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## Diagnosics of Infectious Anemia of Horses and its Distribution in Krasnoyarsk Krai.

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### ABSTRACT

The results of studies have shown that the diffusion precipitation reaction (RDP) remains the standard method for diagnosing infectious anemia (INAN) in horses. The epizootological analysis and analysis of the results of studies of horses on INAN in the RDP on the territory of the Krasnoyarsk Territory for 1996-2016 allowed to identify the source of the infectious agent – sick horses and virus carriers, which later act as a reservoir of infection. The main mode of transmission is transmissible, the disease often occurs in chronic and latent forms. In 1996, in a study of five thousand horses, 7 positive goals (0.14%) were detected in two districts of the region. In 2001, 17.9 thousand heads of horses were investigated and 0.51% of the reacting were identified. In 2004, in the study of 42.4 thousand reacting heads, 528 heads (1.25%) were detected. In 2005-2011, in the study of 42.47 thousand heads, the number of positively reacting annually decreased from 1.25 to 0.39%. After 2013, the number of reactors was maintained at a level of 0.02-0.03%. In 2016, in a study of 42.5 thousand heads, 14 infected heads were identified, which amounted to 0.03%, and only three disadvantaged points were found for INAN horses. Territorially, infected horses were mainly allocated in two zones of the region – Achinsk and Minusinsk. It was less often in the Central and Kan zones, and we did not notice the disease in the northern zones (Yenisei and Boguchanskaya). During the study period, unsuccessful locations were found in 22 districts of the region with varying degrees of infection from 2 to 171 heads per district and from 1 to 46 heads to a disadvantaged item. The work done to identify the epizootic situation in the region according to INAN horses showed that the serological research and the removal of infected animals helped stabilize the situation, reduce the appearance of new disadvantaged items, reduce morbidity, but did not eliminate INAN. Features of the virus, its mobility, and untimely removal of infected horses hinder the improvement of the territory of the region from infectious anemia.

**Keywords:** equine infectious anemia (INAN), transmission pathway of infection, diagnostic methods, blood serum, diffuse precipitation reaction (RDP), virus drift.

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## INTRODUCTION

Equine infectious anemia (IAN) is a viral disease of one-hoofed animals, characterized by recurrent fever, lesion of the blood-forming organs, anemia, and a diverse course (from acute to unsystematic latent and long-term virus-carrying).

The causative agent of infectious anemia is the RNA containing a virus that belongs to the causative agents of cattle leukemia, slow infections of sheep, human AIDS, etc. to the subfamily lentiviruses, the family of retroviruses.

The source of the causative agent of infection is the sick horses and virus carriers, which then act as a reservoir of infection.

Transmission of infection when IAN passes in several ways:

- Transmission: through insect bites (paut and fly-flyer); the infection period is July-August;
- Intrauterine: infection of the foal in utero and through the colostrum, which occur only during the exacerbation (recurrence) of the disease;
- Hematogenous: mechanical during the period of vaccination, blood collection, and during operations;
- Contact: after recurrence of the disease, the virus is released into the external environment and infection is possible through abrasions and wounds.

However, the main transmission mechanism of the IAN virus is the transmission path.

For IAN diagnostics, Japanese researchers have proposed the reaction of complement fixation (RCF) since 1966. However, RCF allowed to detect only early antibodies. In 1972, L. Coggins in the United States developed a test system for the diagnosis of IAN in the precipitation gel reaction (RDP). Then the RDP was determined by the OIE decision as the main test for the IAN diagnosis.

At present, new tests are proposed for the diagnosis of the disease: (a) immunochemical: various modifications of ELISA and (b) molecular genetic: RT-PCR. The advantage of RT-PCR is that the virus can be detected as early as 3 days after infection, while specific antibodies in the RDP are detected only 15-23 days after infection. In practice, the diagnostic value of RT-PCR is lower than the standard RDP, but can be used in the complex of laboratory studies on the IAN [1, 2].

Modern test systems for the IAN diagnosis in ELISA, as a rule, include antigens of recombinant proteins obtained by genetic engineering.

In general, ELISA is a fast-performing method (2 hours), and it is highly sensitive. ELISA results correlate with RDP data, but conflicting results are also possible. In accordance with the recommendations of the OIE, all samples that are positively responsive to IAN in the ELISA should be checked in the RDP.

Immunoblotting is a method by which antibodies to various IAN virus polypeptides are detected, and infected horses can be detected earlier than in the RDP. Thus, the RDP still remains the main method, which allows identifying patients with IAN horses by detecting precipitating antibodies from 14 ... 50 days after the infection and during the whole life [3,4,5,6].

Due to certain features of the causative agent, the means of specific prophylaxis are not developed. According to the International Epizootic Bureau, IAN is found in most countries of the world, Europe (Bulgaria, Hungary, Czech Republic, Slovakia, Germany, France, etc.), as well as Ukraine, Belarus, Mongolia, Russia [6,7,8]. IAN is also registered in the Krasnoyarsk region [9].

## THE PURPOSE OF THE STUDY

The purpose was to study the diagnosis and spread of infectious anemia in horses in the Krasnoyarsk region. The task of the study was to conduct the epizootological monitoring of infectious anemia in horses in the Krasnoyarsk region.

## CONDITIONS, MATERIALS AND METHODS

The work was done at the Department of Epizootology, Microbiology, Parasitology, and Veterinary-Sanitary Expertise of the Institute of Applied Biotechnology and Veterinary Medicine, Krasnoyarsk State Agrarian University, at the Krasnoyarsk Veterinary Laboratory, and at the Krasnoturanskaya Veterinary Laboratory in 2016-2018 (Russia). Studied archival documents and documents of veterinary reporting for 1996-2016 were kindly provided by the service for veterinary supervision of the Krasnoyarsk region.

The object of the study: horses of different breeds, gender, and age groups in the territory of the Krasnoyarsk region.

Biomaterial: serum of horses. It was investigated in the reaction of diffuse precipitation.

We analyzed the 1996-2016 results of research at the Krasnoyarsk Veterinary Laboratory.

We analyzed the 2006-2014 results of research on the RDP and considered the 2015-2018 results of their own research on the RDP, which were received at the Krasnoturanskaya Veterinary Laboratory.

For the formulation of the reaction, we used a set for the diagnosis of infectious anemia of horses in the reaction of diffuse precipitation No. 13-5-02 / 0894 dated January 27, 2004 and approved by the Veterinary Department of the Ministry of Agriculture of the Russian Federation (a set by the Schelkovsky Biokombinat since 2009). The statement of the reaction and the results were recorded in accordance with the instructions for diagnostic sets.

## RESULTS AND DISCUSSION

Infectious anemia of horses in the territory of the Krasnoyarsk Territory was recorded long ago. After the 60-70s of the twentieth century, when horses, in general, ceased to perform corpulent force, many diseases, including infectious diseases, became less likely to diagnose.

Further, in the annual plans of antiepidemiological measures, the study of horses for infectious anemia was not planned.

With the emergence of new rules to combat the INAN, it was planned to conduct an annual study of horses, mainly those coming to the racetrack, training to participate in equestrian competitions and in farms for horse breeding.

In 1996, in order to clarify the epizootic situation in INAN, they began to study horses in ordinary farms and in the private sector. A study of five thousand horses revealed a positively reacting 7 heads (0.14%) in the B.-Murtinsky and Balakhta regions. In 2001, the study covered 17.9 thousand horses and identified a number of reacting heads of 0.51%, and in 2004, with a study of 42.4 thousand heads, there were 528 reacting heads (1.25%).

In the subsequent 2005-2011, in the study within 42-47 thousand heads, the number of reacting annually decreased from 1.25 to 0.39%. After 2013, the number of reacting was maintained at the level of 0.03-0.02%. In 2016, a study of 42.5 thousand heads revealed 14 infected heads, which amounted to 0.03%, and only three unfavorable points were identified.

Summarizing the results of studies and the extent of infection of the INAN horses geographically, infected horses were mainly allocated in the two zones – Achinsk and Minusinsk. It was less often in the Central and Kan zones. And they did not notice the disease in the northern zones (Yenisei and Boguchanskaya).

Thus, in 2004, 89 unsuccessful items on equine infectious anemia were identified in the region, where 528 heads (1.25%) responding to the RDP were detected, of which 38 (42.0%) were in trouble zones: 49 points (55%) in Minusinskaya, and 2 disadvantaged points in the Kan zone.

The degree of infection in the farms of the Achinsk zone was 214 heads (40%). In the Minusinsk zone, there were 314 heads (59%) of all responding in the krai for 2004. From the reacting Achinsk zone, 98 heads (6.4%) were found in the farms of the Nazarovsky district, 35 (4.65%) – in the Birilyussky area, 54 heads (2.04%) – in the Balakhta district, out of 314 reacting heads in the Minusinsk zone, 171 (5.19%) were found in the Yermakovsky District, 121 (4.55%) – in the Karatuzsky District, and 22 (0.97%) – in the Shushensky District of the total number of the studied population in these areas.

The horses responding to INAN for the period 1996-2016 were found in 326 troubled points in 22 districts of the region, with varying degrees of infection from 2 to 171 heads per area and from 1 to 46 heads to a dysfunctional point.

During slaughter, when the internal organs are viewed, degenerative changes were smoothed, clearly expressed signs of anemia, serous and serous-jelly edema in the subcutaneous tissue, the spleen was slightly enlarged, the liver was changed, the kidneys were enlarged, dense, and had characteristic of all stages of INAN horses.

The complexity of the clinical diagnosis of INAN horses lies in the fact that in most cases the infection proceeds in an asymptomatic (latent) form, when the horses carrying the virus remain for a long time (for life) the source of infection, with no visible clinical signs of the disease, and only during relapses they have a fever and fatigue.

Existing methods of laboratory diagnosis of ELISA allows one to install antibodies, and PCR allows to detect the virus genome even at an early stage of the disease. Antibodies (that persist in the body of horses for a long time) allows one to identify the RDP. In the conditions of veterinary laboratories, the standard reaction of diffuse precipitation is mainly used.

One needs to considering the fact that the INAN virus is subject to antigenic drift during the entire reproduction in the body. However, after each recurrence of the disease, new variants of the virus appear. The existing antibodies of the previous type do not work, and the unstable immunity is formed, when antiviral antibodies and newly formed antigen (virus) are in the blood of horses.

With the presence of such antigenic variability of the INAN virus, when a new variant of the pathogen appears, a short-term explosion of the virus reproduction and infection of new cells occurs until the immunological control is restored. During the period of periodic recurrence of the disease, the key role is played by the defectiveness of the reticuloendothelial system and the integration of the virus into the cell genome.

This creates a condition for the formation of immune complexes that cause specific lesions in the liver, spleen, kidneys, and heart.

Conducted work to clarify the epizootic situation in the region on the INAN horses through serological studies and the removal of infected animals stabilized the situation, reduced the emergence of new dysfunctional items, reduced morbidity, but failed to eliminate INAN for the following reasons: among the districts of the region are not fully covered by horse research among owners of all forms of ownership, especially among the private sector, resolved the issue of compensation for the delivery of the horse to the meat factory.

The reported properties of the virus, its mobility and untimely removal of infected horses hinder the improvement of the territory of the region from infectious anemia.

#### CONCLUSION

1. The main sources of infectious anemia of horses are the sick and virus carriers. The main way of infection is the INAN transmission, where *Tabanidae* and *Stomoxys calcitrans* play the main role in the transmission of infection.
2. The disease proceeds mainly in chronic and latent forms, and the RDP remains the standard INAN diagnostic method.

3. In the region, a disease of INAN horses was recorded in 22 districts with a degree of infection from 1 to 46 animals per one dysfunctional point.
4. The basis of prevention is the study of all horses in all farms of different forms of ownership twice a year in the fall and spring.
5. Control methods include research and removal of all horses reacting to INAN, regardless of their value and forms of ownership.

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