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Study of Diagnostic Methods and Control Measures For Listerosis Disease Among Big-Horned Animals in the Ganja-Gazakh Zone

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Abstract. *The purpose of the research is to study the diagnostic methods and control measures for listeriosis among cattle in farms of the Ganja-Gazakh zone. The research was conducted in various farms located in Shamkir, Samukh and Goygol districts, in the laboratory of the Department of "Episootology, Microbiology and Parasitology" of the Faculty of "Veterinary Medicine" of the Azerbaijan State Agrarian University of Veterinary Medicine. Depending on the immune status of the organism and the virulence of the pathogen, the disease manifests itself in acute, and subacute forms among animals. Based on the high efficiency of their application in combination with sulfagin, gentomycin and oxytetracycline for the specific treatment of the disease, we recommend their use for the prevention of listeriosis before vaccination. In case of listeriosis, along with specific treatment, we carried out symptomatic treatment. We carried out disinfection, disinsection and deratization works in livestock farms in an organized manner.*

Keywords: *cattle, disease, listeriosis, bacteriological examination, morphological, cultural, biochemical, antigenic, pathogenic properties*

Introduction

Listeriosis is an infectious disease of animals and humans caused by the bacteria Listeria monocytogenes and is characterized by nervous system disorders, septic conditions, puerperal disease and mastitis. Listeriosis is caused by the bacterium Listeria monocytogenes, which possesses multiple virulence factors that enable it to invade host cells, evade immune responses, and spread systemically (Vázquez-Boland & O'Brien, 2011). An asymptomatic (latent) form of the disease is also observed. Many species of domestic and wild mammals, birds and humans are affected. It can occur as a secondary or mixed infection in a number of infectious diseases in pigs and birds. The disease has been recorded in Azerbaijan (Shirinov, 2002; Eyubov, 2005).

Many scientists have determined that the occurrence of listeriosis in various types of animals and birds depends on the amount of precipitation, the nature of the feed, pH, the degree of contamination of the soil with the causative agent of the disease, the presence of antagonistic microflora in the soil and the degree of virulence of the causative agent of listeriosis. P. R. Lazarev, V. I. Gershun and I.I.Guslavskaya show that incompleteness of the feed ration due to cold and soluble protein, as well as changes in the pH of silage, reduce the overall resistance of animals and increase their susceptibility to listeriosis (Kalinin, 1987).

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Listeriosis causes a decrease in animal productivity, causes metritis during calving, and causes great economic damage. At the same time, it causes a certain amount of financial resources to be spent on various health measures (Mammadli, 2015).

Listeriosis is a zoonotic disease of animals and humans, characterized by polymorphism of clinical signs or asymptomatic carriage and high mortality. M.M. Halimbayov, A.A. Annaghiyev, M.G. Ganiyev, A.M. Khalilov, A. Khankishiyev, H.A. Rahimova, Y. Ahmadov, C.N. Mammadova, A.G. Abbasov, F.M. Gulubayov played a special role in studying the epizootology, pathogenesis and specific prophylaxis of listeriosis in various types of agricultural animals in the Republic of Azerbaijan. In our country, the first vaccine against listeriosis of agricultural animals in world veterinary practice was developed and this preparation was used in the specific prophylaxis of listeriosis of sheep in 1965–1978. In 1980, a colored antigen was prepared for the diagnosis of listeriosis of agricultural animals with AR (A. A. Annaghiyyev, E. A. Aliyev, F. M. Gulubeyov), an author's certificate was obtained for the diagnostic, and it is currently used in both veterinary and medical practice (Aliyev, 2013; Mammadli, 2020).

Listeriosis is an infectious disease characterized by meningoencephalitis, balanitis, metritis, mastitis, septicemia. The incubation period lasts from one week to 1 month. Listeriosis occurs in septic, nervous, genital and atypical forms. Factor (0.3-0.5 and 0.5-2.0 μm) is a bacterium located singly, in pairs or in clusters. In some cases, bacteria occur in polymorphic rod, cocci, vibrio forms. Bacteria stain positively with all aniline dyes and the Gram method. Microbes do not form capsula and spores. In fresh (4-6 hours) broth culture, bacteria are motile. On meat-peptone agar-agar, bacteria form thin, fleshy colonies similar to Pasteurella, with a diameter of 1-1.5 mm (Lysak, 2007).

When studying culture strains obtained from various sources by I.A. Bakulov, O.V. Krivonosov, I.V. Gershun, it was determined that there are both virulent and weakly virulent variants of listeria. In addition, various literature data show that external environmental factors play an important role in the occurrence and course of listeriosis (Bessarabov, 2007).

The disease has a mortality rate of up to 40%, and causes considerable damage to agriculture as a result of reduced productivity and calving in animals. In addition, a system of various health measures is used, which leads to the expenditure of a certain amount of financial resources (Gadimov, 1990).

Materials and Methods

To achieve the set goal, we selected 26 sick animals related to the red desert and local breeds, divided them into 6 groups of 4 animals in each group, and conducted scientific research on them. The research work was carried out in various cattle farms of the Ganja-Gazakh zone (Shamkir, Samukh and Goygol), at the Department of Epizootology, Microbiology and Parasitology of the Azerbaijan State Agricultural University. In recent years, the climate in the Republic of Azerbaijan has changed dramatically, the amount of precipitation has decreased and drought has increased. As a result, the composition of feed in many regions has changed, its quality has deteriorated, and the number of insects and rodents has increased. As we know, the main source of infection is various wild and domestic animals, especially rodents, birds and insects, which carry the causative agent of the disease (Swaminathan & Gerner-Smidt, 2007). The microbe enters the body through the mucous membranes (nose, conjunctiva, mouth) through water, feed, air, dust, etc. It is assumed that infection also occurs through blood-sucking insects, especially ticks.

As a result of all these factors, listeriosis was recorded among cattle in various farms of Shamkir, Samukh and Goygol regions. Based on the appeal of the heads of these farms, employees of the

Department of Epizootology, Microbiology and Parasitology of the ASAU were engaged in studying the occurrence, course, treatment and many other issues of the disease.
It turned out that the farms mentioned were healthy due to listeriosis and had no contact with unhealthy farms. Listeriosis infection was recorded in August-October 2022. The disease was mainly observed in 18-28-month-old heifers, and infection was not detected in the causative bulls. The incubation period of the disease is 7-30 days. In addition to fever, general weakness, and loss of appetite, some animals showed signs of central nervous system dysfunction, including loss of balance, convulsions, severe nervous excitability, paralysis of some muscle groups, neck flexion, and conjunctivitis. In a group of sick animals, blindness, stomatitis, anemia of visible mucous membranes, and comatose state were noted.

Listeriosis mainly has an acute, subacute, and chronic course. Listeriosis occurs in several clinical forms: nervous, septic, mixed, latent-asymptomatic, with damage to the reproductive system (parturition, delayed ejaculation, endometritis, and metritis) and udder (mastitis). In large horned animals, the CNS is more affected. The disease begins with weakness and loss of appetite. Serous - mucous discharge occurs from the nasal cavity, and abundant mucus is secreted from the mouth. After 3-7 days, uncontrolled movements, convulsions, fits of excitement, paresis of individual muscle groups, loss of vision, conjunctivitis, stomatitis are observed. Body temperature rises at the beginning of the disease or remains within the normal range. Listeriosis in calves proceeds in the form of septicemia, sometimes accompanied by damage to the CNS.

Results and Discussion

From the research work we conducted, it became clear that the pathological-anatomical changes in large horned animals were mainly sharp changes in the brain, especially the engorgement of cerebral blood vessels, blood leakages were observed on the brain and cerebellum. In the cranial cavities and ventricles of the brain, a cloudy fluid mixed with pus was seen. To diagnose listeriosis, we took into account its epizootological characteristics, clinical signs and pathological-anatomical changes, used blood culture, bacteriological examination and serological reactions. While the animal was alive, we used the blood of the sick animal (5-10 ml), the discharged pupa, pupal discharge, pupal membrane, nasal passages, conjunctival fluid, and milk taken from the inflamed scrotum. We also sent the brain and spinal cord to the laboratory for examination.

We examined the pathological samples received in the laboratory based on the bacteriological scheme. Bacteriological examinations play an important role in the diagnosis of listeriosis. During microscopy, we prepared a smear from the pathological material and stained it using the Gram method. During microscopy, listeria were found singly or in pairs. In some cases, we determined that listeria were observed in coccus-like, diplobacteria, rod-shaped, and chain-like forms.

We obtained a pure culture of the factor by inoculating the blood of sick animals from the parenchymatous organs of dead animals into various nutrient media, prepared smears on glass slides, examined them under a microscope, and used biological tests. After obtaining a pure culture of the listeriosis pathogen, it was examined for its morphological, cultural, tinctorial, and biochemical properties, and its pathogenicity was determined. It turned out that the pathogen forms a precipitate in liquid nutrient media and, when shaken, rises up like a hair, resembling small, transparent dewdrops in solid nutrient media.

Table 1.
Treatment of cattle with listeriosis

Treatments	Injection method	Dose (once)	Number of patients	Conclusion	
				Survived quantity	%
Sulfagin and gentomis	Perosal and intramuscular	25 g+2.5 ml	4	4	100
Sulfagin and oxytetracycline	Peros	25 g+2.5 ml	4	4	100
Gentomycin	Intramuscular	2.5 ml	4	3	75
Oxytetracycline	Intramuscular	2.5 ml	4	3	75
Sulfagin	Peros	25 g	4	2	50
Control	–	–	4	4	–

As can be seen from Table 1, while sulfagin in combination with gentomycin and oxytetracycline achieves 100% recovery, only 50-75% of animals recover from the use of these drugs alone. Based on the high efficiency of sulfagin in combination with gentomycin and oxytetracycline, we recommend their use for the prevention of listeriosis before vaccination. In the case of listeriosis, along with specific treatment, we carried out symptomatic treatment. The main goal of symptomatic treatment is to stimulate the functional activity of the cardiovascular system and the digestive system. In order to ensure the healthy and vigorous development of large-horned animals, and to prevent them from contracting listeriosis, we set ourselves the goal of timely implementation of preventive measures. One of such measures and the main one is timely and high-quality vaccination of animals with the appropriate vaccine.

According to researchers, individuals who have contracted listeriosis and recovered from it in natural conditions develop relative immunity. Precipitins, agglutinins, and complement-binding antibodies are detected in the blood serum of animals that have recovered from the disease naturally. However, the serum of convalescents does not have therapeutic properties. The first vaccine against the disease was prepared by A. A. Annagiyev from the “A” strain. A dry live vaccine prepared from the “AUF” strain was proposed in 1974 and is used based on the current instructions (Alasgarov, 2016).

We vaccinated all clinically healthy animals 10 days after treatment with a dry vaccine prepared from the AUF strain against listeriosis. We vaccinated 687 cattle on farms according to the instructions. We injected the vaccine intramuscularly twice with an interval of 10 days. We periodically sent samples to the laboratory to determine the quality indicators of feed and water. We applied the feed ration, which is important for feeding large horned animals. We implemented the solution of the following issues in disease prevention. In order to ensure the protection of healthy livestock farms from the disease due to listeriosis:

- We ensured that animals brought to farms were kept in quarantine for 30 days.
- We ensured the systematic elimination of rodents.
- We eliminated blood-sucking insects and ticks.
- Ensuring constant quality control of feeds (especially silage and mixed feed)
- We ensured the conduct of bacteriological examinations in accordance with the relevant requirements.
- In cases of abortion or stillbirth, we implemented a strict approach and sent pathological samples for examination.

We boiled milk from large horned animals (with a positive result in serological reactions) for 15 minutes. We banned the transportation of feed that had been in contact with sick animals. We carried out disinfection, disinfestation and deratization work in livestock farms in an organized manner. We

biothermally neutralized manure on the farm. We disinfected stables and farmyards. For this purpose, we used 3% sodium hydroxide, 2% chlorinated lime solution, and 6% creolin emulsion. We periodically carried out deratization measures on the farm. We lifted the restriction 2 months after the last patient recovered from the farm and after receiving a negative result during serological tests.

Conclusion

The results of the study showed that 100% recovery was achieved when sulfagin was used in combination with gentamicin or oxytetracycline for the treatment of sick animals in unhealthy farms due to listeriosis. During the research work we conducted, we vaccinated all animals susceptible to listeriosis in unhealthy farms with a dry vaccine prepared from the AUF strain according to the relevant instructions. For the prevention of listeriosis, animals should always be brought to the farm from healthy farms. We always monitored the quality of feed and fought against rodents. We used preventive measures when the disease was observed on the farm.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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