have an enhanced microbicidal power.

(ii) kill or suppress the growth of intracellular parasites.

(d) Acquired resistance persists as long as the parasite survives *in vivo* and/or DHS persists.

(e) Antigenically specific acquired immunity and DHS can be recalled rapidly by infection.

Thus activation of macrophages is through the mechanism of DHS.

A large body of evidence in support of the hypothesis that DHS plays a mediator role in cellular immunity has accumulated over the years (Collins, 1971). Collins and Mackaness (1968) and Collins and Mackaness (1970) and Mackaness and Blanden (1967) demonstrated that rodents immunized with facultative intracellular parasites such as *Mycobacterium*, *Listeria*, *Brucella* and *Salmonella* elaborate an immunity which is consistently associated with DHS. Waiyaki (1974) found that maximum DHS to most of *Salmonella typhimurium* antigens occured

at about the same time when there was maximum reduction of Salmonella organisms in the livers and spleens of the infected mice. The study of Osebold et al. (1974) disagrees with the findings of Collins and Mackaness (1968, 1970) and Mackaness and Blanden (1967) with regard to listeriosis.

A question pertaining to the need for the persistence of DHS in the infected host for long term protection remains unanswered. Mackaness (1971) argued that while demonstrable DHS may not persist, a re-infection of the host leads to a rapid stimulation of DHS with a consequent rapid recall of cellular immunity which then protects the animal. Still the question of whether or not cellular immunity really requires persistence of the infecting organism and DHS remains to be answered.

(5) Epidemiology of Brucellosis

Meyer (1964) examined 550 Brucella strains and found that each species of Brucella has a decided host preference which can be presented schematically as:-

Man:- melitensis > suis > abortus

Cattle:- abortus > melitensis > suis

Goat:- melitensis only.

It has been shown that cattle can be infected by way of the conjuctiva (Thomsen, 1918; Schroeder, 1922), by way of the skin (Hardy et al., 1929; Bang and Bendixen, 1931), per vaginam (Bendixen and Bloom, 1947; Manthei, 1950), as well as by injection and it is probable that most species can become infected by any of these routes. Guinea pigs can be infected by inhalation of quite small numbers of Brucella.

Interspecies transmission of brucellosis is well documented in the literature. Br. abortus has been isolated from camels, sheep, domestic buffalo, donkeys and horses while Br. melitensis epizootics have been reported in cattle (FAO/WHO Brucellosis Committee, 1971). Isolations of Br. abortus, Br. melitensis and Br. suis from dogs have also been reported (Salem, 1975). Brucellosis in camels caused by Br. abortus has been reported in the two-hump and one-hump camels and it is especially high in camels living in contact with infected large and small ruminants (FAO/WHO Brucellosis Committee, 1971).

Wild animals especially those of the two families - Bovidae and Carnivora play a large role in the epidemiology of brucellosis. Br. abortus, Br. melitensis and Br. suis have been isolated from wild carnivores, namely wolves and foxes, Br. abortus has been isolated from the African buffalo, waterbuck, impala, and deer while Brucella agglutinins have been detected in many African bovidae such as eland, impala, gazelles and others (FAO/WHO Brucellosis Committee, 1971).

(6) Diagnostic Methods.

6:1. Serological tests for Brucellosis.

The milk ring test (MRT), complement fixation test (CFT), serum agglutination test (SAT) and buffered *Brucella* antigen as card test (CT) or Rose bengal plate test (RBPT) all employ whole cell *Brucella* organisms as antigens.

Evidence as to the efficiency of the various diagnostic tests for detection of *Br. melitensis* infection in sheep and goats is limited (Dept. Vet. Serv., 1971). It has been stressed (Unel *et al.* 1969; Morgan, 1970; Renoux, 1972) that no serological test for brucellosis is exclusively superior to any of the others and that they should be used in conjunction with each other.

The limitations of SAT have been discussed by Renoux (1957) and Unel *et al.* (1969). The CFT is highly specific and sensitive for *Brucella* antibodies. It has higher correlations between infection and positive reactions than the SAT. The RBPT has been reported to pick many false positives in cattle sera (Morgan *et al.* 1969; Davies, 1971). Jones *et al.* (1973) have found that CT was not sensitive enough to detect infected goats. Philpott and Auko (1972) observed that the RBPT failed to detect a number of goats reacting at a titre of 1:5 in the CFT, but negative in the SAT.

The CT has been found to be positive only when the agglutinating titre was high (Varela-Diaz et al., 1973). Corbel (1972) has shown that in cattle sera the reaction to the CFT and the RBPT is mediated by IgG. He suggested that a complete correlation between the results of the two tests should not be expected since RBPT may detect antibody of another class.

6:2. Milk Ring Test (MRT).

The test was first developed in Germany in 1937 and subsequently improved by Danish and Swedish workers. Roepke et al. (1948), Bruhn (1948), Wood (1948), Huddleson and Carillo (1949), Davenport (1950), Hamilton, and Hardy (1950), and Bremer (1950) have all reported favourably upon the use of the test in detecting Brucella infection in dairy herds. Stained antigen milk test has also been used in sheep and goats but not to the same extent as in cattle.

6:3. Complement Fixation Test (CFT).

This test has been used in conjunction with the agglutination test in earlier investigations on specific diagnosis of bovine brucellosis in Denmark (Holth, 1909) and England (MacFeyden and Stockman, 1909). Much has been reported about the sensitivity, reliability and specificity of CFT and its advantages compared to

other serological tests for diagnosis of brucellosis in cattle (Larsen, 1912; Boerner and Stubbs, 1942; Rice et al., 1952; Jones et al., 1963; Mylrea, 1972) and sheep and goats (Gaumont, 1963; Alton, 1969; and Unel et al., 1969). The CFT has been found to be more sensitive than the SAT in revealing reactors in camels (FAO/WHO Brucellosis Committee, 1971).

Renoux et al. (1971) found results obtained in microtitre plate CFT identical to those obtained by the tube CFT for brucellosis. Alton (1967) found the CFT particularly useful in differentiating the vaccinal titres from the infection titre in adult animals.

6:4. Serum Agglutination Test (SAT).

This was originally developed by Wright and Smith (1897) and was later applied in conjunction with the CFT for diagnosis of bovine brucellosis (Grinsted, 1909; Holth, 1909). Both tests were found reliable in detecting infected animals.

Brucella infection in goats was first detected using the agglutination test (Zammit, 1905). The SAT proved to be consistent and reliable (Polding, 1937; Polding, 1939). Currently, the SAT is perhaps the most widely used serological test for the diagnosis of brucellosis and for assessing eradication campaigns (Morgan, 1967).

The SAT cannot be used for individual animal diagnosis of brucellosis because a negative reaction does not indicate freedom from Brucella infection (Renoux and Alton, 1955; Nicoletti and Muraschi, 1966; Morgan et al., 1969). Furthermore, in the incubative and chronic stages of the disease there is a difficulty in differentiating antibodies induced during infection from those induced by vaccination (Morgan, 1967). For instance, goats which are vaccinated with live Br. melitensis, Rev 1 or killed Br. melitensis H38 adjuvant vaccine may have suspicious or positive titres several years after vaccination (Alton, 1967).

6:5. Rose Bengal Plate Test (RBPT).

This test is the same as the acidified plate test except that the antigen is stained and concentrated to increase its sensitivity. To inhibit the activity of non-specific agglutinins the antigen is acidified. It is a comparatively new test which is simple, rapid and sensitive (Dept. Vet. Serv., 1971). Reports on the use of RBPT for diagnosis of brucellosis in sheep and goat are few. However, the test has been commonly used for the diagnosis of cattle, pig and human brucellosis.

A close correlation has been reported between results obtained with SAT, CFT, and RBPT when bovine, ovine, caprine, and human sera are tested for brucellosis (Morgan et al., 1969; Nicoletti and Fadai-Ghotbi, 1971; Ocmen and Waghela, 1974). In infected cattle herds

the RBPT apparently can identify infected animals at an earlier stage than the SAT and is often positive along with CFT when SAT is negative or inconclusive. However, some workers have found the RBPT to be over-sensitive compared with SAT or CFT (Rep. Anim. Hlth. Serv., 1970; Davies, 1971).

6:6. Slide Agglutination Test.

This test is performed with whole blood and blue-stained antigen for quick screening of animals. It was introduced by Brumpt (1940) and Castaneda (1951).

6:7. Surface Fixation Test.

This was developed by Castaneda (1951). It detects antigen-antibody complexes on filter paper.

6:8. Additional Serological Tests.

The acidified plate antigen test (Rose and Roepke, 1957; Lambert and Amerault, 1962) and heat inactivation tests (Hess, 1953; Morse et al., 1955; Amerault et al., 1961) have been employed in order to differentiate non-specific reactions from reactions resulting from Brucella infection. There is as yet no consensus as to the value of these tests and they must always be used in conjunction with the established methods.

The use of mercaptoethanol to differentiate various classes of immunoglobulins has been widely employed in research. The significance of the different

classes of immunoglobulins at varying stages of Brucella infection is under investigation at the present time. The application of mercaptoethanol in routine diagnosis must await further study (Anderson et al., 1964; Rose and Roepke, 1964; Reddin et al., 1965).

The modified Coomb's Test has been widely used in the diagnosis of human and bovine brucellosis. It has the advantage of detecting both complete and incomplete antibodies.

6:9. Evaluation of the Serological Tests.

Schaap et al. (1956) compared CFT, SAT and Coomb's test in a series of 211 persons and concluded that CFT rendered the most reliable results. Their findings were supported by those of Nicoletti (1969) who evaluated the results of various tests and interpreted their relative values as diagnostic tests in percentages:-

SAT	61%
Slide Agglutination Test (pH 6.4 to 7)	66%
Heat Inactivation Test	87%
Acidified Plate Test	96%
CFT	98%

3. MATERIALS AND METHODS

3:1. Samples.

Seven hundred serum samples were collected at random from the three districts of North Eastern Province. Most of the blood was collected by bleeding the animals from the jugular vein while a few were collected from slaughterhouses. The blood was left to stand overnight at room temperature. The seven hundred serum samples comprised of 174 camel sera, 220 bovine sera, 145 caprine sera and 161 ovine sera. Each of the seven hundred serum samples was analysed for Brucella antibodies using RBPT, CFT and SAT.

3:2. Rose Bengal Plate Test (RBPT).

a) Equipment:

(i) Test plate

(ii) Good quality serological pipettes.

(iii) Antigen dropper.

(iv) Applicator sticks.

(v) Mechanical rotator.

b) Reagents:

i) Antigen - 8% concentrate of Brucella cells stained with Rose bengal and suspended in buffer at pH 3.65. The antigen was prepared from Br. abortus strain 99 according to the method recommended by Agricultural Research Service, United States, Department of Agriculture (USDA), Ames, Iowa, U.S.A., (quoted in

Alton and Jones, 1967). It was standardised against a reference antigen obtained from the Central Veterinary Laboratory, Weybridge, England.

(ii) Phenol saline for rinsing the antigen dropper.

(c) Test procedure:

The antigen and test sera were warmed to room temperature 30 minutes prior to the test. Test sera (0.03ml.) were arranged in a row in a white enamel test plate. Equal amounts (0.03ml.) of antigen were added to each sera and the reactants mixed with an applicator stick. The plate was rocked for 4 minutes and the test read against a white background. For each test, a known positive and negative sera were included as controls.

(d) Interpretation of the Results:

Little or complete clumping was taken to indicate a positive reaction while no clumping indicated a negative reaction.

3:3. Complement Fixation Test (CFT).

a) Equipment:

(i) Microtitre plates

(ii) 0.5ml. dropping pipettes.

(iii) 0.025ml. dropping pipettes.

(iv) 0.025ml. microdiluters.

(v) Blotting paper.

(vi) 10ml., 5ml. and 1ml. pipettes.

(vii) Bijoux and universal bottles.

(viii) Concave mirror on stand.

(ix) Colorimeter.

b) Reagents:

(i) Veronal buffer, pH 7.4 for diluting the sera.

(ii) Guinea pig complement prepared from male guinea pigs starved overnight.

(iii) Hemolytic serum (hemolysin) for sensitising sheep erythrocytes.

(iv) Sheep erythrocytes from donor sheep of known packed cell volume and hemoglobin concentration.

(v) Brucella antigen from the Veterinary Research Laboratory, Kabete. This was originally obtained from Max von Pettenkofer Institute in Berlin. It was used at a dilution of 1:100 as recommended by Max von Pettenkofer Institute, Berlin, West Germany.

(c) Heating of sera:

All sera were diluted at 1:2.5. Bovine and camel sera were inactivated at 56°C. for 30 minutes. Sheep and goat sera were inactivated at 62.5°C. for 30 minutes. Inactivation of sera was done in order to eliminate anticomplementary activity.

(d) Preparation of complement:

This was prepared from male guinea pigs following the method of Alton and Jones (1967). Animals were starved overnight before bleeding. Complement was stored at -20°C. until used.

(e) Sensitising of sheep red blood cells:

A 2% suspension of sheep erythrocytes was prepared and mixed with equal volumes of the appropriate dilution of hemolysin and allowed to remain at room temperature for 10 minutes. The mixture was then incubated at 37°C.

(f) Test procedure:

The test was based on the microtitre system and was similar to the one described for use by the United States Department of Health, Education and Welfare (Monograph, Public Health No.74, 1965). The test was a six well test with serum dilutions ranging from 1:2.5 to 1:80. The test included one volume (0.025ml.) of serum diluted in veronal buffer, pH 7.4, complement (five minimum hemolytic doses) and one volume of sensitised sheep red blood cells. The usual complement,

antigen, anticomplementary, negative and positive serum controls were included in the test.

(g) Recording of the results:

The results were recorded according to the degree of fixation at a particular titre. One hundred (100%) per cent fixation was recorded as 4, 75% as 3, 50% as 2 and 25% as 1.

3:4. Serum agglutination test (SAT).

a) Equipment:

(i) Racks for test-tubes.

(ii) Test-tubes.

(iii) An indirect source of light with a black background.

(iv) Incubator at 37°C.

b) Reagents:

(i) Phenol saline diluent:

For bovine and camel sera the diluent contains 0.85% sodium chloride in 0.5% phenol. For sheep and goat sera, the diluent contains 5% sodium chloride in 0.5% phenol.

(ii) Antigen for SAT was prepared following the method of Alton and Jones (1967) from smooth Br. abortus strain. 99 grown on serum dextrose agar. The antigen was standardised against the International Standard for anti-Brucella abortus Serum (ISAbS).

An antigen dilution which gave 50% agglutination at 1:500 final dilution of ISAbS was selected for use.

(iii) ISAbS for standardising the antigen.

c) Test procedure:

The SAT was a five-tube test beginning at a serum dilution of 1:6.25. Doubling serum dilutions were made in 0.5ml. of 0.5% phenol in 5% saline and 0.5ml. antigen was added to each tube. Tubes were shaken and incubated at 37°C. for 18 hours. Controls included a known positive serum and a negative serum. The end-point prepared by adding 0.25ml. antigen to 0.75ml. phenol saline to give 50% clearing was also included in the test. The highest serum dilution showing 50% agglutination was taken as the end-point of the serum.

d) Recording of the results:

The results were recorded in International Units (I.U.)/ml. obtained by multiplying the reciprocal of the titres by 2.

3:5. Interpretation of Results (SAT and CFT).

(a) Sheep and Goats

Interpretation	SAT	CFT
Suspicious	25 i.u./ml.	2:2.5 to 1:5
Positive	50 i.u./ml. and over.	2:5 and over

(b) Cattle (non-vaccinated)

Suspicious	40-50 i.u./ml.	1:20
Positive	80-100 i.u./ml.	1:40

Cattle over 30 months old vaccinated with S19 at calf-hood.

Suspicious	100 i.u./ml.
Positive	200 i.u./ml. and over

(c) Camels:

No guidelines on interpretation of results exist. Therefore any camel showing a titre is regarded as positive.

(d) Reactors = Positives + Suspicious for SAT and CFT.

Reactors = Positives for RBPT.

3:6. Statistical Analysis:

It was thought necessary to discover whether the three test procedures differed significantly in any way in the degree of positivity to the sera of the four species examined.

The 2 x 2 table was chosen to compare any set of two procedures at a time. Since the procedures were carried out on the same serum samples, the usual Chi-square test cannot be used in analysis of data obtained. This follows from the violation of the basic assumption that n observations in the full table must be independent. The latter is not the case here since the sampling unit was the animal, whose serum yielded three parts apportioned respectively for each of the three test procedures. Hence, the animal was being sampled thrice, once for each procedure.

To overcome this problem, the McNemar's test for correlated proportions was used (Remington and Schork, 1970). The data were appropriately recast so as to count each animal only once (Table 12). If we let the index value 1 to denote positive, and 2 to denote negative, then the proportion of positives to the first procedure is:-

$(O_{11} + O_{12})/n$, and the

proportion of positives to the second procedure is:-

$(O_{11} + O_{21})/n$., then the null hypothesis (Ho) is:-

$$(O_{11} + O_{12})/n = (O_{11} + O_{21})/n$$

and the alternative hypothesis is:-

$$(O_{11} + O_{12})/n \neq (O_{11} + O_{21})/n.$$

The test statistic is:-

$$\chi^2 = \frac{(O_{12} - O_{21})^2}{O_{12} + O_{21}}$$

(O = Observed).

with one degree of freedom and tested at 5% and 10% levels of significance. The results of Chi-square calculations are shown against each small table (Table 12). The results were interpreted by comparing the χ^2 calculated with the χ^2 critical of 2.71 at 10% level of significance and χ^2 critical of 3.84 at 5% level of significance. Any values less than 2.71 were considered to indicate that there was no significant difference between the tests at 10% level of significance. A similar interpretation was made at 5% level of significance if the calculated χ^2 was less than 3.84.

χ^2 calculated values greater than 2.71 indicated a significant difference at 10% level of significance.

χ^2 calculated values greater than 3.84 indicated a significant difference at 5% level of significance.

It should be noted that for purposes of statistical analysis suspicious titres were considered as negative results.

4. RESULTS

In appendices I, II, III and IV are shown the complete raw data for the different serological tests performed on the various animal sera. Based on these data the results were interpreted and presented on Tables 9, 10, 11 and 12. In Table 9 is shown the tabulation of results from testing camel, bovine, caprine and ovine sera by RBPT, SAT and CFT while Table 10 shows the relationship between positive RBPT and SAT and CFT reactors in the four species. Table 11 shows the positive percentages and reactor rates which are the indicators of infection rates in the various species. Finally Table 12 shows the statistical analysis of the three test procedures using McNemar's test for correlated proportions.

Table 9: Tabulation of Results of Testing Camel, Bovine, Caprine and Ovine Sera by RBPT, SAT and CFT.

Species	RBPT		SAT			CFT		
	+	-	+	S	-	+	S	-
Camels	8	166	18	-	156	17	-	157
Bovine	17	203	10	8	202	7	4	209
Caprine	10	135	5	8	132	3	0	142
Ovine	11	150	10	7	144	5	6	150

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Table 10: Relationship of Positive RBPT to SAT and CFT Reactors in the 4 Species.

Species	Reactors	RBPT(+) Sera	RBPT(-) Sera	Total Reactors
Camels	SAT	8	10	18
	CFT	8	9	17
Bovine	SAT	10	8	18
	CFT	6	5	11
Caprine	SAT	6	7	13
	CFT	1	2	3
Ovine	SAT	9	8	17
	CFT	4	7	11

Table 11: Positive Percentages and Reactor Rates in the 4 Species.

Species	Total No. of Animals	Positives	Reactors	Positives%	Reactor Rate %
Camels	174	RBPT-8	RBPT-8	4.60	4.60
		SAT-18	SAT-18	10.34	10.34
		CFT-17	CFT-17	9.77	9.77
Bovine	220	RBPT-17	RBPT-17	7.73	7.73
		SAT-10	SAT-18	4.55	8.18
		CFT-7	CFT-11	3.18	5.00
Caprine	145	RBPT-10	RBPT-10	6.90	6.90
		SAT-5	SAT-13	3.45	6.97
		CFT-3	CFT-3	2.07	2.07
Ovine	161	RBPT-11	RBPT-11	6.83	6.83
		SAT-10	SAT-17	6.21	10.56
		CFT-5	CFT-11	3.11	6.83

Table 12: Comparison of the three serological tests

Using McNemar's test for correlated proportions.

I) Camels: 174 Serum Samples

a) SAT versus CFT:

		CFT +	CFT -	Total
SAT	+	10	8	18
	-	7	149	156
Total		17	157	174

χ^2 Calculated = 0.07, Probability (P) = 0.05.

χ^2 Calculated = 0.07, P = 0.10

b) RBPT versus CFT

		CFT +	CFT -	Total
RBPT	+	8	0	8
	-	9	157	166
Total		17	157	174

χ^2 Calc. = 9.00, P = 0.05.

χ^2 Calc. = 9.00, P = 0.10

c) RBPT versus SAT

		SAT +	SAT -	Total
RBPT	+	8	0	8
	-	10	156	166
Total		18	156	174

χ^2 Calc. = 10.00, P = 0.05.

χ^2 Calc. = 10,00, P = 0.10

II: Bovine . 220 Serum Samples

a) SAT versus CFT

		CFT +	CFT -	Total
SAT	+	2	8	10
	-	5	205	210
Total		7	213	220

χ^2 Calc. = 0.69,
P = 0.05.
χ^2 Calc. = 0.69,
P = 0.10

b) RBPT versus CFT

		CFT +	CFT -	Total
RBPT	+	4	13	17
	-	3	200	203
Total		7	213	220

χ^2 Calc. = 6.25,
P = 0.05
χ^2 Calc. = 6.25,
P = 0.10

c) RBPT versus SAT

		SAT +	SAT -	Total
RBPT	+	7	10	17
	-	3	200	203
Total		10	210	220

χ^2 Calc. = 3.77,
P = 0.05
χ^2 Calc. = 3.77,
P = 0.10

III) Caprine 145 Serum Samples

a) SAT versus CFT

SAT \ CFT	+	-	Total
+	1	4	5
-	2	136	140
Total	3	142	145

χ^2 Calc. = 0.67,
P = 0.05.
χ^2 Calc. = 0.67,
P = 0.10

b) RBPT versus CFT

RBPT \ CFT	+	-	Total
+	1	9	10
-	2	133	135
Total	3	142	145

χ^2 Calc. = 4.45,
P = 0.05.
χ^2 Calc. = 4.45,
P = 0.10

c) RBPT versus SAT

RBPT \ SAT	+	-	Total
+	2	8	10
-	3	132	135
Total	5	140	145

χ^2 Calc. = 2.27,
P = 0.05
χ^2 Calc. = 2.27,
P = 0.10

IV) Ovine 16 Samples

a) SAT versus CFT

		CFT +	CFT -	Total
SAT	+	3	7	10
	-	2	149	151
	Total	5	156	161

χ^2 Calc. = 2.78,
P = 0.05

χ^2 Calc. = 2.78,
P = 0.10

b) RBPT versus CFT

		CFT +	CFT -	Total
RBPT	+	2	9	11
	-	3	147	150
	Total	5	156	161

χ^2 Calc. = 3.00,
P = 0.05

χ^2 Calc. = 3.00
P = 0.10

c) RBPT versus SAT

		SAT +	SAT -	Total
RBPT	+	6	5	11
	-	4	146	150
	Total	10	151	161

χ^2 Calc. = 0.11,
P = 0.05.

χ^2 Calc. = 0.11,
P = 0.10

5. DISCUSSION

A high reactor rate was found in the sera of the four species (Table 11). Reactor rates are higher than percentage positives except in camels since reactor rates include animals with suspicious titres. In the absence of guidelines for the interpretation of results in camels, every camel showing a titre was considered positive. This probably accounts for the high reactor rate. SAT reactors occured in approximately equal proportions in RBPT positive and RBPT negative sera. A similar finding was found with CFT reactors (Table 10). This makes it necessary to do SAT and CFT on sera regardless of whether the RBPT is positive or negative. This is a major limiting factor in remote areas with poor laboratory facilities since any meaningful survey must involve all the three tests.

A good correlation between RBPT and both SAT and CFT has been reported (Morgan *et al.*, 1969; Davies, 1971). Oomen and Waghela (1974) and Oomen (1975) found RBPT to be a highly reliable test for diagnosis of human brucellosis and recommend it as

adequate for remote areas with poor laboratory facilities. However, this study has found RBPT to be inadequate on its own (Table 10) and should be used in conjunction with the other two tests in control and/or eradication programmes. The difference in efficiency of the test in the diagnosis of human and animal brucellosis is probably because animals are routinely vaccinated against brucellosis while humans are not.

The three tests were compared for their ability to pick positives using McNemar's test for correlated proportions (Table 12). This was done by comparing two tests at a time at levels of significance of 5 and 10% allowing one degree of freedom. SAT versus CFT, CFT versus RBPT and SAT versus RBPT were tested at levels of significance of 10% and 5% in the four species. χ^2 critical at 5% level of significance is 3.84 while χ^2 critical at 10% level of significance is 2.71. χ^2 calculated values are shown in Table 12. Any values greater than 2.71 are considered significant at 10% level of significance while those greater than 3.84 are considered significant at 5% level of

significance. In camels SAT and CFT were found to pick more positives than RBPT. There was no significant difference between SAT and CFT (χ^2 calculated = 0.07, Probability = 0.05; χ^2 calculated = 0.07, P = 0.10). Therefore in diagnosis of camel brucellosis, SAT or CFT could be chosen depending on convenience. Some workers have found CFT to be the most reliable test in the diagnosis of camel brucellosis (FAO/WHO Brucellosis Committee, 1971) but in this study it was found that SAT and CFT have an approximately equal reliability. In bovine sera RBPT was found to pick more positives than SAT and CFT with no significant difference between SAT and CFT (χ^2 = 0.69, P = 0.05; χ^2 = 0.69, P = 0.10). This cannot be accepted as conclusive in the absence of vaccination history since it is known that in cattle vaccinated with Br. abortus strain 19 but in which infection with field strains cannot be demonstrated, the number of false positive reactions may be very high (FAO/WHO Brucellosis Committee, 1971). Besides, RBPT has been reported to pick many false positives in cattle sera (Morgan et al., 1969; Davies, 1971). The above two reasons might

account for the above finding while it is known that CFT is the most reliable test in the diagnosis of bovine brucellosis (Larsen, 1912; Boerner and Stubbs, 1942; Rice et al., 1952; Jones et al., 1963; Mylrea, 1972).

In caprine sera RBPT was found to pick more positives than SAT and CFT with no significant difference between SAT and CFT (χ^2 = 0.67, P = 0.05; χ^2 =.0.67, P = 0.10). There are only a few reports on the efficiency of RBPT in the diagnosis of caprine brucellosis and most workers have found CFT to be the most reliable test (Alton, 1967). Waghela, (1975) found that RBPT failed to detect a number of CFT and suspicious reactors. He also found RBPT to pick many false positives in caprine sera and that a combination of RBPT and CFT would detect most caprine reactors. Limited studies have shown that RBPT may prove valuable in the diagnosis of caprine brucellosis (FAO/WHO Brucellosis Committee, 1971). This study supports this report. Evidence as to the efficiency of the various serological tests in goats is somewhat limited. Positive serological reactions are common in goats from which no Brucella organisms can be isolated at autopsy, yet animals shown to be infected may have

interpretation of results and the FAO/WHO Brucellosis Committee, (1971) recommends that a positive reaction in a goat should make the whole herd to be considered potentially infected and the appropriate control and/or eradication measures should be applied to the whole herd.

In ovine sera RBPT and SAT were found to pick more positives than CFT with no significant difference between RBPT and SAT (χ^2 = 0.11, P = 0.05; χ^2 = 0.11, P. = 0.10). Review of literature shows that a large number of infected sheep fail to react to any serological test and this is a major hindrance to diagnosis. It has also been reported that CFT and SAT detect less than 70% of infected sheep unless diagnostic criteria are adopted to classify as infected many animals known to be unexposed (Brucellosis, 5th Report, 1971). From the foregoing, it appears that interpretation of results in sheep is also a problem and should be done as in goats.

In this study the percentage positives on SAT in cattle is approximately a half of the one found by Wegener (1968-1969) while the reactor rates agree

very closely with that obtained in the survey quoted in the Dep. Vet. Serv. Annual Report (1947). The survey quoted in the Dep. Vet. Serv. Annual Rep.(1947) found a reactor rate of 5.75% and the area covered by the survey included Marsabit, Samburu, Isiolo, Garissa, Mandera and Wajir which is far larger than the present North Eastern Province. Therefore a complete agreement with the finding is not expected. The percentage positives of 3.45% in goats on SAT is in close agreement with that of Philpott and Auko (1972) who found 2.25%. However, complete agreement is not expected since Philpott and Auko surveyed 89 goats from Garissa only while this survey includes goats from all over the North Eastern Province. This study disagrees with the findings of Cooke (1956) who reported a very high percentage positives in sheep and goats. As for camels, this is the first investigation of camel brucellosis in the North Eastern Province and it has been found that there is a close relationship between the reactor rates in cattle, sheep, goats and camels.

With the proved efficiency of CFT and the short-comings of SAT and REPT in serological diagnosis of brucellosis, the results obtained using CFT in this

study are considered the most reliable. However, as has been stressed by some investigators (Unel et al., 1969; Morgan, 1970; Renoux, 1972), that no serological test is exclusively superior to any of the others, a true indication of the infection rate can only be attained by subjecting every animal to the three tests, namely, RBPT, SAT and CFT. This should be coupled with bacteriological isolations.

The livestock potential for North Eastern Province is very great (Spinks, 1956; East African Livestock Survey, 1967; Watson, 1972) and this region is the major supplier of animals for the K.M.C. abbatoirs. With passing years prolonged drought in the province has caused serious shortage of forage. This has resulted in emaciation and death of many animals. In addition it has made controlled grazing difficult. This problem has been complicated by the fact that most of the people living in the North Eastern Province are nomads. This way of life has been a major handicap to disease control and this is probably why there is such a high reactor rate in the province.

With better water supply the province could become a very important area for beef production with organised livestock management in ranches (group, co-operative and commercial ranches). Sheep and goats could also be raised intensively in fenced farms. With organised animal husbandry, disease control especially brucellosis would be possible. The Kenya Government in a joint project with the United States Agency for International Development (USAID) is to spend K£s.180 million in water development in the province (Kenya News Agency, March 1977). This will be a major step to opening the province for economic livestock production with better disease control possibilities. However, even with better facilities for livestock production,diseases such as brucellosis will continue to be a major limiting factor. With organised ranches and farms, it will be easier to locate infected animals and to carry out intensive control and/or eradication programmes.

In this study a high reactor rate was found in the four major domestic species in the province. The review of literature has shown the epidemiological

significance of wild animals especially those of the two families Bovidae and Carnivora. Interspecies transmission has been well documented with Br. abortus isolation from sheep and Br. melitensis epizootics in cattle (Brucellosis, 5th Report, 1971). Br. abortus, Br. suis, and Br. melitensis have also been isolated from dogs while Br. abortus has been isolated from camels (FAO/WHO Brucellosis Committee 1971). From the foregoing, it is suggested that any meaningful control and/or eradication programme in North Eastern Province should involve the four major domestic species including wild animals especially those belonging to the two families - Bovidae and Carnivora. Since there are reports of isolations of Br. abortus from camels and this study has shown the presence of Br. abortus antibodies in camels, it is recommended that a study should be undertaken to determine the efficiency of Br. abortus S19 vaccine in camels in view of the importance of these animals as a source of food and transport and their involvement in the epidemiology of human and animal brucellosis in the province.

6. CONCLUSIONS

There is a high reactor rate of brucellosis in cattle, sheep, goats and camels. Serum agglutination test (SAT) reactors occured in approximately equal proportions in Rose bengal plate test (RBPT) positive and Rose bengal plate test (RBPT) negative sera. A similar finding was found with complement fixation test (CFT) reactors.

Rose bengal plate test (RBPT) was found to pick more positives than SAT and CFT in bovine and caprine sera. In camels SAT and CFT were found to pick more positives than RBPT with no significant difference between SAT and CFT. In ovine sera, SAT and RBPT were found to pick more positives than CFT with no significant difference between SAT and RBPT.

Accurate diagnosis is the basis for control and/or eradication programmes and these programmes in North Eastern Province will remain handicapped until the area is opened for organised livestock production so that infected animals can be traced to their respective farms/ranches and herds of origin.

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Appendix I: Tabulation of results of testing of camel sera by RBPT, SAT and CFT

Sample No.	Test Results RBPT	SAT	CFT
1	Positive (+)	+	+
2	Negative (-)	-	+
3	-	+	-
4	+	+	+
5	-	+	-
6	-	+	-
7	-	+	+
8.	+	+	+
9	+	+	+
10	-	-	+
11	+	+	+
12	-	+	-
13	-	-	+
14	+	+	+
15	-	-	+
16	-	+	-
17	-	+	-
18	-	-	+
19	-	+	+
20	-	-	+
21	-	-	+
22	-	+	-
23	-	+	-
24	+	+	+
25	+	+	+
26-174	-	-	-
	Total P = 8 " N = 166 P + N = 174	Total P = 18 " N = 156 P + N = 174	Total P = 17 " N = 157 P + N = 174

Appendix II: Tabulation of results of testing of bovine sera by RBPT, SAT and CFT

Test Results

Sample No.	RBPT	SAT	CFT
1	+	-	+
2	+	+	+
3	+	+	Suspicious (S)
4	-	-	S
5	-	+	-
6	-	+	-
7	+	-	-
8	+	-	-
9	-	-	S
10	-	-	+
11	+	+	+
12	+	+	S
13	+	-	-
14	+	-	-
15	-	+	-
16	+	+	-
17	+	+	-
18	+	+	-
19	+	-	+
20	-	S	-
21	-	S	-
22	+	S	-
23	+	S	-
24	+	-	-
25	+	S	-
26	-	-	+
27	-	S	-
28	-	S	+
29	-	S	-
30-220	-	-	-
	Total P = 17	Total P = 10	Total P = 7
	" N = 203	" S = 8	" S = 4
	P + N = 220	" N = 202	" N = 209
		P+N+S = 220	P+N+S = 220

Appendix III: Tabulation of results of testing caprine sera by RBPT, SAT and CFT

Sample No.	Test Results RBPT	SAT	CFT
1	+	-	-
2	-	+	-
3	+	+	+
4	-	-	+
5	-	S	-
6	-	S	-
7	-	+	-
8	-	S	+
9	+	S	-
10	-	S	-
11	+	-	-
12	+	-	-
13	+	S	-
14	-	+	-
15	+	-	-
16	+	S	-
17	+	+	-
18	+	S	-
19-145	-	-	-
	Total P = 10 " N = 135. P + N = 145	Total P = 5 " S = 8 " N = 132 P+N+S = 145	Total P = 3 " N = 142 P + N =145

Appendix IV: Tabulation of results of testing ovine sera by RBPT, SAT and CFT

Test Results

Sample No	RBPT	SAT	CFT
1	+	+	+
2	+	S	-
3	+	S	S
4	-	-	S
5	-	S	-
6	-	+	S
7	-	+	+
8	-	+	-
9	+	-	-
10	+	S	-
11	+	+	-
12	+	+	-
13	-	-	S
14	-	S	-
15	-	+	-
16	+	+	-
17	-	S	-
18	-	-	+
19	+	+	-
20	-	-	S
21	-	-	S
22	-	-	+
23	+	+	+
24	-	S	-
25	+	-	-
26-161	-	-	-
	Total P = 11 " N = 150 P + N = 161	Total P = 10 " S = 7 " N = 144 P+N+S = 161	Total P = 5 " S = 6 " N = 150 P+N+S = 161