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The present investigation was undertaken to assess the incidence of Brucellosis in man and animals in Gujarat State with special reference to foetal loss. From man, samples of blood, and from animals, samples of blood and milk were collected for this purpose and various serological techniques were used to ascertain the antibodies titres. In addition to this study, attempts were made to isolate Brucella organism from milk of cows and buffaloes, and from blood, placenta and urine of aborted women.

The meagre data available regarding the incidence of Brucellosis in women with abortion and in animals in Gujarat State, necessitated the work on the subject of this thesis.

Gujarat is one of the important milk producing States in India. Hence the study on the incidence of Brucellosis in this State is most important; since Brucellosis is a Zoonotic disease, there is possibility of its transmission from animals, probably through the consumption of raw milk or by direct contact with animals; as Gujarat is also one of the
important agricultural States, so farmers come in contact with animals, as they keep them for their income through the milk production or through ploughing the land.

The disease may be transmitted from man to man by the blood transfusion carried out in the medical hospitals. Because of the inherent hazards that might be caused by the transfusion of blood, the testing of the serum samples to detect the presence of Brucella agglutinin may be preferred. This may reduce the hazard among human population. Besides, the reduction in the incidence of the disease in animals will, no doubt, protect the human population from this infection, although Indians have the habit of drinking mostly boiled milk; but their contact with the animals in one way or other, is very common. But, the eradication of Brucellosis in our country, as a whole, is quite impossible; whereas in other countries, the eradication is carried out by the slaughter of the infected animals or by calfhood vaccination, but in India, the slaughter is banned because of the religious reasons, and the calfhood vaccination is not satisfactory. Hence the disease is perpetuated in animals and thereby the
possibility of the incidence of the disease in Indian population is always there, because of their contact with animals through the agricultural profession.

INCIDENCE OF BRUCELLOSIS IN MAN AND ANIMALS IN GUJARAT STATE

Since there were no recorded evidences of Brucellosis by tube agglutination test in aborted women and insufficient reports regarding its prevalence among animals in Gujarat State, this problem was undertaken with a view to throw some light to both medical and veterinary professions about the prevalence of this Zoonotic disease.

Mathur (1960) was able to conclude that there is possibility of the human Brucellosis in Orissa, as there was the record of high incidence of Brucellosis among animals.

From this present study, it is clear that 8.51 percent of patients of different groups showed positive (30 I.U./ml. and above) Brucella agglutinin in Gujarat (Table III). However, the percentages of positive incidence of Brucellosis in syphilitic and pyrexia of unknown origin (PUO) cases were 24.47 and 4.37 respectively. But Phadke and Phadke (1974)
reported 21.8 and 17.4 percent respectively in Poona. The prevalence of Brucellosis was 11.33 percent in Karnataka (Stephen et al., 1973). Randhawa et al. (1974) recorded an incidence of 15.1 percent in 200 cases and cited that, in India, it ranged from 0.9 - 6.4 percent. Besides, Mathur (1963 b) found that 43 percent of sera from syphilitic patients showed Brucella agglutinin in Karnal. Shukla (1962) also found Brucella agglutinins in 92 percent of human sera tested in Baroda.

There is an incidence (6.46%) of Brucellosis in aborted women in this present investigation (Table III). This confirms the serological finding (4.2%) of Randhawa et al (loc.cit.). In this study, suspected cases of Brucellosis showed an incidence (14.14%) of Brucellosis, but Randhawa et al (loc.cit.) claimed 100 percent of infection in Haryana and Punjab. During the serological study, much attention was paid to the cross agglutination with Vibrio Cholerae. Hence the history about the Cholera vaccination and outbreak of cholera was taken. Besides the income and occupation of the patients were also taken into account. Almost all of them had a low income. They were mostly farmers coming from rural areas. However,
only two women had symptoms of abortion similar to those of animals. The unhygienic conditions prevailing in villages may be one of the reasons for the prevalence of Brucellosis among farmers who had a low standard of living and some who had usually the habit to relax under the shady tree where the animals also used to rest or graze. The incidence in such human beings may, most probably, be through the indirect or direct contact with the animals. Brucellosis is prevalent in sheep (11.3%) and goats (10.6%) in Gujarat State as indicated in the present study. Ovine Brucellosis is also prevalent in the country of Mediterranean and the Middle East and in the U.S.S.R. (FAO/WHO Expert Committee on Brucellosis, 1964). In our Country, people drink the goat's raw milk during summer due to cooling effect. The incidence in goats might cause a great hazard in human beings, because most infected goats excrete *Brucella melitensis* in their milk (FAO/WHO Expert Committee on Brucellosis, 1964). Thus people may contract the infection. Another possibility of contracting the infection may be due to the handling of meat before cooking. The possibility of contracting the infection by the rural population is less, because they consume mostly pasteurised milk.
supplied by the municipal dairies. On the contrary, they may contract the infection by consuming milk products like cheese or ice cream (Mathur, 1964). In the present study, more number of animals and men had low agglutinin titres. The simple deduction is that they might have acquired the low titres due to their close association with the infected animals or they may try to resist the infection, or due to the past infection.

Mathur (1963) pointed out that the goat was the important reservoir of infection for the human being and the reports of Brucellosis were found to be more common among people who keep goats than among those who rear cows or buffaloes. The adulteration of cows or buffaloes’ milk with that of goats due to the scarcity of milk during summer should, therefore, be considered detrimental to the public health. Therefore, people should pay more attention to drink boiled or pasteurised milk in order to safeguard them from hazard of Brucellosis.

The percentage of incidence of Brucellosis is more or less similar in both buffaloes and cows in Gujarat State (Table X). In this connection, much
attention should be paid by the animal breeders in carrying out the natural service or artificial insemination lest the infection may not spread from animal to animal through the intensive cattle breeding programme undertaken by many States of India. Instead of producing better breeds of animals, the animal breeders may indirectly infect the animal population and thus cause economic loss to the nation. From the present investigation, it is clear that the infection is prevalent in Gujarat, besides further personal enquiry from the farmers who came to supply the milk to the municipal dairy revealed that there were abortions in their animals. Very recently, the National Dairy Development Board, Anand (Gujarat) has undertaken a prestigious "Operation Flood" (White revolution) project to flood milk throughout the country. Under this project, the farmers are encouraged to import animals from other States to boost the milk production. Farmers of Gujarat State are not exempted from this project. As a result of which, the animals were imported from other States like Punjab, Haryana, where the infection is prevalent according to Mathur (1959), Panjarathinam and Gulrajani (1974). Hence, there is the possibility of constant movement of animals throughout all States.
of India, thus the infection may, most probably, be perpetuated among Indian animals, thereby, exposing the human being to the risk of infection. In India, the eradication of Brucellosis in animals is a never ending problem, due to many reasons like economy, religion etc.

All the available tests were employed to evaluate the incidence and the critical comparison of the tests was attempted at considerable detail.

Tube agglutination test

All the serum samples were tested by the tube agglutination test using Brucella plain antigen. The results obtained in the present investigation proved that this test is the test of choice of detecting serologically cases of Brucella infection and can be employed for the evaluation of the incidence of Brucellosis in man and animals. Serum agglutination reactions were attributed to IgM antibody (Chappel et al (1973)).

Complement fixation test

In the complement fixation test, Brucella (Weybridge) plain antigen was used and found quite suitable. The work of Alton (1961), Panjarathinam
and Gulrajani (1975) regarding the use of plain antigen was confirmed by this study, though it was already found suitable by Burki (1961) and Bertachinger (1961). The test was found to be more cumbersome and time consuming and it is not considered for routine survey work. Stockman (1914), Carpenter et al (1930), Wise et al (1924), Schoenoers et al (1953) and Roux (1979) were of the same opinion. Clapp et al (1954) fixed 1:10 titre to be taken as positive for Ovine Brucellosis. The complement fixation test can be recommended for the additional use, when the results with the agglutination test are doubtful, as opined similarly by Feils (1955), Burki (1956), Jones et al (1963), Wisniowski (1964) and Roux (1979). This test can further detect IgG antibodies as reported by Poole (1975).

In this study, many serum samples having agglutinin titres agreed with complement fixation test (Tables XIV, XIV (A) and XIV (B) and further confirmed the opinion of Roux (1979).

**Indirect bacterial Haemagglutination test**

The observations of Macpherson et al (1953), Noter et al (1954), Fins et al (1955), Landy et al (1953), Davies et al (1953) were found to be in confirmative with the observation of author as the
alkali treatment of the trichloracetic acid extract obtained from acetone dried cells increased its absorption on erythrocytes. The results obtained in this study (Tables XIV, XIV (A) and XIV (B) indicated that the HA test can be adopted as a supplementary test to confirm the infection. However, Veraiilova et al (1974) found that the diagnostic efficacy of HA test was 20-30 percent greater than that of agglutination test.

**Gel diffusion test**

An interesting feature of the study was that the extract of the whole cell was obtained from *Brucella abortus* (strain 99) by autoclaving for 3 hours for 3 consecutive days and was found to produce precipitation lines in the modified Ouchterlony technique with the sera having Brucella agglutinin titres (Table XV).

There is no record in the available literature on the precipitation reaction with 3 hours autoclave extract of *Brucella abortus* (Strain 99). Because of its easy method of extraction and subsequent encouraging results being obtained, it is recommended for use in gel diffusion technique.
A factor that it is not destructible by heat, and treatment with acid is suggested in the present study.

Huston et al (1934) could extract a precipitating substance "S" from *Brucella melitensis*, but not from *Brucella abortus* or *Brucella suis*.

Ouchterlony (1958) and Redfearn et al (1960) used 1.5 percent agar in their technique. The present observation also claims that 1 percent agar can be used to get precipitation lines and 0.35 percent sodium chloride did not interfere with the result.

In the present study, it was found that the lines were more prominent at incubation temperature ($37^\circ C$) than at room temperature ($23^\circ C$) but no line appeared at refrigeration temperature ($4^\circ C$).

Distilled water of pH 7.0 was suitable in the preparation of agar gel. The distance of 0.4 cm. between the central well and six peripheral antisera wells was found satisfactory for the antigen antibody system, as it was found by Bruce et al (1958) whereas Redfearn et al (1960) were satisfied with the distance of 0.5 cm. The results of gel diffusion test were in agreement with those of tube agglutination test as observed by McNahon et al (1979).
Rose Bengal agglutination test in Brucellosis

Since Morgan et al. (1969) and Diaz et al. (1976, 1978) made use of this test for bovine and human Brucellosis, an attempt was made, in the present investigation, to utilise Rose Bengal test for the diagnosis of Brucellosis in human beings, sheep and goats, it was found that the results of Rose Bengal test were similar to those of tube agglutination test (Table VII, VIII and XVII) as reported by Diaz et al. (1976).

The antoglobulin (Coombs') test for Brucellosis

In the present study, Coombs' test could detect positive cases thereby eliminating the fallacy of blocking antibodies as observed by Jones and Wilson (1951), Schuhardt et al. (1951) and Wilson and Merrifield (1951). This AHG test is useful to detect non-agglutinating antibodies, either IgG or IgA (Poole, 1975). Coombs' test can confirm the results of tube agglutination test (Schoutens et al., 1978).

Serum plate agglutination test

The rapid plate test using coloured antigen (Tables IX, XVII) gave comparatively less number of
positive cases than the tube agglutination test which contradicted the report of Moreira-Jacob (1955). This test can be considered as rapid and helpful adjunct to the tube test.

The results obtained in the present study clearly showed that the presence of agglutinins, complement fixing antibodies, haemagglutinins or precipitins in the test sera was of great value to detect the infection. When all these tests were employed and compared, it was always possible to find out the existence of the infection.

C-reactive protein determination

Since the preliminary studies of Stollerman et al. (1953) indicated that the test is positive in bacteremic patients with Brucellosis, an effort was made in the present investigation to determine the C-reactive protein in aborted cases with agglutinin titre ranging from 30 to 640 I.U./ml. and it was observed that out of 52 cases, 16 were weakly positive and 19 were positive indicating the presence of active infection as suggested by Stollerman et al. (1953).

Milk Ring Test

The survey work on Brucellosis among buffaloes and cows carried out by employing Milk Ring Test (Table XVIII)
brought into evidence that 31.76 percent of buffalo milk and 31.42 percent of cow milk samples were positive on Milk Ring Test. The results gave an idea that Brucellosis is equally prevalent in buffalo and cow in Gujarat State.

Hence, people should pay more attention to drink boiled or pasteurised milk in order to safeguard them from hazard of Brucellosis.

**Isolation of Brucella organism from human placenta, blood and urine**

The efforts to isolate the micro-organisms from the placenta, blood and urine of cases with abortion, did not come out with success. Perhaps, the micro-organisms were not present in the placenta, because of absence of erythritol, Keppie et al. (1965), in human placenta; failure to isolate Brucella from blood or urine of aborted women may, most probably, be due to the treatment with antibiotics in the early stage.

**Isolation of Brucella from milk**

Milk samples of buffaloes from 425 cans and samples of cows from 175 cans were subjected to the culture test. Out of 425 cans, only one can gave
positive cultural test i.e. Brucella organism was isolated. It shows that Brucella are rarely excreted through milk; Brucella isolated may, probably, be from one buffalo or many, because each can contained usually milk from 10-20 animals.

In the present study, many animals were positive on Milk Ring Test; thus serologically positive animals may not always excrete Brucella organisms through milk secretion. However, Karstan (1932) and Singh (1965) proved that the aborted animals had disseminated the micro-organisms in their milk secretion.

Brucella organism isolated from milk of buffaloes was Brucella abortus biotype 1 (Tables XIX and XXI).

**Brucellosis in women with abortion**

The Indian available literature indicated that only Randhawa et al (1974) reported the serological evidence of Brucellosis in aborted women and no attempt of isolation of Brucella was made. Hence in the present study an attempt was made to report the serological evidence of Brucellosis in aborted women and the isolation of Brucella. The present finding showed the serological incidence (6.46%) of Brucellosis in aborted women in Gujarat, similar to the finding of Randhawa et al (loc.cit.), but there was failure in isolation of
Brucella from aborted women, whereas Poole et al. (1972) reported a case of abortion due to *Brucella abortus* biotype 2 in England.