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Article

Study of Antibiotic Sensitivity of Pathogenic and Vaccine Strains of Brucella

Zamira **Mamatova[1], *Odil* Achilov[2], *Adkham* Khudjamshukurov[3], *Farangiz* Yuldasheva[4]**

1. PhD, Associate professor, Department of Birds, Fish, Bees and Fur-bearing Animals Diseases, Samarkand State University of Veterinary Medicine, Livestock and Biotechnologies, Samarkand, Uzbekistan
2. PhD, Associate professor, Samarkand State University of Veterinary Medicine, Livestock and Biotechnologies, Samarkand, Uzbekistan
3. PhD, senior teacher, Department of Epizootology and Infectious Diseases, Samarkand State University of Veterinary Medicine, Livestock and Biotechnologies, Samarkand, Uzbekistan
4. PhD, Assistant, Department of Pathological Physiology, Samarkand State Medical University, Samarkand, Uzbekistan

* Correspondence: -

Abstract: Antibiotic resistance of infectious disease pathogens to antibiotics prescribed to combat them is a pressing issue for researchers – microbiologists in terms of determining the correct treatment protocol for many human and animal infections. Irrational use of antibiotics leads to the emergence of new mutated strains or antibiotic-resistant pathogens. This article presents the results of a comparative analysis of antibiotic sensitivity of pathogenic Br. abortus 99 and vaccine Br. abortus 19 strains of Brucella to 15 antibiotics of various origins. This article presents the results of a comparative analysis of the antibiotic sensitivity of pathogenic Br. abortus 99 and vaccine Br. abortus 19 strains of Brucella to 15 antibiotics of various origins. It was established that the vaccine Br. abortus 19 and pathogenic Br. abortus 99 strains of Brucella, which are not exposed to environmental factors, factors of the body's immune system and the surrounding microbiological biotome of the body, exhibit sensitivity to all antibiotics used in the experiment, and also that the vaccine strain Br. abortus 19 demonstrates greater sensitivity to the 6 antibiotics used in the experiment (rifampicin, ciprofloxacin, sisomycin, ofloxacin, ceftriaxone, azithromycin) out of 15 than the pathogenic strain Br. abortus 99.

Keywords: brucellosis, antibiotics, antibiotic resistance, vaccine strain, pathogenic, virulent.

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1. Introduction

Brucellosis is an acute and chronic disease of agricultural, domestic, wild, exotic and ornamental animals, as well as humans. New cases of brucellosis in animals should be reported to the World Animal Health Information System, as the spread of the disease in animals leads to an increase in the incidence of the disease in humans. The highest incidence rates are observed in the Middle East, the Mediterranean region, sub-Saharan Africa, China, India, Peru and Mexico, with a significant increase in cases in Central and South-East Asia. In contrast, countries in Western and Northern Europe, Canada, Japan, Australia and New Zealand are considered free of the infectious agent[1].

The view that poultry are immune to the disease is not entirely true. Brucellosis is a bacterial, rarely seen in birds, infectious and allergic disease accompanied by septicopoiesis. In natural conditions, the disease is extremely rare in chickens and even

rarer in turkeys. Birds of prey - scavengers found on the territory of Uzbekistan: black vulture, white-headed vulture, Himalayan vulture, bearded vulture and Egyptian vulture, feeding on meat of animals fallen from the disease, aborted fetuses for a long time in their bodies can retain pathogens of many infections, including brucellosis, transport, spread it for many kilometers around the focus of infection, which in many cases causes the emergence of brucellosis infection in previously brucellosis-free areas.

For clinical signs of the disease to appear in the organism of a bird (rather resistant to infection, which is carried out mainly orally), a rather large dose of the pathogen (Br. abortus, Br. suis, Br. melitensis) is necessary[2]. The clinic of brucellosis in birds is most often manifested by the following signs: decreased appetite, anemia of beards and scallops, swellings in the area of eyes and joints, arthritis, decreased egg production, deterioration of shell quality and appearance of shellless eggs, emaciation of birds of various degrees of severity.

Patanatomic signs of brucellosis in birds are expressed by emaciation of carcasses, anemia and serous-fibrinous arthritis. Spleen is enlarged, pale coloured and thickened, with small necrotic nodules. Similar changes are also found in the liver. Sometimes there are dystrophic and inflammatory changes in the ovaries, oviducts and testes.

The disease development is also favoured by a decrease in resistance of the birds' organism. No special seasonality in registration of infection in birds was observed, although brucellosis of birds is more severe in winter[3].

During serological monitoring by microagglutination reaction in poultry in Africa in 36% of cases antibodies in titre 1:40 and higher to Br. abortus were detected, which indicates the role of birds in the emergence and spread of infection in nature.

In many cases, the disease is caused by disease in dogs in previously brucellosis-free areas.

Canine brucellosis is an infectious disease caused by the microorganism Brucella canis, which is a frequent cause of reproductive disorders. B. canis is the sole species of Brucella peculiar to canines. Furthermore, this species has been observed to have a detrimental effect on wild canines such as foxes and coyotes, as well as on primates including chimpanzees, and on rodents such as mice, rabbits and guinea pigs[4].

Clinically, the disease manifests itself as abortion (miscarriage), stillbirth, epididymitis, orchitis, and abnormalities in spermatogenesis. In castrated animals, clinical signs may include eye lesions and discospondylitis.

In chronic cases, atrophy of the testes and infertility in male dogs have been observed. Lymphadenitis is a prevalent condition in canines. In the case of an oral infection, the pharyngeal lymph nodes are enlarged, while in the event of a vaginal infection, the external inguinal and external iliac lymph nodes are enlarged. Generalised lymphadenitis has also been observed.

Brucellosis, a disease caused by the bacterium Brucella, has the potential to affect canines of all breeds. The occurrence of animal fatalities is an infrequent phenomenon, with the exception of foetuses and newborn puppies. B. canis has been found to be excreted with semen, with the highest concentrations observed during the first six months following infection[5]. It is important to note that other potential sources of the pathogen include the foetus, placenta, amniotic fluid, vaginal discharge following abortion or stillbirth (for several weeks), and normal vaginal discharge, especially during heat.

The bacterium may be found in various bodily fluids and secretions, including urine, milk, saliva, nasal and eye discharge, and faeces. In the majority of cases, infection is transmitted either sexually or through contact with an aborted foetus and its membranes. Infection in puppies can occur in the intrauterine environment or via the milk. It is important to consider the potential for infection through blood transfusions and contaminated syringes as a separate, possible route of transmission[6].

The transmission of Brucella to dogs can occur through mechanical means. The organism under scrutiny can persist for months in water, aborted foetuses, on equipment

and clothing; it can survive desiccation, especially in organic substrates. The persistence of brucellosis is also favoured by low temperatures and a lack of sun exposure.

The diagnosis of brucellosis in animals can present a significant challenge, therefore the employment of multiple diagnostic methods is recommended, encompassing bacteriological examination, PCR and serology. The glass and in vitro agglutination reaction is the most common method of determining antibodies. ELISA, complement binding, immunofluorescence, immunochromatography and immunoelectrophoresis are, however, utilised with less frequency.

Serological diagnostics have been shown to be a valuable tool in the diagnosis of brucellosis, especially in resource-constrained settings. These assays permit rapid and relatively inexpensive detection of antibodies directed against brucellae, although they may raise issues of sensitivity and specificity[7-8].

The polymerase chain reaction (PCR) has been shown to be a highly effective method of detecting abortive Brucellae, due to its high levels of sensitivity and specificity. The insertion sequence IS711, which is specific to the genus Brucella, facilitates accurate identification and differentiation of species within this genus, thus serving as a valuable instrument for diagnostic purposes and genetic characterisation. The Bruce ladder, a multiplex PCR method developed for the identification and differentiation of Brucella species, including those used for vaccines, was first described in detail by Lopez-Goni et al. in 2008.

Brucellosis is a zooanthroponotic disease; in many cases, the source of infection for humans is animals and products obtained from them. People can also become infected during the handling of sick livestock, processing meat from sick animals.

The ongoing challenge of brucellosis underscores the necessity for enhanced collaboration between public health and veterinary sectors. The non-implementation of mandatory vaccination programmes on certain farms, particularly within the private sector, in conjunction with inadequate biosecurity measures, substandard farm infrastructure, constrained resources, and unregulated movement and insufficient testing, represent significant contributing factors to the epizootiology of brucellosis[9]. The limited awareness among rural populations further complicates the effective control of the disease.

The prevention and control of brucellosis should be based on the implementation of a set of veterinary, sanitary and medical-sanitary measures aimed at reducing and eliminating the incidence of brucellosis in farm animals. Particular attention is paid to proper and timely removal of afterbirths and aborted foetuses, as well as manure.

Treatment of farm animals shall be carried out in a non-targeted manner. Treatment of brucellosis of other animal species is complicated, complex and prolonged. The main method of brucellosis treatment is etiotropic antibacterial therapy, e.g., for dogs a combination of two different antibiotics is used. In parallel, non-steroidal anti-inflammatory drugs, antihistamines, B vitamins and physiotherapy are prescribed.

In most cases, antibiotics are used inappropriately and irrationally, sometimes without determining the antibiotic sensitivity of the isolated microbe to antibiotics, which leads to the emergence of antibiotic-resistant strains of pathogenic microbes. The situation is aggravated by the fact that in veterinary medicine antibiotics are used to increase the weight of fattened animals and poultry, which leads to the emergence of new mutated strains or antibiotic-resistant pathogens of infectious diseases[10-11]. At present, antibiotic resistance (insensitivity or resistance of infectious agents to antibiotics prescribed to combat them) is an urgent problem not only for veterinary, medical specialists and scientists - biologists, but also for representatives of pharmaceutical industry.

Taking into account the relevance of the above-mentioned, we aimed our research at comparative determination of antibiotic sensitivity of pathogenic and vaccine strains of brucellae deposited in the museum of the Regional Diagnostic Laboratory of the Veterinary Research Institute.

2. Materials and Methods

The work was conducted by the regional diagnostic laboratory of the Veterinary Research Institute. The strain Br.abortus 19 was selected as a vaccine strain, and Br. abortus 99 as a pathogenic strain. Their morphological, tinctorial, culture, biochemical, serological and pathogenic properties were studied according to standard methods. Brucellae were grown in complex enriched nutrient media (meat-peptone-liver-glucose-glycerol broth and agar at pH 6.8 - 7.2, temperature - 35 - 37 °C.

Considering that museum strains of B. abortus were used, the cultures were grown under aerobic conditions. Biochemical activity was studied in relation to D-ribose, alanine, glutamine, asparagine, urease, lipase, amylase, catalase and hyaluronidase.

Antibacterial activity was studied in relation to tetracycline drugs - doxycycline, rifampicin, chloramphenicol; fluorinated quinolones - fleroxacin, pefloxacin, ofloxacin, ciprofloxacin, lomefloxacin, ceftriaxone, as well as gentamicin, sisomycin, monomycin, streptomycin, clotrimoxazole and other aminoglycosides; betalactam carbapenem antibiotic meropenem; azathromycin, an azalide macrolide[12].

Antibacterial activity was studied by disc method, for which discs impregnated with different antibiotics were used, which were placed on the sown brucella culture on a Petri dish at an equal distance from each other, placed in the thermostat at a temperature of - 35 - 37 ° C for 2 - 3 days. The result was recorded by the appearance of a lysis zone around the antibiotic discs and the lysis diameter in cm was estimated.

3. Results

Classical Brucella species include B. abortus, B. melitensis, B. suis, B. canis, B. ovis and B. neotomae, while recently identified species include B. ceti, B. pinnipedialis, B. microti, B. inopinata, B. papionis and B. vulpis. Brucella abortus is predominantly prevalent in cattle, B. suis in pigs and B. canis in dogs. These species are traditionally divided into the following biovars: B. melitensis includes three biovars (1, 2, and 3); B. abortus contains eight (1, 2, 3, 4, 5, 6, 7, and 9); and B. suis contains five. The genus Brucella is remarkably homogeneous, showing more than 90% genetic homology between different species of the same genus.

It is classified on the basis of various phenotypic, genotypic and ecological characteristics. Bacteriological culture is considered the gold standard for the diagnosis of brucellosis because of its high specificity and ability to identify different species and biovars. This method is crucial for epidemiological studies as it helps to monitor the spread of the disease within a region and identify potential sources of infection[13-14].

Microscopic, bacteriological comparative research of pathogenic and vaccine brucella strains gave only confirmatory answer about their adherence to the bovine brucella species and the indices were identical in both species Br. abortus 99 and 19.

In the event of a positive agglutination reaction on a degreased slide, following a period of 1-3 minutes in a drop of positive serum, the formation of flakes, lumps, and lucidity of the liquid was observed, a phenomenon that was especially evident when the glass was subjected to agitation[15]. Conversely, in the control experiment, the addition of negative serum in equivalent dilutions resulted in uniform turbidity, devoid of any discernible flakes. At the same time, the positivity of in vitro RA with Br. abortus 99 was higher than with Br. abortus 19 (1:200 and 1:80).

As can be seen from the presented data, the vaccine and pathogenic Brucella strains do not show any difference in morphological, culture and immunogenic properties.

Table 2. Results of comparative study of antibiotic resistance of pathogenic and vaccine strains of brucellae stored in the museum strain collection of the regional diagnostic laboratory of the Research Institute of Uzbekistan

1. №	Antibiotics	Br. abortus 99	Br. abortus 19
1	Doxcycline	D = 20 mm	D = 20 mm
2	Rifampicin	D = 22 mm	D = 23 mm
3	Chloramphenicol	D = 20 mm	D = 20 mm
4	Fleuroxacin	D = 20 mm	D = 20 mm
5	Pefloxacin	D = 20 mm	D = 20 mm
6	Ofloxacin	D = 20 mm	D = 22 mm
7	Ciprofloxacin	D = 15 mm	D = 16 mm
8	Lomefloxacin	D = 20 mm	D = 20 mm
9	Ceftriaxone	D = 18 mm	D = 20 mm
10	Gentamicin	D = 14 mm	D = 14 mm
11	Sisomycin	D = 19 mm	D = 20 mm
12	Monomycin	D = 20 mm	D = 20 mm
13	Streptomycin	D = 20 mm	D = 20 mm
14	Clotrimoxazole	D = 20 mm	D = 20 mm
15	Azithromycin	D = 22 mm	D = 24 mm
		Average D=19,33	Average D=19,93

As can be seen from the results of the study, the vaccine and pathogenic strains of brucellae, unaffected by environmental factors, factors of the immune system of the organism and the surrounding biotome of the organism showed almost the same antibiotic sensitivity. The difference was one step in three cases (Br. abortus 99 showed rifampicin sensitivity of 22 mm, Br. abortus 19 - 23 mm, against ciprofloxacin 15,16 mm; against sisomycin 19;20), and in three cases two steps (against ofloxacin 20;22 and ceftriaxone 18;20 with azithromycin 22;24).

In all cases, the vaccine strain showed greater sensitivity to all 15 antibiotics used in the experiment than the pathogenic strain (the vaccine strain Br. abortus 19 had a group mean D=19.93 and the pathogenic Br. abortus 99 had a mean D=19.33, respectively), which can be explained, apparently, by the greater virulence of the pathogenic strain [16-17].

4. Conclusion

Based on the research conducted, the following conclusions were drawn:

1. Vaccine Br. abortus 19 and pathogenic Br. abortus 99 strains of Brucella do not show any difference with respect to morphological, culture and immunogenic properties.
2. Vaccine Br. abortus 19 and pathogenic Br. abortus 99 strains of Brucella, unaffected by environmental factors, factors of the immune system of the organism and the surrounding microbiological biotome of the organism show sensitivity to all antibiotics used in the experiment.

The vaccine strain Br. abortus 19 shows greater sensitivity to 6 antibiotics (rifampicin, ciprofloxacin, sisomycin, ofloxacin, ceftriaxone, azithromycin) out of 15 used in the experiment than the pathogenic strain Br. abortus 99.

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