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Heterological serological diagnostics of nodular cattle dermatitis

Annotation. *The article is devoted to the current problem of laboratory diagnosis of nodular dermatitis in cattle. Specific tools and methods for diagnosing this disease have not yet been developed. There is evidence of an antigenic relationship between the causative agent of nodular dermatitis of cattle and the causative agent of sheep pox. The possibility of using means and methods of serological diagnosis of sheep pox for the diagnosis of ND cattle is being considered. Reducing the timing of diagnosis helps to increase the effectiveness of ongoing therapeutic and antiepidemiological measures.*

Key words: *Complement Fixation Test (CFT), Cattle, Nodular dermatitis, Diffusive Precipitation Test (DPT).*

Introduction. Nodular dermatitis of cattle (infectious nodular dermatitis, skin tubercle, nodular exanthema, skin-nodular rash, “scrapie disease of skin”) - a cross-border, emergent infectious disease of cattle (Cattle), manifested by persistent fever, damage to the lymphatic system, edema of subcutaneous fiber and internal organs, formation of skin nodes (bugres), damage to the eyes and mucous membranes of respiratory and digestive organs, loss of productivity and living body weight.

Outbreaks of nodular dermatitis are sporadic, dependent on animal movement, their immune status, and wind and rain regimes that affect vectors. The main transmission method is considered to be mechanical, i.e., arthropod carriers.

Currently, the disease is found in 34 countries in Africa and Asia. Nodular dermatitis (LSD) was first discovered in Zambia in 1929, then it spread to Botswana by 1943 (Haig, 1957) and then to South Africa, where it affected more than eight million cattle, resulting in heavy economic losses. In 1957, he entered Kenya, where he was linked to an outbreak of sheep smallpox (Weiss, 1968). Nodular dermatitis spread north, to Sudan, and by 1974 to the west, to Nigeria, and was reported in Mauritania, Mali, Ghana, and Liberia in 1977. Another epizootic of nodular dermatitis occurred between 1981 and 1986 and struck Tanzania, Kenya, Zimbabwe, Somalia and Cameroon, with a mortality rate of 20 % affected by CRS. The appearance of nodular dermatitis north of the Sahara Desert and outside the African continent was first confirmed in Egypt and Israel between 1988 and 1989, then again reported in 2006 [1]. According to official IEB reports from 2013 to 2015, the disease spread widely in the Middle East. According to the data of the national veterinary services in 2014, the disease of CRS with the LP virus was detected in Turkey - 230 hotbeds, Lebanon - 32, Azerbaijan and Iraq - 16, Egypt and Iran - 6 hotbeds. In 2014-2015, the disease was diagnosed in Cyprus and Greece. In 2015, the first cases of ND in Russia were registered in the territory of the Republic of Dagestan and the Chechen Republic. During the second half of 2015, 17 outbreaks of the disease in 3 regions were detected in the Russian Federation. During the period of 2016, 313 outbreaks were notified in 16 regions of the Russian Federation. In 2017, 43 outbreaks were registered in 6 regions of the Russian Federation. This fact indicates that the disease will continue to spread to the new territories of the Russian Federation. The first case of nodular dermatitis of cattle in Kazakhstan was recorded in 2016 in the west of the country, with further spread throughout the country, which leads to serious social and economic consequences for domestic livestock production [2].

ND is considered a particularly dangerous animal disease capable of causing epizootics and causing economic damage. According to new classification, it is included in the list of the diseases IEB (International Epizootic Bureau) which is a subject to the obligatory notice (notification) in category "Diseases and Infections of Cattle".

The disease causes significant economic damage in cattle breeding without having a specific incubation period in the field, but after inoculation, it takes 6-9 days before the onset of fever. It causes a significant decrease in milk weight, loss of living body weight, damage to skin quality. In steel animals, abortions are noted; bulls can become temporarily or permanently sterile. It is possible to kill sick animals in case of acute form of disease development, or complication with second-time infection [1, 2].

The agent of nodular dermatitis (ND) is a DNA-containing shell virus belonging to the Neethling group, the genus Capripoxvirus, of the family Poxviridae. Neethling virus is a prototypical agent of LP. This pathogen has an antigenic relationship with sheep smallpox virus [1, 2].

Poksvirusa (Poxviridae family; from lat. pox empty, ulcer) is a family of large DNA-containing viruses.

Poxviruses were previously called smallpox viruses. Poxviruses are the largest viruses. Two subfamily have been isolated in the family Poxviridae: Chordopoxvirinae (animal smallpox viruses) i Entomopoxvirinae (insect smallpox viruses).

The subfamily Chordopoxvirinae consists of 8 genera: Orthopoxvirus, Parapoxvirus, Leporopoxvirus, Capripoxvirus, Suipoxvirus, Molluscipoxvirus, Yatapoxvirus, Avipoxvirus, which differ from each other in terms of DNA content and properties, Members of each genus have extensive antigens and are capable of genetic recombination.

Orthopoxvirus (from Greek Orthos-correct). It includes vaccine (prototype virus) and natural smallpox (variola) viruses, cow smallpox viruses, camels, stripe raccoons, African gerbies, monkeys. Buffalo and rabbit smallpox viruses are subspecies of vaccine virus. Virions are brick-shaped, measuring 200-250 × 250-300 nm. The genome of these viruses consists of 185 thousand nucleotide pairs (n.p.), the proportion of G (guanine) + C (cytosine) 36 %. Viruses have hemagglutinating activity. Genetic recombination occurs between different species. Viruses are similar in antigenic structure and give cross-serological reactions [3].

The genus Capripoxvirus includes sheep smallpox virus (BOO), goat smallpox virus (VOC), and nodular dermatitis virus (ND). The genome consists of 150-160 thousand n.p. Viruses are sensitive to the ester and antigenically related to each other. Members of the genus are closely related, as proved by immunological analysis and genome structure.

To date, a small number of domestic publications have been submitted on the study of the biological virus ND of cattle. At the same time, most of the research was carried out with only one reference field virulent strain-prototype "Neethling" of ND of cattle virus. Because of studying its properties, sensitivity of virus to different cell cultures is established, optimal conditions of its cultivation are determined. Studies of the clinical picture of the disease have found that the ND exciter of the cattle causes an increase in temperature, viremia and the formation of beads on the skin followed by necrosis. In intravenous infection it causes generalized infection, and in intracutaneous - the disease flows in soft form. However, the results of the study of clinical signs caused by experimental infection of animals with the "Э-95" ND virus strain suggest the possibility of causing the disease of naturally susceptible animals with characteristic clinical signs when the agent is administered intracutaneously.

Due to insufficient study of the biological properties of the virus, diagnostic methods are limited solely to molecular approaches.

In connection with the above, the study of biological properties of ND of cattle virus in order to develop new means of diagnosis and prevention of the above-mentioned disease is a pressing task of veterinary science and practice [2].

Antigenic immunological relationship between members of the family Poxviridae, a genus of Capripoxvirus, is known [3]. It includes sheep smallpox, goat smallpox and cattle nodular dermatitis. Kits for serological diagnosis of sheep smallpox are produced by biological industry of the Republic of Kazakhstan.

In view of the above, one of the objectives of our research was to develop a method of serological diagnosis of ND of cattle using preparations for the diagnosis of sheep smallpox. The use of antigenic kinship for diagnostic purposes is used as a basis for preparing monospecific polyclonal antisera to antigenically related proteins, which allowed to obtain an immune response to all both weak and strong antigenic determinants. In order for related proteins to be distinguished among themselves, antisera to them must contain antibodies to the largest number of different antigenic determinants. Both weak and strong antigens can distinguish related proteins, so it is important to have antisera with this set of antibodies to solve the problem of separately determining these proteins. Such antisera can be used for differential determination of related proteins in human blood serum in the diagnosis of a number of diseases and in biological preparations by immunoenzyme test systems or radial immunodiffusion [4].

Materials and methods. In our research used sets of diagnostics for CFT and DPT in smallpox of sheep produced by Research Institute of biological safety problems (Otar station, Zhambyl region). Under conditions of district, veterinary station of Aksu of Pavlodar region the material from cattle with clinical signs of nodular dermatitis was selected. Preliminary diagnosis for the presence of pathogen - ND of cattle virus - was made by viroscopy (silver by Morozov). To carry out diagnostics, samples of affected areas of skin were taken, from which 50 % organoxane suspension was prepared on physiological saline, which was examined in CFT and DPT in dilutions from whole to 1:16 for detection of viral antigen. For intravital diagnosis, blood serum samples were taken from the sick animals at various stages of the infectious process (initial period, marked signs of disease, convalescence).

To control the specificity of the method, a material obtained from healthy animals and animals with similar clinical signs of skin damage but no positive viroscopy reaction was used.

The test material was tested in an Ouchterlony DPT using 1 % Diphco agar while holding the reaction at $(37 \pm 1) ^\circ\text{C}$ for 24 hours.

When examining samples of organoreconilant material, diagnostic serums for sheep smallpox in whole form were introduced into central wells, and dilutions of suspension of tested organoreconilant material were introduced into peripheral wells.

When examining blood serums, diagnostic antigens of sheep smallpox virus in dilution 1:2 were introduced into central wells, and in peripheral test blood serums in whole form.

When setting the reaction, controls were set to check the specificity and activity of the diagnostic preparations.

CFT was carried out in the modification of long-term complement binding. The test material was titrated in dilutions from 1:2 to 1:32.

Results. The results of studies of samples of organotissue material are presented in table 1.

Table 1 – The results of studies in the RPD and RBC samples of organotissue material

Material	Number of tests	Serum for sheep smallpox	
		RLBC	RPD
Skin of cattle with signs of dermatitis:			
Virusoscopy-confirmed presence of smallpox virus	4	1:4-1:8	1:2-1:4
Negative viroscopy	4	-	-
Skin of healthy animals	4	-	-

More relevant is the issue of retrospective diagnosis of ND of cattle. To this end, in order to detect virus-specific antibodies in the blood serum of sick animals, a reaction was set up using diagnostic antigens of sheep poxvirus. The research results are presented in table 2.

Table 2 – Results of serological reactions in blood serum samples of sick animals

Test sera	Number of samples	Sheep smallpox virus antigen	
		CFT	DPT
Sick cattle			
The expressed signs	12	1:2-1:8	W-1:2
Reconvalescence	4	1:4-1:16	1:2-1:4
Animals with dermatitis without positive virology	10	-	-
Healthy cattle	6	-	-

W - Whole.

Discussion. As can be seen from the data shown in Table 1, when specific serum was used for sheep smallpox virus, ND of cattle antigens were found in skin samples in titers from whole to 1:4 in DPT and in dilutions up to 1:16 in CFT. A study to control the specificity of cattle skin preparations obtained from sick animals with other skin diseases and healthy animals showed a negative reaction result, indicating the specificity of the method. As can be seen from the data presented in table 2, virus-specific antibodies related to the sheep poxvirus antigen were detected in the blood serums of sick animals during various periods of the disease. Blood serums from animals with other diseases and from healthy cattle showed a negative reaction, which indicated the specificity of the method.

Conclusion. Thus, because of the conducted research, the possibility of setting serological reactions for the diagnosis of nodular dermatitis in cattle using sheep pox diagnostics was established.

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Ірі қара малдың нодулярлы дерматитінің гетерологиялық серологиялық диагностикасы

Мақала ірі қара малдағы нодулярлы дерматитті зертханалық диагностикалаудың өзекті мәселесіне арналған. Бұл ауруды диагностикалаудың арнайы құралдары мен әдістері әлі әзірленбеген. Ірі қара малдың нодулярлы дерматитінің қоздырғышы мен қой жұқпасының қоздырғышы арасында антигендік байланыс бар екендігі дәлелденген. Ірі қара малдың диагнозы үшін қойдың жұқпасын серологиялық диагностикалаудың құралдары мен әдістерін қолдану мүмкіндігі қарастырылуда. Диагноз қою уақытын қысқарту жүргізіліп жатқан терапиялық және эпизоотияға қарсы шаралардың тиімділігін арттыруға көмектеседі.

Түйін сөздер: комплемент фиксациясының ұзақ мерзімді реакциясы, комплемент фиксация реакциясы, ірі қара (ірі қара), түйінді дерматит, диффузиялық жауын-шашын реакциясы.

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Гетерологичная серологическая диагностика нодулярного дерматита крупного рогатого скота

Статья посвящена актуальной проблеме лабораторной диагностики нодулярного дерматита крупного рогатого скота. Специфические средства и методы диагностики данного заболевания до настоящего времени не разработаны. Имеются данные об антигенном родстве возбудителя нодулярного дерматита КРС и возбудителя оспы овец. Рассматривается возможность применения средств и методов серологической диагностики оспы овец для диагностики НД КРС. Сокращение сроков диагностики способствует повышению эффективности осуществляемых лечебных и противоэпизоотических мероприятий.

Ключевые слова: Реакция длительного связывания комплемента (РДСК), реакция связывания комплемента, крупный рогатый скот, нодулярный дерматит, реакция диффузионной преципитации.