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Epizootological Monitoring of Glanders in the Republic of Kazakhstan

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ABSTRACT

This research aimed to study the epizootological characteristics of the territory of the Republic of Kazakhstan on glanders and to develop an advance forecast of the incidence of zoonotic infections. Epizootic situation by glanders in the world and in Kazakhstan was studied by analyzing the data of reports of the Veterinary Control and Supervision Committee of the Ministry of Agriculture of the Republic of Kazakhstan, and to the World Organization for Animal Health. In addition, articles on the prevalence of glanders in the world and countries bordering with Kazakhstan were examined. OIE data indicate the circulation of the glanders pathogen in some countries of Eastern Europe, Asia and Africa over the past ten years. Special prevention measures against the glanders, carried out in the territory of veterinary and sanitary well-being, which is Kazakhstan, include the basic requirements for preventing the introduction of the pathogen into the country, such as quarantine, clinical examination and negative results of malleinization of imported animals. However, under conditions of intensive development of horse breeding and close economic ties of Kazakhstan with neighboring regions and countries, including those unfavorable for glanders, there is still a risk of introduction of the pathogen into the territory of Kazakhstan. In these conditions, it is necessary to conduct epizootological monitoring with short-term and long-term forecasting and analysis of veterinary and sanitary measures against glanders.

Key words: Glanders, Horses, Monitoring, Malleinisation, Epizootic situation.

INTRODUCTION

Glanders is a contagious and fatal zoonotic disease affecting horses, donkeys and mules, as well as humans, resulting in nodules and ulcers in the upper respiratory tract and lungs (Domnitsky et al. 2019). The disease is caused by *Burkholderia mallei*, which belongs to the β-subclass of Proteobacteria, family Burkholderiaceae, genus Burkholderia (Khan et al. 2013; Van Zandt et al. 2013; Zakharova et al. 2018).

B. mallei is Gram-negative bacteria, immobile, non-spore-forming bacillus, facultative intracellular pathogen. It is 1-5μ long, 0.3-0.8μ wide, and stains with common dyes, with the granularity of the bacterial cell protruding. *B. mallei* grows on common nutrient media (Van Zandt et al. 2013; Jupina 2015).

The source of the infectious agent is a sick animal. The pathogen is excreted into the external environment with nasal discharge, sputum from coughs and pus from skin ulcers and with faeces if sputum is swallowed. It is also known that the disease is transmitted mainly through the digestive tract by eating feed or water contaminated with sick horses that are constantly excreting glander pathogens (Neverov et al. 2023). Contact infection through damaged skin and mucous membranes is also possible (Wernery et al. 2011).

In horses, the disease is chronic and can last up to several years. Depending on the localization of the pathological process, the disease is divided into nasal, pulmonary and cutaneous forms (Knox et al. 2023). In the acute course, there are septicemia, prolonged and unilateral nasal discharge, swelling of lymphatic vessels, submandibular, inguinal, subcutaneous lymph nodes with the formation of nodules and then ulcers. In chronic course, susceptible animals lose weight, cough, and have mucopurulent nasal discharge (Tnu.in.ua n.d.).

The main methods of intravital diagnostics are allergic test (malleic test). Mallein was prepared in 1891, which is now used all over the world for allergic diagnosis of glanders. Ophthalmomalleinization (conjunctival test) is performed twice, with an interval of 5-6 days. The first

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study is carried out early in the morning: 4-5 drops of mallein are injected into the conjunctival sac with a sterile pipette. The reaction to mallein occurs after 2-3 hours and lasts for several hours; delayed reactions after 12-24 hours are rarely observed. The reaction is checked after 3-6-9 hours and the next day (in 24 hours) (Melnikova et al. 2016).

The detection of specific antibodies is essential for correct diagnosis and initiation of timely treatment. The search for antibodies in the sera of patients with glanders of people and animals is carried out in paired sera obtained with an interval of 2 to 3 weeks. Increasing titers of specific antibodies confirm the correctness of the preliminary diagnosis. The main methods for detecting serum antibodies are Rheumatoid arthritis, Rapid slide agglutination test and Enzyme-linked immunosorbent assay. Upon receiving the material, a series of diagnostic procedures are conducted, including bacterioscopy of smears using both light and luminescent microscopes. Furthermore, a bacteriological examination is performed with the objective of isolating the glanders microbe culture. To enhance precision and ensure purity, a biological examination is carried out as an additional step. Additionally, molecular techniques such as polymerase chain reaction (PCR) are employed as part of the diagnostic process (Currie 2015).

Glanders has been known since antiquity and has caused great economic and social damage at all times. Glanders occupies a special place among infectious diseases due to the variety of clinical and pathological manifestations and the damage to various organs. Lethality in septic and pulmonary forms of melioidosis exceeds 90%. Despite the use of the most modern drugs for treatment, the mortality rate is more than 40% (Sharrer 1995).

The disease is endemic to humid tropical and subtropical regions of Southeast Asia, Northern Australia, West Africa and Latin America. Quite a large number of sporadic cases of the disease associated with importation from endemic regions has recently been noted for a number of countries of the temperate climatic zone (Choy et al. 2000; Elschner et al. 2014; Benoit et al. 2015; Currie 2015; Berger 2018). The development, continuous improvement and implementation of the system of antiglanders activities allowed localisation and elimination of this disease in the regions of the former Commonwealth of Independent States (CIS).

Given our close links with farm, domestic and wildlife animals, zoonoses are a major public health problem worldwide. They can also lead to disruptions in the production and trade of animal products intended for food and other uses (Chewapreecha et al. 2017; Elschner et al. 2017).

In this regard, prediction of the next rise in incidence will allow timely implementation of preventive measures, which is much more effective and cheaper than the use of emergency measures to eliminate sudden outbreaks at the top of the wave of the focus activity (Alikhanov et al. 2023).

This research aimed to study the epizootological characteristics of the territory of the Republic of Kazakhstan on glanders and to develop an advance forecast of the incidence of zoonotic infections.

MATERIALS AND METHODS

To determine the epizootological characteristics of glanders over the last 14 years in the world and on the territory of the country, a literature and information analysis was carried out, official data of veterinary reports and data of the International Epizootic Bureau (OIE) were studied (OIE 2018, 2020, 2023).

The analysis of the effectiveness of veterinary and sanitary measures against glanders in Kazakhstan was based on the study of official data from veterinary reports of the Veterinary Control and Supervision Committee of the Ministry of Agriculture of the Republic of Kazakhstan, the Republican Veterinary Laboratory, and the National Veterinary Reference Centre (Bureau of National Statistics 2023).

The study of regulatory documents was carried out: The Law of the Republic of Kazakhstan "On Veterinary", veterinary rules for the implementation of measures to prevent and eliminate glanders (from 17.01.2012 No 10-1/18), veterinary (veterinary and sanitary) rules (from 29 June 2015 No 7-1/587), Sanitary and Epidemiological and Veterinary and Sanitary Rules and Regulations "Organization of epidemiological and epizootological surveillance and carrying out sanitary and anti-epidemiological (prophylactic) and veterinary and prophylactic (anti-epizootic) measures for glanders in the Republic of Kazakhstan" (Approved by joint order of the Ministry of Health of the Republic of Kazakhstan and the Ministry of Agriculture of the Republic of Kazakhstan from 16 August 2007 No 507) (Vuzlit.com n.d.; Donchenko and Samolovova 2016; Kettle and Wernery 2016; Nurzhigit et al. 2021).

RESULTS

Over the last 15 years, there has been an increasing trend in the number of outbreaks of glanders in thoroughbred horses and other farm and wild animals. As a consequence, the outbreaks are now categorized as "re-emerging infections". Outbreaks of glanders have been reported in several regions of Africa, Asia, the Near and Middle East, Central and South America, covering 22 countries. In Asia (China, Turkey, Iran, Iraq, Lebanon, Syria, UAE, Kuwait, Bahrain, Mongolia, India, Pakistan), Africa (Zimbabwe) and Central and Latin America (Brazil).

Monitoring studies have shown that Iran ranks first in terms of glanders prevalence, with cases reported annually from 2013 to 2023 (Table 1). In India and Brazil, over the past 10 years, outbreaks of glanders have been recorded annually from 2013 to 2021.

In Pakistan, horses infected with glanders were detected for 3 years from 2013 to 2015 (OIE 2018). The 2019 outbreak of glanders in Turkey was suspected to be from horses imported from Brazil (OIE 2020). In 2018, 4 cases of glanders in Arabian horses imported from Russia were investigated in Mongolia.

In 2018-2019, cases of glanders were detected in China. It is noteworthy that already in 2020-2021, there have been outbreaks of glanders in Nepal, presumably from horses imported from China and India, where uncontrolled movement of animals is observed in border areas (Donchenko and Samolovova 2016).

Table 1: Global prevalence of glanders (OIE data from 2013 to 2023)

No.	Countries	Years										
		2013	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023
1	Afghanistan	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
2	Brazil	++	+	+	NR	+	NR	+	++	+	NR	NR
3	Guinea-Bissau	NR	NR	NR	NR	+	NR	+	NR	NR	NR	NR
4	Germany	NR	+	+	NR	NR	NR	NR	NR	NR	NR	NR
5	Hong Kong	NR	++	NR	NR	NR	NR	NR	NR	NR	NR	NR
6	Eritrea	NR	NR	NR	NR	NR	NR	+	NR	NR	NR	NR
7	Zimbabwe	NR	NR	+	NR	NR	NR	NR	NR	NR	NR	NR
8	Iran	NR	NR	+	+	+	+	+	+	++	++	++
9	Iraq	++	NR	NR	++	++	++	++	++	NR	NR	NR
10	India	++	++	++	++	++	++	NR	NR	NR	NR	NR
11	China	NR	NR	NR	NR	NR	+	+	+	+	NR	NR
12	Kuwait	NR	NR	NR	NR	NR	NR	+	NR	NR	NR	NR
13	Mongolia	NR	NR	NR	NR	NR	+	NR	NR	NR	NR	NR
14	Nepal	NR	NR	NR	NR	NR	NR	NR	+	+	+	NR
15	Pakistan	+	++	++	NR	NR	NR	NR	NR	NR	NR	NR
16	Russia	+	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
17	Türkiye	NR	NR	NR	NR	+	NR	+	+	NR	NR	NR

Note: + indicates the disease was registered once a year; ++ indicates the disease was registered 2 times a year, and NR = not reported

According to the official data of the Federal Service for Veterinary and Phytosanitary Supervision (Russian Agricultural Supervision), by 2023 in Russia, the disadvantaged regions for horse glanders are the Republic of Buryatia, the Trans-Baikal Territory.

Based on official sources of the authorized veterinary authority (Veterinary Control and Supervision Committee of the Ministry of Agriculture of the Republic of Kazakhstan) and data of the World Organization for Animal Health (OIE) in the territory of the Republic of Kazakhstan, outbreaks of horse glanders have not been registered since 1939.

In Fig. 1 shows the annual increase in the horse population from 2013 to 2023, with percentage increases ranging from 6.79 to 11.20%. The rate of increase has been relatively stable, increasing by approximately 9% on average. By 2022, the horse population has increased by more than 100% compared to 2013.

To date, according to the Bureau of National Statistics of the Agency for Strategic Planning and Reforms of the Republic of Kazakhstan, the number of horses is 4 million 436 thousand heads (Fig. 1).

In the last decade in the Republic of Kazakhstan there has been a positive trend of growth in the number of horses. The analysis of horse population growth in the period of the last 10 years is presented in the form of Fig. 2.

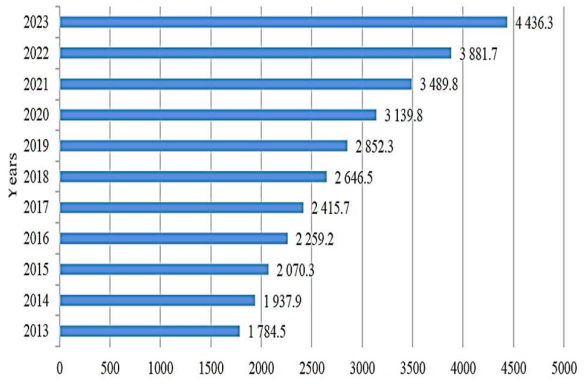

Fig. 1: Growth in the number of horses from 2013-2023.

When analyzing the risks of pathogen introduction from neighboring regions, special attention should be paid to the number of horses in the border regions. As we can see in Fig. 2, Turkestan region is the leader among the regions with 476 144 horses. The second place is occupied by Karaganda region with 409 190 heads of horses. In East Kazakhstan, Pavlodar, West Kazakhstan, Kyzylorda, Almaty and Akmola regions, the number of horses is between 226 and 302 thousand heads.

In Kazakhstan, all economic entities annually conduct routine allergic testing of animals for horse glanders by diagnostic test with mallein once a year. To date, the risks of horse glanders are present when transporting sport horses for participation in competitions, during sale and inter-farm exchange of animals. In this case, sport horses should be serologically tested negative for glanders within the last 6 months before travelling to competition.

In accordance with the Terrestrial Animal Health Code, Chapter 2.1, the equine import risk analysis aims to reduce the likelihood that an imported horse will result in the release and/or spread of the causative agent of glanders. Analyses of horse imports for the 4 years from 2019 to 2022 are presented in Fig. 3.

As can be seen from the Fig. 3 for 4 years, there is a sharp increase in the import of horses from other countries from 280 to 3901 head (Fig. 3). The Russian Federation (Altai Territory, Novosibirsk, Namangan region, Orsk city, Bashkortostan) ranks first in terms of imports, followed by Kyrgyzstan (Chui, Talas, Naryn regions, Bishkek). From these two neighboring countries, horses are constantly purchased for breeding and slaughter. In 2022, a large purchase of 707 horses for slaughter was made from Azerbaijan, the horses were supplied to West Kazakhstan region and Aktau. From other countries we see one-off purchases of thoroughbred horses for breeding. In 2021, 60 heads of horses were imported to Shymkent from Turkey, where in 2020 there were cases of horse glanders. There are certain risks here, given the fact that glanders migrates steadily in latent form, in some cases with a negative reaction to mallein.

The leader in horse procurement is East-Kazakhstan region, followed by Almaty, Zhambyl, Turkestan regions, West Kazakhstan region, Shymkent city, Aktobe city.

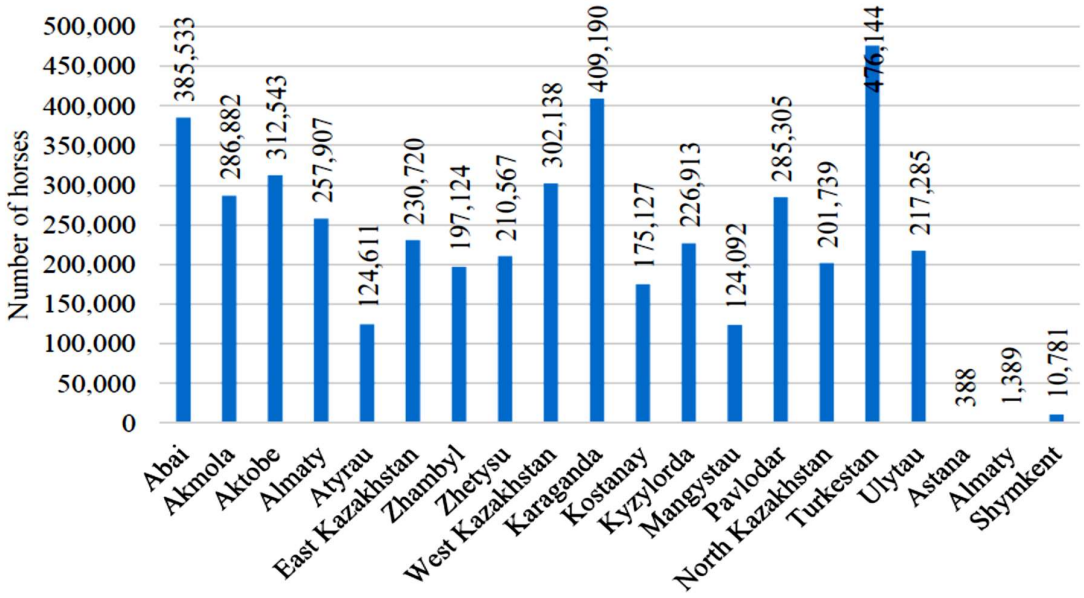

Fig. 2: Horse population in the regions of Republic of Kazakhstan in 2023.

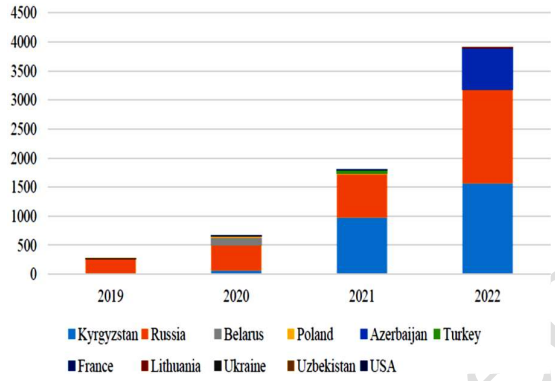

Fig. 3: Import of horses to Kazakhstan from 2019 to 2022.

According to the statistical data of veterinary reporting of the Veterinary Control and Supervision Committee of the Ministry of Agriculture of the Republic of Kazakhstan, the territory of the Republic of Kazakhstan on the presence of the pathogen, as the basic principle of regionalisation, is classified as safe (free) for glanders.

The regionalization of the risk of the disease introduction from other countries is based on the analysis of literature review data on the presence of outbreaks in Central Asia and the Middle East. Based on the analysis of the available data, regionalization by level of risk the disease introduction from other countries shows that the potential threat of glanders infection in horses may come from Russia, Mongolia and China. Also, given the geographical and economic links, caution should be exercised with countries such as Pakistan, India, Iran, Iraq, Turkey, where outbreaks of glanders have been reported more than once (Fig. 4).

Fig. 4 shows the countries with more than one case of horse glanders (yellow) and more than 5 cases (orange). The presence of a common border and close economic ties act as a risk factor for the introduction of the pathogen into the country.

The land border between Kazakhstan and Russia is 7598.8km. In Russia, outbreaks of horse glanders were registered in 2007, 2013 and the last case was detected in February 2023, which with close trade and economic ties creates a threat of introduction of the pathogen into the territory of our country. In addition, over the last 4 years, the largest number of horse purchases is also made from Russia.

The total length of the borders with China is 1,783 kilometers, where according to official data outbreaks of glanders were recorded in 2018 and 2019. If there is uncontrolled movement of horses (no official data available) into East Kazakhstan and Abai regions, there is a risk of introduction of the pathogen.

In Mongolia, the most recent outbreaks of glanders were reported in 2018 and included Bayan-Ulgii District, which is 40km from the border with Kazakhstan, which also poses a risk of introduction of the disease.

Kazakhstan's close economic ties with neighboring regions and geographical location in the center of the Eurasian continent, acting as a transboundary region, retain the risk of pathogen introduction and outbreaks of horse glanders in the country. Thus, the implementation of planned veterinary and sanitary measures, border and customs control, scientifically based forecasting with regionalization will allow to maintain the well-being of the Republic of Kazakhstan on horse glanders.

DISCUSSION

Burkholderia mallei is naturally present in certain populations of ungulates, predominantly horses (Zakharova et al. 2018). It becomes pathogenic after weakening of the obligate host organism due to overexploitation and underfeeding, as well as after penetration into the organism of potential hosts, which include carnivores and humans (Karmaliyev et al. 2023). The trigger mechanism that forms the manifestations of the epizootic process of infectious diseases of this group is caused by internal or external stress effects on animals (Cárdenas et al. 2019).

Fig. 4: Countries posing a threat of horse glanders entering the territory of the Republic of Kazakhstan.

Taking into account that on the territory of the Republic of Kazakhstan, outbreaks of horse glanders have not been registered since 1939, the methods and principles of forecasting should be aimed at identifying the potential threat of introduction of the infectious agent from outside (Sattarova et al. 2023). Forecasting epizootic activity is the basis for planning preventive measures (Ivanov et al. 2021; Turgenbayev et al. 2023). When predicting glanders, it is necessary to be based on the data that over the last decade the latent form of the disease without obvious clinical signs and in some cases lack of response to mallein are widespread. In this case, we consider the investigated process (risk of pathogen introduction) based on factors affecting the investigated process, such as:

1) Tthe welfare of cross-border zones, taking into account the analysis of the length of the common border and trade and economic relations.
2) Control of import and export of horses in cross-border zones, transport of horses participating in equestrian competitions.
3) Zoographic factor (horse population with consideration of housing density).
4) Stress factor, which weakens immunity in the transport of horses.

The use of factor analysis makes it possible to identify the leading factors, as well as their combinations that determine the intensity of the disease manifestation. In order to do this, a number of factors that have presumably the greatest influence on the occurrence of the disease should be selected based on epizootological indicators (Karmaliyev et al. 2023; Sattarova et al. 2023). These two methods are convenient for the collection of primary information and operate with a well-developed mathematical apparatus, but have time limitations, i.e., the accuracy of the forecast depends on the duration of information collection. For example, according to our results, cases of horse glanders in Nepal, due to the uncontrolled movement of horses from China, have caused the spread of the disease (Koirala et al. 2022).

Planning of veterinary and prophylactic measures enables the agro-industrial complex of the Republic of Kazakhstan to preserve the epizootic well-being of horse glanders, allows to optimize anti-epizootic measures, reduces the costs of their implementation, increases their efficiency. Risks in the implementation of veterinary preventive measures for glanders are based on such features as:

- Long incubation period of the pathogen,
- Low sensitivity and specificity of available diagnostic tests, and
- Possible infection of clinically healthy animals in latent course of the disease.

In the long term, spanning 5 years or more, there is a risk of introducing horse glanders into the Republic of Kazakhstan, particularly in regions like East-Kazakhstan, Almaty, Zhambyl, and Turkestan. This risk is influenced by factors such as the increasing horse population in Kazakhstan, the growth of equestrian sports, the presence of glanders outbreaks in neighboring countries like China and Mongolia, and the significant import of horses from countries such as Turkey. East Kazakhstan region is in the risk zone, noting such factors as:

- Border zone with Russia, China, proximity to Mongolia,
- First place in the number of horses – 545,012 thousand heads, and
- First place in import of horses from abroad (Russia).

Conclusion

The territory of the Republic of Kazakhstan is safe concerning glanders. Preventive veterinary and sanitary measures show their effectiveness to date, but there are still risk factors for the introduction of the pathogen, therefore, it is necessary to conduct epizootological monitoring with forecasting. The epizootological characteristic of the territory of the country on horse glanders for the last 14

years was determined. Literature and information analysis and research on the prevalence of horse glanders in the world and countries bordering Kazakhstan were carried out. The epizootic situation of horse glanders was studied by analyzing data from veterinary reports of the Veterinary Control and Surveillance Committee of the Ministry of Agriculture of the Republic of Kazakhstan, as well as from OIE data. In some countries in Europe, such as France, there are isolated cases of glanders in imported horses, indicating the need for careful examination of all imported animals. However, the disease should also be investigated in countries where it has disappeared to prevent its reintroduction from other areas of the globe.

Authors' Contribution

KA, AA and AS – coordination of material collection and work in the field; SN and BY and NS coordination of manuscript preparation; AA, BY and KA - DNA extraction and sequencing; AS, SN and NS - data processing and analysis and manuscript preparation; KA, AA, AS, SN, BY and NS – project supervision. All authors have read and agreed to the published version of the manuscript.

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