SERO-EPIDEMIOLOGICAL STUDY OF BRUCELLOSIS AMONG GOATS AND SHEEP AT PESHAWAR DISTRICT

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Summary

Sero-epidemiological study was carried out to observe the prevalence of brucellosis in 500 slaughtered as well as in 500 healthy animals in Peshawar district of N.W.F.P. All serum samples were subjected to four serological tests i.e. Standard Plate Test (SPT), Standard Tube Test (STT), Rivanol Test (RV) and 2, Mercapto-Ethanol Test (2, ME). The incidence of disease in 500 healthy animals tested by standard plate test, standard tube test, rivanol test and 2, Mercapto-ethanol test, was 2.8%, 1.8%, 1.6% and 1.2% respectively. While the incidence of brucellosis in 500 slaughter animals from Peshawar abattoir was 3.0%, 2.2%, 2.00% and 1.2% by standard plate test, standard tube test, rivanol test and 2, Mercapto-ethanol test The disease prevalence was higher in slaughtered animals as compared to healthy animals. The disease was more common in goats than sheep, also more prevalence in aged female than younger stocks. The efficacy of SPT was found more effective as compared to STT, RV, and 2, ME tests both in slaughtered as well as apparently healthy animals at Peshawar district. Standard Plate test detected 2.9%, Standard Tube test 2.0%, Rivanol test 1.8% and 2, Mercapto-ethanol test detected 1.2% positive cases in slaughtered as well as in healthy animals. So the Standard Plate Test was found to be more reliable, sensitive, and easy to performed.

(**Key Words** : Brucellosis, Sheep and Goats, SPT, STT, RV and 2ME Tests, Peshawar)

Introduction

Brucellosis as a serious economic disease of animals producing abortion, Still-birth, repeat breeding, loss of calf and milk production in aborted animals i.e. 10% or more. New epidemiological evidence has shown that not only the Cattle, Goats, and Swine are carrier of casual agents but other domestic animals like dogs, horses, and poultry may also harbour and spread brucellae. Reservoir of brucellae may also exist in wild animals (Hurvell, 1982). The incidence of disease varies considerably among breed, areas, and countries. Brucellae are usually found in the uterus during pregnancy and latter in the mammary gland. When the organism invade a dissimilar host, it tends to spread or localize in the mammary glands and reticuloendothelial system. Carrier animals usually secrete the organisms in the milk, rather then in the uterus and foetal membranes (Meyer, 1964). In females the organisms localized in the gravid uterus and cause abortion, endometritis, retention of placenta, repeat breeding and infertility problems. In males the seat of prediction is testes where it cause orchitis, epididymitis, seminal visiculities and necrosis of one or both testes (Trichard, 1982). Transmission of the disease occurs more frequently by ingestion of infected materials and or through inhalation of infected dust containing viable bacteria, through mucus membrane of the eyes, abraded or intact skin and also through infected semen during natural and artificial service. In human beings the disease is called Undulant or Malt fever, and the humans gets infection through contact with infected animals, infected materials and unpasteurized milk from diseased animals. The veterinarian, dairy workers and butchers are always at higher risk.

It is hoped that the findings of this study will help in formulating a programme for controlling of brucellosis in Peshawar district.

Materials and Methods

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Blood collection

A total of 500 serum samples from goats, and sheep of both sexes (as detailed in table 1) slaughter at Peshawar abattoir, and 500 serum samples from animals (table 2) maintained in the precinct of Peshawar city were used as a source of serum samples for this study. Blood from the slaughtered animals were collected from the jugular vein/ carotid artery in a clean, sterilized plastic bottles at the time of their slaughter and allowed to clot for half an hour in a slanting position. Then it was brought to the Microbiology Laboratory of Veterinary Research Institute Peshawar and kept at room temperature. The next day the sera were pipetted out in a clean sterilized screw capped plastic vials, labelled it carefully and stored in the freezer at −20℃ till to use.

The blood from the 500 live animals were collected in a sterile disposable syringe from the jugular vein (by pricking the needle), approximately 10 ml blood was drawn into the syringe and was allowed to clot. The rest of the procedure was same as for the blood collected from slaughtered animals.

TABLE 1. ANIMALS SLAUGHTERED AT PESHAWAR ABATTOIR

S. NO	Species	Female	Male	Total
i	Goats	240	10	250
ii	Sheep	240	10	250

TABLE 2. ANIMALS LIVING IN THE PRECIENCT OF PESHAWAR CITY

S. NO	Species	Female	Male	Total
i	Goats	240	10	250
ii	Sheep	240	10	250

Antigens

The brucella abortus plate and Tube test antigens, Rivanol antigen, and 2, Mercapto-ethanol antigen imported from United State Department of Agriculture (USDA) and National Animals Disease Centre (NADC) were used.

Serological work

The standard serological procedure of Brown (1974), was used for Standard Plate, and Standard Tube tests, Hafeez (1985) for Rivanol and 2, Mercapto-Ethanol tests for the detection of brucella antibodies in the serum of diseased animals. For 2, Mercapto-Ethanol the procedure is as follows.

Put four tubes in a rack for each serum sample and delivered to it 0.08, 0.04, 0.02, 0.01 ml of serum. add to it 1 ml of 0.1 m solution of 2, ME and mixed by shaking the rack (0.1 M sol of 2, ME was prepared by adding 3.57 ml of 2, ME to 496.43 ml of 0.85% NaCl sol and stored in the refrigerator until use). After the addition and mixing of 1 ml of brucella tube test antigen (with out phenol), The final dilution obtained was 1:25, 1:50, 1:100, 1:200 respectively. After overnight incubation at 37℃, the tubes were observed closely for agglutination against a dull black back ground with light coming from behind the tubes and the degree of agglutination were recorded as follows.

a: Complete agglutination and sedimentation with 100 % clear supernatant ---------- + + + +.

b: About 75% agglutination and sedimentation with slightly cloudy supernatant ---------- + + +.

c: Approximately 50% agglutination and sedimentation with moderatly cloudy supernatant. ---------- + +.

d: If 25% of the organism clumped and the supernatant fluid was cloudy ---------- +.

e: When their is no agglutination and supernatant remains cloudy. ---------- (-ve)

Results

In this study, a total number of 1,000 animals composed of 500 goats, and 500 sheep were examined for the evidence of brucellosis by Standard plate Test, Standard Tube Test, Rivanol Test, and 2, Mercapto-Ethanol Test. The sera giving positive brucellosis reaction by different tests were 29 (2.9%), 20 (2.0%), 18 (1.8%) and 12 (1.2%) respectively by Standard plate Test, Standard Tube, Rivanol, and 2, Mercapto-Ethanol Tests.

While in 240 live sheep gives 4 (1.66%), 2 (0.83%), 1 (0.41%), and 1 (0.41%) positivie cases of brucellosis respectively. Whereas in blood samples from 240 slaughtered sheep brucella antibodies were detected in 6 (2.50%), 6 (2.50%), 5 (2.09%) and 2 (0.83%) by Standard plate Test, Standard Tube, Rivanol, and 2, Mercapto-Ethanol Tests (table 3). Among the 10 live rams brucella positive reaction was seen in 1 (10%) sample only by Standard plate test while the other three tests were found negative. (The same result was obtained by 10 slaughtered male) The incidence of disease in 240 live goats was 8 (3.33%), 7 (2.91%), 7 (2.91%), and 5 (2.08%) positive for brucellosis by standard plate, standard tube, rivanol and 2, Mercapto-ethanol tests, While in 240 serum samples from slaughtered goats the prevalence of disease by these tests was 7 (2.91%), 7 (2.91%), 7 (2.91%) and 5 (2.09%)

respectively (table 3). Among 20 male goats 10 live and 10 slaughtered brucella positive reaction was seen in 1 (10 %) sample only by Standard plate test whereas the other three tests were found negative. (table 4).

The disease was higher in live goats as compared to slaughter animals, While this was found more in slaughters sheep than live sheep, more common in goats than sheep.

The sex based comparison of prevalence indicated that out of 480 female goats and 20 male goats examined for brucellosis reveals that 15 (3.12%), 12 (2.50%), 12 (2.50 %), and 9 (1.87%) goats were found positive by SPT, STT, RT, and 2, ME tests (table 4), but only 2 (5%) male shows positive result by standard plate test, while the other three test were found negative. However the sex base comparison for the prevalence of brucellosis in 480 sheep and 20 rams examined for brucellosis, the number of positive cases by different tests was 10 (2.08%) by standard plate test, 8 (1.66%) by standard tube test 7 (1.45 %) by rivanol test and 3 (0.62%) by 2, Mercapto-ethanol test. where as 2 (5%) rams were recorded positive by standard plate test, while the other three test were found negative (table 4).

The age group relation-ship of brucellosis in goats observed that out of 400 old goats (above 11 months) 6 (1.50%), 5 (1.25%), 5 (1.25%), and 4 (1.00%) were positive by SPT, STT, RT, and 2, ME test. The sero-positivity in 400 old sheep (above 11 months) was 8 (2.0 %), 7 (1.75%), 5 (1.25%) and 3 (0.75%) respectively by the afore said tests, Whereas in 100 goats and 100 sheep (below 11 months) age were all negative for brucellosis (table 5).

TABLE 3. BRUCELLOSIS IN FEMALE LIVE AND SLAUGHTERED ANIMALS IN PESHAWAR

Spp.	No. of animals tested	SPT		STT		RV		2, ME	
		+ve	%	+ve	%	+ve	%	+ve	%
Goats									
(Live)	240	8	3.33	7	2.91	7	2.91	5	2.08
(Dead)	240	7	2.91	5	2.09	5	2.09	4	1.67
Sheep									
(Live)	240	4	1.66	2	0.83	1	0.41	1	0.41
(Dead)	240	6	2.50	6	2.50	5	2.09	2	0.83

TABLE 4. SEX-WISE SERO-PREVALENCE OF BRUCELLOSIS IN ANIMALS

Spp.	Sex.	No. of animals tested	SPT		STT		RV		2, ME	
			+ve	%	+ve	%	+ve	%	+ve	%
Goats	F.	480	15	3.12	12	2.50	12	2.50	9	1.87
	M.	20	2	10.0	0	0.00	0	0.00	0	0.00
Sheep	F.	480	10	2.08	8	1.66	7	1.45	3	0.62
	M.	20	2	10.0	0	0.00	0	0.00	0	0.00

F = Females. M. = Males. SPT (Standard plate test) STT (Standard tube test), RV (Rivanol test) 2, ME (2, Mercapto-Ethanol test).

TABLE 5. AGE-WISE SERO-PREVALENCE OF BRUCELLOSIS IN ANIMALS

Spp.	Age group	No. of animals tested	SPT		STT		RV		2, ME	
			+ve	%	+ve	%	+ve	%	+ve	%
Goats	O.	400	6	1.50	5	1.25	5	1.25	4	1.00
	Y.	100	0	0.00	0	0.00	0	0.00	0	0.00
Sheep	O.	400	8	2.00	7	1.75	5	1.25	3	0.75
	Y.	100	0	0.00	0	0.00	0	0.00	0	0.00

+ Young (Y) up to 11 months. Old (O) above 11 months.

Efficacy among various tests

A total of 1,000 serum samples, collected from animals were subjected to four serological test i.e. SPT, STT, RV, and 2, ME tests and gave 29 (2.9%), 20 (2.0%), 18 (1.8%) and 12 (1.2%) of brucella positive cases (figure 1), While figure 2 and 3 shows the brucellosis in apparently healthy and slaughtered goats and sheep.

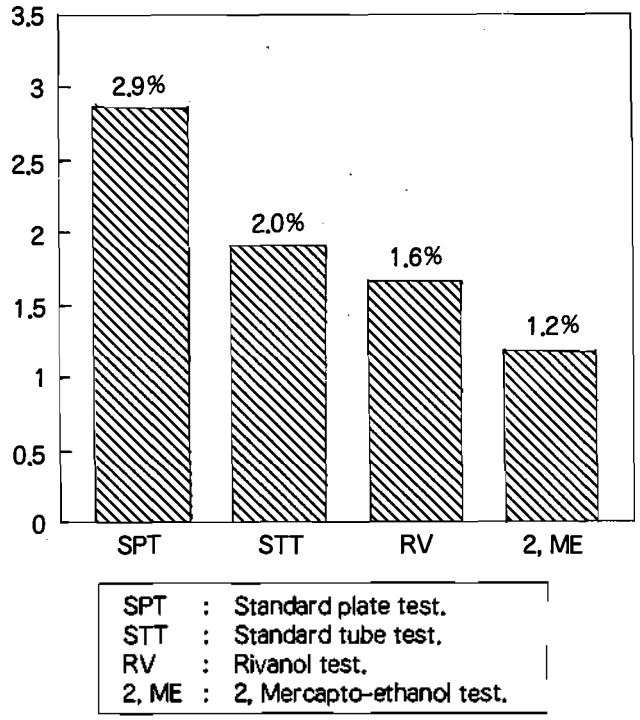

Figure 1. Efficacy among various tests.

Discussion

The disease brucellosis can affect the economy of a country by inflecting heavy losses to the livestock and dairy industry. Three species of the Genus Brucella i.e. Brucella Suis, Brucella melitensis, and Brucella abortus have been reported to produce disease in pigs, sheep and goats, and cattle and buffaloes. So the proper diagnosis of the disease can only be achieved by some rapid, reliable, and sensitive diagnostic procedure. The most commonly tests used are Standard Plate Test, Standard Tube Test, Rivanol Test, and 2, Mercapto-Ethanol Test, Milk ring test, complement fixation test, Enzyme Linked Immunoassorbent Assay Technique (ELIZA) (Xie xin, 1982. Heck et al., 1982, Hobbs, 1985). The Tube and Plate agglutination test have been the standard serological procedure for the diagnosis of brucellosis since the inception of its serology (Biegleisen et al., 1962; Davies 1971; Contini et al., 1973) and these tests have further

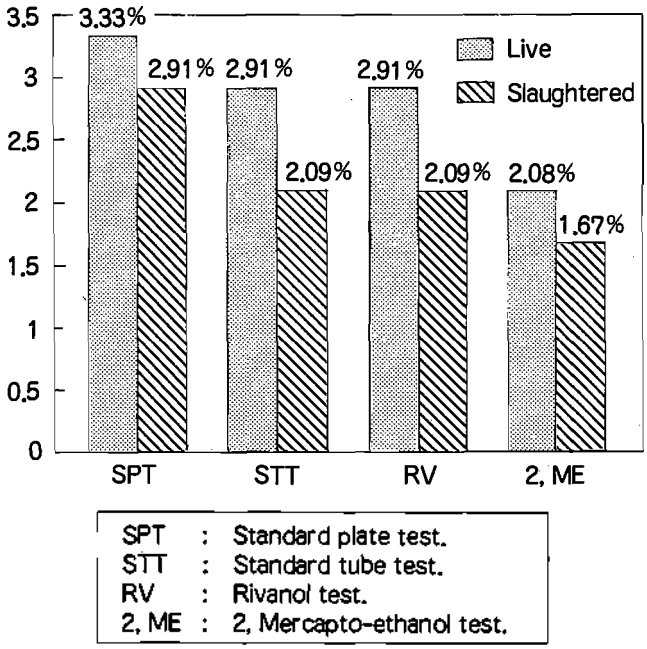

Figure 2. Brucellosis in live and slaughtered.

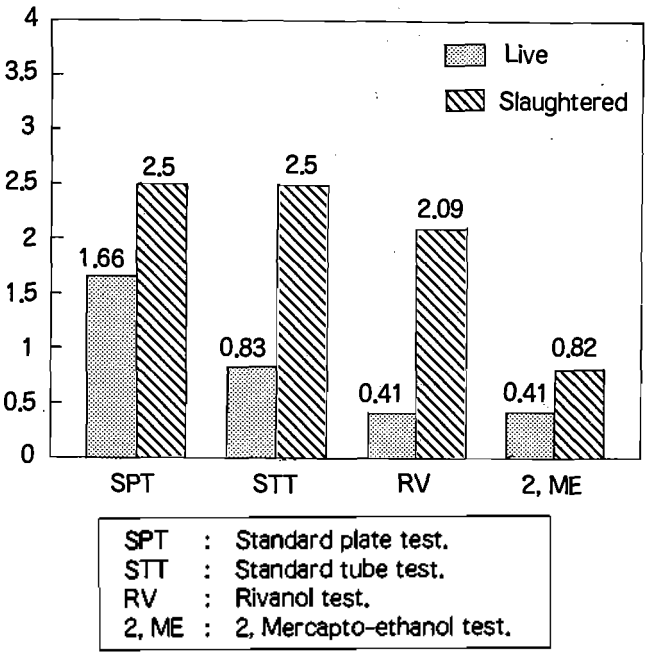

Figure 3. Brucellosis in live and slaughtered sheep.

been endorsed by Brown (1974), Oomen and Waghela (1974) and Stemshorn et al. (1985). In this study Standard Plate Test, Standard Tube Test, Rivanol Test, and 2, Mercapto-Ethanol Test were used for detecting the presence of brucella antibodies in the blood of goats and sheep in peshawar district of N.W.F.P (Pakistan) The incidence of disease in 1,000, animals was 2.90% by Standard Plate Test, 2.0% by Standard Tube Test, 1.8%

by Rivanol Test, and 1.2% by 2, Mercapto-Ethanol Test. The standard plate test gave invariably a high result in test animals being 3.4% in goats and 2.4% in sheep, followed by standard tube test 2.4% and 1.6%, Rivanol test 2.4% and 1.2% and finally by 2, ME test 1.8% and 0.60% respectively. The result obtain by the 2, ME test showed a lower incidence in sheep 0.60% than goats 1.8%, this difference may be due to non-specific agglutination materials for brucella present in the serum of animals with no history of brucellosis (Hess and Reopke, 1964) This was confirmed later to have characteristics similar to those of IgM class of antibodies (Rose, Roepke and Briggs, 1964), The result of this work were consistent with the findings of Ahmad (1984), Maqsood (1986), and Masoumi (1986). The sex based analysis of prevalence indicates that higher prevalence in females may be due to the natural service, artificial insemination, where a single infected male can introduce the disease to a large number of females, These findings are in agreement with those of Pandle (1969), Nuru (1975), and Maqsood (1986). However this study did not agree with the work of Qureshi and Bhatti (1968), Who observed that the incidence of disease is higher in males than females. In 1979 Baby and Paily found out that both the sexes are equally effected by brucellosis.

The tube agglutination test detect a prevalence of 0.83 % in sheep and 2.92% in goats regardless of their age and sex, The data of this study is in agreement with the finding of Mausomi (1986), and Ajmal et al. (1989). Similar incidence in sheep has been reported by Sen et al. (1971), Kulshreshtha et al. (1976) and also by Aquino et al. (1981). A similar prevalence in contrast to sheep was reported by Dafaala (1962), Sheikh et al. (1968), Randhawa et al. (1974), Kulshreshtha et al. (1978), Bale et al. (1982), and Hashim et al. (1987).

Sex based comparison of the disease by tube agglutination test recorded a higher prevalence in sheep 1.6% as compared to rams of which no positive case was observed. These findings were in agreement with those of Ghani et al. (1983), and Masoumi (1986).

The age group relationship of the disease recorded an incidence of 1.75% and 1.25% in sheep and goats respectively (Above 11 months age) by tube aggtutination test but there was no evidence of the disease in the young (Below 11 months). This total negativity of young animals may be due to the non availability of erythritol, which stimulate the growth of brucelae. The role of erythritol in the pathogenesis of brucellosis has been suggested by Keppie et al. (1965).

The finding of this study reveals that the incidence of brucellosis in our goats, and sheep in Peshawar district are at low proportion, but still various epidemiological factors comprising Ages, Sex, Breed, Lactation numbers, Herd size, and living condition was found to influence the disease incidence.

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