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DIAGNOSIS OF ANIMAL BRUCELLOSIS BY ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA) METHOD

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Annotation: In the Turg'unboy Shokirov livestock-specialized farming enterprise located in Asaka district of Andijan region, a total of 158 out of 750 cattle were tested for brucellosis using the ELISA (Enzyme-Linked Immunosorbent Assay) method. As a result, 23 blood samples tested positive, while 128 samples were found to be negative. Seven blood samples were hemolyzed and deemed unsuitable for testing.
Keywords: cattle, infection, brucellosis, serology, farm, positive, negative, blood, region, disease.

Relevance of the Topic: Brucellosis is a chronic, acute zoonotic infectious disease. The causative agent of the disease is a bacterium belonging to the *Brucella* genus, which is highly resistant to environmental factors. Animals become infected through damage to the mucous membranes of the skin, digestive, and respiratory tracts. Humans are commonly infected by consuming unpasteurized animal products (such as milk and meat) or through direct contact with infected animals. The disease is characterized by lesions in the reproductive, nervous, cardiovascular, and skeletal systems, and is difficult to treat.

Currently, the World Organisation for Animal Health (WOAH) requires the use of rapid and accurate diagnostic methods such as ELISA (Enzyme-Linked Immunosorbent Assay) to assess the health status of animals with respect to brucellosis. Therefore, it is crucial to ensure adequate supply of these diagnostic tools. Brucellosis poses a significant threat to the livestock sector. It is well known that brucellosis in farm animals causes major economic losses in agriculture and is a pressing issue due to the risk it poses to human health. In the field of veterinary medicine, insufficient diagnostic testing and a shortage of brucellosis diagnostic tools exacerbate the problem, contributing to the wider spread of the disease and increasing the risk of transmission to humans.

Materials and Methods: The ELISA tests were conducted at the Brucellosis Laboratory of the Veterinary Research Institute and the Laboratory for the Development of Veterinary and Animal Husbandry in the Andijan region. Blood samples were collected from 158 cattle out of a total of 750 (including 42 calves) at the "Turg‘unboy Shokirov" livestock farm in the Andijan region. The samples were analyzed in the laboratory using the standard "sandwich" ELISA method.

The detection principle is based on the indirect ELISA (IFT) technique for identifying IgG antibodies against *Brucella* antigens in cattle. *Brucella* antigens were immobilized on the inner surfaces of the microplate wells. The antibodies in the blood samples of the cattle bind to these antigens. The resulting immune complex is detected using a conjugate of mouse monoclonal anti-bovine IgG antibodies and horseradish peroxidase (HRP). This forms an enzyme-linked "sandwich" structure that is bound to the plate.

During incubation with the substrate solution tetramethylbenzidine (TMB), a color change occurs in the wells. The intensity of the color is directly proportional to the amount of specific IgG antibodies against the *Brucella* antigen present in the sample.
Any version of the ELISA reaction includes three essential stages:

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1. **Binding stage** – Specific antibodies in the test sample form an immune complex with the corresponding antigens.
2. **Conjugation stage** – The immune complex binds with the conjugated antibodies or enzyme complexes.
3. **Enzymatic reaction stage** – Complex protein molecules (enzymes) accelerate chemical reactions and generate a detectable signal, providing information about the presence of the target antibodies. This is the general principle of the ELISA method and was strictly followed throughout the procedure.

The samples were tested at the Veterinary and Animal Husbandry Development Laboratory of Andijan region. The testing was performed under controlled laboratory conditions with ambient temperatures ranging from +18°C to +25°C. Prior to testing, the ELISA kits, which had been stored unopened in a refrigerator at +4°C to +8°C, were brought to room temperature (+20°C to +25°C) and left for 30 minutes.

From the 158 cattle, 5 ml blood samples were collected and sera were separated. The detection of *Brucella* infection and evaluation of immune response were conducted based on the indirect ELISA principle using IgG and IgM antibodies. In the tested samples, antigens in the microplate wells bound with antibodies specific to *Brucella* found in the serum. In cases of infection, the first detectable antibody in the cattle serum is IgA. Among ELISA techniques, the sandwich method is considered the most widely used and effective for brucellosis diagnosis [28; pp. 34–38, 29; pp. 3–42].

Research Results At the Brucellosis Laboratory of the Veterinary Research Institute and the Laboratory for the Development of Veterinary and Animal Husbandry in the Andijan region, blood samples were collected from 158 cattle out of a total of 750 (including 42 calves) at the "Turg‘unboy Shokirov" livestock farm located in the Asaka district. The laboratory testing of these samples yielded the following results (Table 1).

Diagnosis of brucellosis in cattle at the Turg‘unboy Shokirov farm in Andijan region using the ELISA method

1-Table

T/R	Name of the farm	Headcount of inspected cattle	Inspection results					
			Against brucellosis					
			Positive result		Negative result		Hemolysis	
			Number of heads	%	Number of heads	%	Number of heads	%
1	Andijon Turg‘unboy Shokirov	158	23	14,5	128	81,0	7	4,43

According to the analysis of the results from Table 1, in Andijan region, from the cattle of the Turgunboy Shokirov farm, blood samples were taken from a total of 158 out of 750 cattle.

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When IFT analysis was conducted on these samples, 23 samples were found to be positive, which accounted for 14.5%. Negative results were found in blood samples taken from 128 cattle, making up 81.0%. Seven blood samples were found to be unsuitable for testing due to hemolysis, accounting for 4.43%.

Conclusions

1. When 158 blood samples from the Turg‘unboy Shokirov farm in Andijan region were tested using the IFT method, 23 samples tested positive, indicating a result of 14.5%.
2. It was found that 128 of the blood samples taken from the cattle of this farm were negative, which corresponds to 81.0%.
3. It was identified that 7 of the blood samples had undergone hemolysis.

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