

CENTO UNCLASSIFIED
ANNEXE «P» TO
EC/9/AG/D3

Diagnosis Of Brucellosis In Goats And Sheep

Paper Submitted By Mrs. Mesadet Doğuer
Etlik Bacteriological Institute, Ankara

After my brief sketch, with which this talk is to begin, to point out the importance, as a first class source of livelihood, of goats and sheep in this country's economic life, it will seem essential that Brucellosis reactors in these animals should be quickly, easily and safely spotted at the least cost.

(1) Goats and sheep are the most rewarding dairy animals providing livelihood to 80 % of this country's entire population.

(2) The same animals are also important for the economic life of Turkey with meat, milk, wool and mohair which they produce.

(3) Peasants who are in close contact with these animals and the population in cities who consume their direct or indirect produce are under the imminent or immediate menace of Brucellosis.

(4) Since these animals, with the exception of large herds, share their rural shelter with cattle, they become dangerous carriers of the disease. This shows the important part they play in the eradication of cattle Brucellosis.

There are no provisions within the present Regulations, in use since 1942, to govern procedures for the detection of Brucellosis, preventive and protective measures to be taken and the fight to be conducted in the case of goat and sheep Brucellosis. Similar provisions have only just been introduced into the Veterinary Control and Animal Health consultant committee. There exists vital need or codification and enforcement of Regulations covering goat and sheep Brucellosis, similar to the one revised for cattle which is to

come into force in the near future. Since there is no effective vaccine yet available, the method presently followed by us in the subject consists of diagnosis by slaughtering and this is more than enough to throw light on the importance of devising a detection method as quickly as possible. There is no record yet, in various countries, of a positive result having been obtained through a single method of diagnosis in goat and sheep Brucellosis.

Methods of Diagnosis of Goat and Sheep Brucellosis : (1)

1. Sero - Agglutination test in tubes.
2. Plate - Agglutination test.
3. Complement - Fixation test.
4. Flocculation test.
5. Anti - Globulin test.
6. Allergic tests.
7. Stained antigen test for milk.
7. Stained antigen test for milk.
8. Whey test.
9. Culture.

Methods which have been referred to in Turkey, up to the present, and results obtained :

1. Sero - Agglutination Test in Tube :

Diagnosis of Brucellosis in sheep by this method has been referred to by Köylüoğlu and Aktan (13). In his serological research Golem (9) has proved that agglutinations for Brucellosis by sheep and goat sera were 13.3% and 50 % respectively. In a total of 48,360 serum tests (44,525 seep and 3,835 goat serum) carried out at our laboratory within the last seven years, exclusive of 1957, the proportion of positive or suspected reaction was discovered to be one percent. In these tests antigens prepared at our Etlik and Pendik Institutes were used. Technique used is described in our Regulations. Dilution percentages of sera are 1/25, 1/50 and 1/100. Rations of 1/50, 1/100 and 1/200 are obtained by adding 1 ml. antigen to 1 ml. serum dilution. Regulation at hand accepts 1/100 and above reactions in cattle as positive, and since there exists no provision for sheep, 1/50 agglutination is adopted as a positive criterium. Dr. Stableforth's method is adopted in the new Regulation with its full phases; and when the Regulation comes into force all laboratories in Turkey will follow it in their researches (2). Beşe (6) has

tested 479 sheep sera from Ankara Slaughter House and State Animal Husbandry positive, 3.96 % suspected cases.

2. *Plate - Agglutination Test :*

Our only source in this kind of work seems to be that of Beşe in his test with the USA Agricultural Department's Plate-Antigen in 179 sheep, whereas his figure for Tube-Agglutination was 44.6 %. This shows a better approximation in Tube-Agglutination Method. Erdöl (8) reported that 96 % agreement between this test and Tube-Agglutination Test, as a result of his test with cattle sera.

3. *Meinicke Test :*

We hear that in certain countries, including Germany, this test is still being used. In spite of its advantage in making the results available in a time as short as 45 minutes, it has never been used in Turkey either for cattle or for sheep due to the difficulty experienced in the preparation of a lipoid antigen.

4. *Complement - Fixation Test :*

According to my findings this test not been in use in our laboratories since 1942 with goat and sheep sera, but occasional references to this test in the case of cattle Brucellosis have been reported by Erdöl (8). Beşe (6) reported that a hundred percent agreement of Complement-Fixation Test agglutination test in every test he tried.

5. *Allergic Test :*

Başkaya (4) and Golem (10) reported good results in tests on cattle with allergens they themselves prepared: Aygün (3) reported that this test is easy to carry out also on sheep and humans and that is a reaction giving quick and accurate results. Beşe (6) obtained 67.3 % positive reaction on 179 infected sheep through intradermal injections of allergens prepared by Aygün (3), whereas his report of tube-agglutination reaction test, on the same animals, gives us 45.1 % recorded positive reaction. This indicates that in sheep allergic reactions carry more weight than the tube agglutination. In his tests with his own K. No. 12 allergen Beşe advises us to prefer intradermo-palpebral reactions to intradermal reactions for the following reasons :

- (a) Stronger formation of intradermo-palpebral allergic reactions in the case of sheep Brucellosis.

(b) Longer duration of allergic reactions.

(c) Convenience in comparative study and observation of the reaction by sight and easy reading and recording.

He also observed a slight increase in agglutination titres on the infected sheep which were injected with the same K. No. 12 allergen, a six-week-long persistence of agglutinin formation in healthy sheep due to allergen injected; and complete disappearance thereof at the eighth week. In agglutination and intradermo-palpebral allergic tests on 120 healthy sheep in a State Farm, Beşe recorded 3.30 % positive reaction by agglutination test as against 34.16 % infected sheep by allergic reaction. Studies and works based on these figures show us the superiority of allergic reaction in sheep Brucellosis to detection through blood sera.

6. Ring Test with Stained Antigen :

In his Brucellosis test by Ring Test on sheep, goats, cattle and water buffaloes in Bursa and Balıkkesir regions, as well as at two State Farms, Yalinalp (18) reports a larger amount of infection encountered amongst sheep. In a test, together with Duruşan (7), we found the ratio of determining the infection by Ring-Test in cows'milk to be eighty-one percent. I am of the opinion that, since we cannot blood-test all of the 55 million goats and sheep of this country, their herd-test using the Ring-Test, for the time being, will be of benefit in the eradication of Brucellosis. I also stick to the idea that goat and sheep milk, together with their derivatives, should be sample-tested at dairy farms in our Mediterranean and Aegean Coasts where these items are generously consumed; and findings in percentage of infection should be added to figures to be obtained through herd-test in order to get an idea of the extension and percentage amongst goats and sheep of Brucellosis in Turkey. I would like to state that my laboratory is ready to produce Ring-Test antigen sufficient in quantity for a country-wide survey and dispatch the same all over Turkey, if the higher authorities deem it proper that, pending the preparation of Brucellosis Regulation for goats and sheep, we adopt Dr. Bendsten's (5) antigen preparation and standardization method. I would also like to mention, in the meantime, the new research work carried on by Dr. Norrung (15) which is also based on Ring-Test and displays great advantages for use by practising veterinarians, being easy in application and giving quick results. Ring-Test antigen, which contains Tetrazolium is painted on a piece of card-board, stirred with a tooth-pick in saline and serum to be tested. The result is obtained within one minute.

7. Whey Test :

Golem (10) Reported that in his tests he observed the superiority of blood serum agglutinations to those of milk serum. I tested both by a method which I followed while in Dr. Morgan's (14) laboratory at Weybridge Institute and through lacto-sero Tube-Agglutination; the sample of milk belonged to certain sheep and cows whose blood sera I used in agglutinations without definite diagnosis because of zone phenomena and I observed the same result being produced by both reactions, and found out that these same animals were infected.

8. Culture :

Berke, Ozcebe, Erdöl, Golem (11) in Turkey are pioneers in isolating, for the first time, *Brucella* organisms from sheep, cattle and goats. In recent years, according to our laboratory records, we have been successful in isolating a total of 86 *Brucella abortus* and *melitensis* strains :

- 25 *melitensis* strains from aborted sheep fetuses
- 4 *melitensis* strains from sheep's milk
- 50 *abortus* strains from aborted cow fetuses
- 5 *abortus* strains from cow's milk
- 1 *melitensis* strain from aborted goat foetus
- 1 *abortus* strain from aborted water-buffalo foetus

Although I have not been able to isolate any *abortus* strain from sheep Brucellosis yet, I have isolated *Brucella melitensis* strain from two cow fetuses. The medium I used in isolating all these 86 *Brucella* strains is agar containing 10 % horse serum, dextrose and tryptose. In the cultural examination of milk and dirty-looking harmful materials we are using a medium containing crystal violet to a final concentration of 1/700,000. Volume of carbon dioxide is 10 %. Due to insufficient apparatuses and chemicals in our laboratories for years, we are not, unfortunately, in a position to use different media in our daily tests. In spite of all these, I see no objection to continuing with this medium, since there has been no case where we could not have isolated *Brucella* organisms from infected material belonging to fetuses, when the mother's blood serum proved to be infected or suspected. Beşe reports isolation of 8 *Brucella melitensis* strains in 179 infected sheep.

9. *Opsono-cyto-pathogenic Test :*

Beşe (6) reports 7.4 % positive reaction in tests on 189 sheep and leaves out this test as a complementary method for Brucellosis diagnosis in sheep.

10. *Vaginal Mucus Agglutination Test in Sheep :*

I propose to offer a brief summary of Keskin-tepe's doctorate thesis, which is yet to be published, as the latest research work in this country on sheep Brucellosis. Keskin-tepe (12) reports that at the onset of Brucellosis in the reproductive tract of sheep, the cells of these organs produce high titre agglutinin and these antibodies remain, for a short time, in the blood circulation at low titre. Antibodies produced in blood serum also pass into the vagina and uterus at low titre. In the vaginal mucus tests of 136 aborted sheep, whose tube-agglutination reaction by research blood serum proved to be negative, 8.1 % positive and 2.2 % suspicious reactions are recorded. It is, therefore, deemed important that mucus agglutination tests be carried out together with tube-agglutination, using serum, in herds of sheep for the eradication of Brucellosis. It may be thought, therefore, that the application of two tests in herds of sheep at State Breeding Farms may be troublesome; and that in order to diagnose reactors at the early stage of the infection, it is better to refer to agglutination test with sera and to subject the rest of the herd to mucus agglutination tests after the positive or suspected ones have been separated.

11. *Concentration of Salty Water (Saline) to be Used in Serological Tests :*

We have found, together with Durusan (7), 2 % zone phenomena in tube-agglutination tests on 401 cows sera. I obtained good results at zone phenomena which I came across also in sheep when I used 5% salty water as recommended by Renoux (16). Beşe (6) reported that in comparative agglutination tests with various salty water concentrations on 320 sheep sera best results were obtained by 6% salty water. In Brucellosis diagnosis amongst sheep, serum agglutination tests using 6% salty water should be carried out first and then a second blood test should be carried out two months after the injection of allergen.

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