

Detection and control of measures of Brucella from Zhan Hua sheep in Bin Zhou, Shandong Province

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Abstract: In order to find out the epidemic situation of Brucella sheep in Binzhou county, the serum samples collected from 13 large-scale sheep farms in Zhanhua District of Binzhou City were tested for brucellosis, and a questionnaire was conducted to investigate the breeding situation and the epidemic situation of brucellosis in the breeding farm. It provides strong support for the prevention and control of cloth disease in sheep farm in Zhanhua area. Brucellosis is a serious zoonosis caused by brucellosis, which is infected with sheep, cattle, pig, human and so on. Can cause pregnant female stillbirth or abortion, male animal orchitis. Causes intermittent fever, insomnia, general weakness, joint pain, muscle pain, neck inguinal lymph node swelling, testicular swelling. Women Patients may develop ovarian inflammation, mastitis, endometritis, pregnant women can have abortion [1].

1. Materials and methods

1.1 Investigation content

Serum samples were collected from 13 sheep farms and 100 sheep were collected.

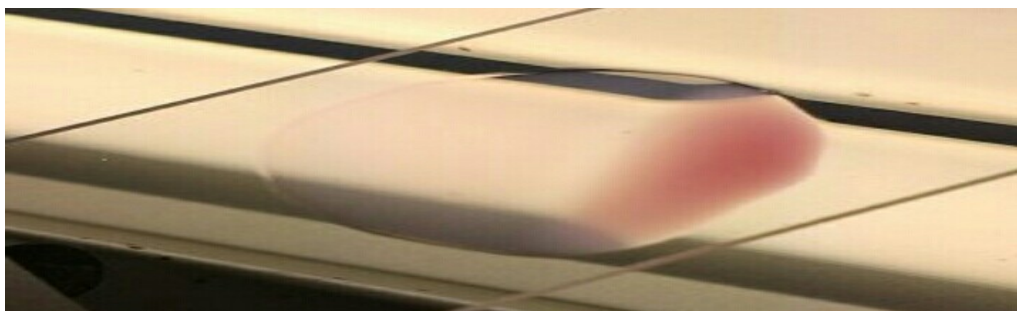
1.2 Tiger red plate agglutination test

The principle of Tiger Red plate agglutination experiment is to detect unknown antibodies in liquid by using known antigens. The specific binding of antigens and antibodies in vitro presents visible reactions [2]. Specific steps: centrifuge the blood with 4000r/min for 5 minutes, grab and add the serum tested by 25ul to the clean glass with moving liquid, shake the tiger red antigen reagent before use, then add the tiger red plate antigen of 25ul, and shake well with the cotton swabs. The results were determined in 5min. Results criteria: uniform pink, no agglutination, negative. There is obvious agglutination, the liquid between the agglutination blocks is cool and positive.

The following image shows positive and negative control pictures.



Positive (with agglutination granules)



Negative (uniform pink)

Fig.1

1.3 Test tube agglutination test

1.3.1 Dilution of tested serum

Diluent preparation: with Carbonic acid and sodium chloride solution to prepare 0.5% normal saline carbonate.

Each serum was labeled with 4 test tubes, numbered, first tubes added with 1.15ml diluent, and 2-4 tubes were added with 0.5ml diluent.

0.5ml is absorbed from the first tube and added to the second tube. After mixing evenly, the 0.5ml is added to the third tube from the second tube, so the ratio is diluted to the fourth tube, and the 0.5ml from the fourth tube is discarded and diluted.

After dilution, the dilution of serum from tube 1 to tube 4 was 1: 12.5 and 1: 25: 1: 50 to 1: 100, respectively.

Awareness, After full oscillation, the tube was placed in 37 °C incubator for 20 h.

The highest dilution of serum agglutination was serum agglutination and the titer of serum agglutination was above 1:50.

1.4 Immune colloidal gold assay

The observation results of sample dilution solution within 10-20 min were not effective after 20 min.

Positive: a red line appears in T (test line) and C (quality control line).

A red line C (quality control line) showed no color in T (test line).

no color on T (test line) or C (quality control line).

Below: positive and negative controls



Fig.2

2. Result analysis

20 positive samples were detected by the tiger red plate test, and 18 positive samples were detected by colloidal gold method. 80 negative samples were detected by 90% tiger red plate negative test. 80 negative samples were detected by colloidal gold method. 18 positive samples were detected by 100% test tube agglutination test, and 18 samples were detected by colloidal gold method, and the coincidence rate was 100%.

3. Differences of three inspection methods

The results of three test methods showed that the results of colloidal gold assay were the same as that of test tube agglutination test, and the results of tiger red plate test and colloidal gold method were close. Tiger red plate agglutination experiment operation is simple, the price is cheap, can detect quickly, suitable for the quarantine of large population. The test tube agglutination test is one of the commonly used serological diagnostic methods, its sensitivity and specificity are high, but the operation steps are complicated. And the test tube agglutination experiment and the tiger red plate experiment can not be detected in situ, but the colloidal gold method can be detected in the field, which saves time and improves the test result. And has good specificity, stability, sensitivity and other advantages [3].

4. Bacterial culture and drug sensitivity test

4.1 Isolation and culture of bacteria

Both the tiger red plate test and the colloidal gold test showed positive blood 15mL. The blood culture bottle was cultured in the blood culture analyzer. The blood culture analyzer alarm indicated the presence of bacteria in the blood. When the blood was inoculated on the blood Agar plate and cultured in the CO2 incubator for 48 hours, the smooth colony was formed with gray circle and tiny protuberance.

4.2 Gram staining and oil microscopy

Dip physiological saline with inoculation ring on the clean slide, then take a single colony on the slide and smear it on the slide to form about broad bean film. Dry and fix it over the flame. Wash the crystal violet for one minute. Lugo's iodine coal was stained with water for one minute, 95% ethanol was decolorized for 30 seconds, then diluted and restained for one minute. After drying, it showed Gram-negative streptobacillus red and other bacteria showed blue.

4.3 Drug sensitivity test.

4.3.1 Preparation of bacterial solution

A single bacterial colony was dipped in sterile saline with sterile cotton swab. The bacterial concentration was measured by WGZ-2XJ turbidimeter produced by Shanghai Xin Rui instrument Co., Ltd.

4.3.2 Bacterial inoculation

Dip the sterile cotton swab into the surface of MH Agar with bacterial suspensions, dry for 3 minutes, and then stick the drug sensitive paper. The paper was not less than 15mm from the edge of the culture medium, and the distance between the paper and the paper was not less than 24mm. The paper was incubated in a constant temperature incubator at 37 °C for 18 hours, and the results of drug sensitivity were observed [4].

4.3.3 Drug sensitivity test

All 12 kinds of drug sensitive paper tablets are purchased from Hangzhou microbes with a diameter of 5mm. They are: cephalosporin, kanamycin, streptomycin, gentamicin, chloramphenicol, erythromycin, tetracycline, cephamycin, cephalosporin, penicillin, ciprofloxacin, Doxycycline.

Table 1 Comparison of susceptibility of 18 strains of *Brucella* sheep to 12 antimicrobial agents

medicinal	sensitivity		intermediary		drug		bacteriostasis (mm)	circle
	Plant number	%	Plant number	%	Plant number	%		
cephalosporin	10	55.6	5	27.8	3	16.7		20.8
kanamycin	14	77.8			4	22.3		24.3
streptomycin	16	88.9	1	5.56	1	5.56		18.9
gentamicin	16	88.9	2	11.1				20.8
Chloramphenicol	18	100						31.2
erythromycin	15	83.3	2	11.14	1	5.56		22.5
tetracycline	16	88.9	1	5.56	1	5.56		27.8
cephamycin					18	100		0
cephalosporin	12	66.7	3	16.7	3	16.7		17.0
penicillin			1	5.56	17	94.4		4.25
ciprofloxacin	10	55.6	5	27.8	3	16.7		23.5
Doxycycline	16	88.9	2	11.1				22.6

Sensitive: The diameter of antimicrobial circle was $\geq 20\text{mm}$; Medium sensitivity: the diameter of medium sensitivity was $10\text{mm} \sim 20\text{mm}$; Drug fast: the diameter of antibiotic circle was $\leq 10\text{mm}$.

The results showed that all strains were sensitive to gentamicin, chloramphenicol and doxycycline, and the average diameter of inhibition circle was 24.5. Tetracycline, streptomycin, kanamycin, ciprofloxacin, erythromycin and highly sensitive bacteria were all above 80.5.

Four antimicrobial agents (gentamicin, chloramphenicol, doxycycline, tetracycline) were selected to form six combinations of antimicrobial agents (gentamicin, chloramphenicol, doxycycline and tetracycline). No synergistic effect was found in other combinations, especially chloramphenicol and tetracycline, chloramphenicol and doxycycline. In order to improve the curative effect, antibiotics with good synergistic effect can be used in clinic.

5. Suggestions and countermeasures

After investigation, it was found that the incidence of brucella in sheep and human beings in Zhan Hua District of Bin Zhou occurred from time to time, and had a rising trend year by year, which seriously endangered the lives and property of the people. Therefore, the prevention and control of brucellosis has become urgent. The following suggestions are put forward.

5.1 Strengthen government supervision.

In order to further improve the national brucellosis prevention and control work, the National Health and Family Planning Commission formulated the National Brucellosis Control Plan. The notice clarifies the principles, techniques and management measures for brucellosis control. However, the implementation of regional programs is not strict, resulting in brucellosis, seriously endangering people's lives and property safety. Therefore, the local government should strengthen the supervision of brucella, carry out the measures stipulated by the state, and ensure the safety of people's life and property.

5.2 Strengthen the construction of grass-roots epidemic prevention team, carry out epidemic situation monitoring and incinerate and eliminate dead animals.

Through the investigation, it was found that the primary epidemic prevention station was in a semi-paralyzed and unmanaged state, which caused frequent movement of unquarantined livestock and caused brucellosis to pick up and spread. Therefore, it is imperative to expand the ranks of basic-level epidemic prevention personnel and increase the welfare of basic-level epidemic prevention personnel at the same time. Strengthen team building, hold regular training courses every year, invite experts to answer questions on the spot and improve the technical skills of practitioners. The sheep were quarantined regularly and the brucellosis positive cattle were culled and burned so as to cut off the route of transmission and avoid the outbreak.

5.3 Carry on face-to-face propaganda and education to raise people's awareness of disease prevention.

While investigating the incidence of sheep, by talking to herdsmen, we found that many herdsmen had been infected or were being infected with brucella, but their awareness of disease prevention was extremely poor and they did not take medicine or take medicine intermittently according to the course of treatment provided by the doctor. So that drug resistance, cause the onset of prolonged, long-term treatment, many herdsmen caused serious joint pain sequelae, perennial suffering from pain. Through various channels of propaganda, television broadcast, medical staff went to the countryside to talk about the harm of brucella, improve the awareness of disease prevention among the masses, so as to consciously cooperate with the work of prevention and control.

5.4 Strengthen the management of the herding market and improve the quarantine system.

Sheep imported from Inner Mongolia Autonomous region, without quarantine and quarantine directly into the local sheep, resulting in cross-infection. Therefore, the newly introduced sheep must be quarantined for at least 30 days and then mixed with local sheep after quarantined. [5]

5.5 Doctors, veterinarians help each other and work together.

As brucellosis is a serious zoonosis, the key to control it is to prevent the disease, so doctors and veterinarians should cooperate with each other. Animal husbandry and veterinary departments do well in animal brucellosis vaccination, epidemic detection, incineration and elimination of dead animals. Health personnel propagandize herdsmen's awareness of disease prevention, wear work clothes, gloves, face masks, hand washing and disinfection in contact with livestock, and strengthen personal protection is also an important measure to prevent brucellosis. At the same time, the serological test of herdsmen, brucella positive people must be actively treated, accept the regular drug taking procedure, to avoid the spread and spread of the disease.

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