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РЕЗЮМЕ

Домашние животные, особенно находящиеся на пастбищах, сталкиваются с природными очагами болезней (грызуны, кровососущие насекомые, дикие животные, птицы и др.). Эти контакты являются обычными. Изменение численности диких животных и птиц и их миграция способствуют изменению интенсивности проявления эпизоотического процесса в природном очаге. Они обеспечивают сезонные и периодические колебания заболеваемости не только диких, но и домашних животных.

Самым опасным инфекционным заболеванием для животного в древности является пастереллез. Эта эпидемия является инфекционным заболеванием, характерным не только для сайгаков, но и для человека, других млекопитающих и птиц. Новые результаты, полученные в этом направлении, несомненно, найдут применение в разработке мероприятий по предотвращению массового вымирания животных от болезни пастереллеза и внесут свой вклад в защиту не только домашних животных, но и людей от эпидемии зооантропоноза.

Решить проблему борьбы с пастереллой пока сложно, поскольку длительное присутствие патогенной пастереллы связано с формированием устойчивого эпизоотического очага не только в организме здоровых и больных животных, но и в организме синантропных животных.

В статье авторы провели полный анализ динамики сезонного наблюдения за пастереллезом животных в Западно-Казахстанской области и на основании результатов предложили меры по эффективной борьбе с этой инфекцией.

Данное исследование способствует решению вышеперечисленных проблем и позволяет ветеринарному врачу правильно выбрать методы борьбы с пастереллезом животных.

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DIAGNOSTICS OF TRIPANOSOMOSIS OF CAMELS AND CONDUCT OF EPISATOLOGICAL MONITORING IN TURKESTAN REGION

ANNOTATION

The article discusses a study conducted in the Turkestan region focusing on camel trypanasomiasis. This study utilized serological tests with antigens derived from a local strain to enhance disease detection. Trypanasomiasis is not only a concern for camels but also affects horses, donkeys, and various laboratory animals like dogs, rabbits, guinea pigs, and white mice. This waterborne disease is prevalent in Central Asian republics and Kazakhstan, particularly in areas around the Turkestan, Kyzylorda, Atyrau regions, and along the Ural and Syrdarya rivers. Insects such as flies and mosquitoes are known carriers of this disease, which is typically transmitted through vector-borne routes. The incidence of the disease tends to spike in autumn, with covertly infected animals often being the primary source of new infections.

Given the serious implications of the disease, it's crucial to regularly monitor camel populations in affected areas for early detection and prevention of widespread outbreaks. The use of antigenic preparations from local parasite strains has been highlighted as critical for the rapid diagnosis of trypanasomiasis. Additionally, to safeguard livestock, it is recommended to utilize pastures that are distant from mosquito breeding grounds. In light of these challenges, there is an increased emphasis on developing camel farming in the Turkestan region. This development aims to maximize the production of camel products, including meat and fur. However, to successfully expand camel numbers, it is essential to ensure they are healthy and free from diseases like invasive trypanasomiasis.

Key words: *Immune Reactor, Carrier-transmitted, Dromedary, Strain, Sleeping Sickness, Pervasive.*

Introduction. Trypanosomes, classified within the fibrous protozoa group and related to the kinetoplastidae order, encompass over ten identified types. These microorganisms inhabit the blood, spinal fluid, and bodily tissues of humans and other vertebrates. They derive nutrients through osmosis and certain species produce harmful substances that lead to the destruction of red blood cells, causing conditions like blood clots. These particular species are responsible for spreading trypanosomiasis among humans, as well as wild and domestic animals. The diseases triggered by trypanosomes vary by host: they cause "sleeping sickness" in humans, "karakaptal" in tadpoles, a condition known as "water disease" in camels, horses, donkeys, and dogs, and "Nagana" in cattle. The transmission of these pathogens is primarily through insect vectors such as the tsetse fly, Kandala, Sona, among others. Infected individuals typically experience symptoms including muscle and joint pain, anemia, edema, swelling of lymphatic vessels, and in severe cases, limb numbness and paralysis.

In our country, livestock farming is a significant part of the agricultural sector. Livestock provide meat, milk, and wool. In Kazakhstan, two types of camels are raised: the single-humped dromedaries and the double-humped Bactrian camels. Currently, 12.5 million camels are bred worldwide, of which 1.7 million are in our country. To develop the domestic camel industry, it's important to protect the livestock from various infectious and parasitic diseases [1, 2].

Trypanosomiasis impacts various animals including horses, camels, donkeys, and mules. It also affects laboratory animals such as dogs, domestic rabbits, guinea pigs, and white mice, making them prone to the disease. Trypanosomiasis is found in the republics of Central Asia and Kazakhstan, predominantly in the regions of Turkestan, Kyzylorda, Zhambyl, Mangystau, and Atyrau, especially along the basins of the Ural and Syr Darya rivers. Dormice and certain types of flies act as vectors for the disease. The disease spreads transmissively, and by autumn, the number of affected animals rises. Camels with a latent form of trypanosomiasis are a primary source of the infection. In farming practices where protective and sanitary-veterinary measures are not adequately implemented, camels are more susceptible to trypanosomiasis. To combat this disease, it's vital to provide timely treatment and proper care, as affected livestock don't recover on their own [3].

In the initial stages of the illness, affected camels exhibit symptoms such as fever, diminished appetite, heartburn, and swelling of the lymph nodes. As the disease progresses, noticeable signs include tearing, nasal discharge extending to the forehead, general apathy, and physical decline characterized by weakness, weight loss, paleness of mucous membranes, reduced red blood cell count (leading to anemia), dullness of the coat, trembling limbs, and difficulty breathing. The fatality rate among adult camels suffering from this condition varies between 40% and 70%. In its chronic form, the disease often leads to fatal outcomes due to significant weight loss.

For treatment, a medication named naganin, specifically formulated for camels, is employed. Naganin effectively eradicates trypanosomiasis in camels, particularly when administered in the early stages of the disease. It is dissolved in a 20% saline solution and injected intravenously, dosed at 0.04-0.07 g per kilogram of the camel's body weight. A follow-up dose may be administered after a week if necessary.

In instances where the disease reappears in previously treated camels, the dosage of naganin is increased to 0.09 g per kilogram of body weight. Additionally, furacelin (a yellow powder) is recommended for two consecutive days, and Peros, a suspension mixed with water, is dosed at 0.1-0.2 g per kilogram of body weight.

Another treatment option includes Anthracide (pyraldine), which is administered subcutaneously in a distilled water suspension at a dosage of 0.01 g per kilogram of body weight, serving both therapeutic and preventive purposes.

To safeguard healthy camels against the disease, a preventive dose of naganin is given at 0.035 g per kilogram of body weight. The drug is injected into the median jugular vein, about one-third down the neck. This preventive measure offers protection against the disease for up to 45 days, after which a booster shot is recommended. During treatment campaigns, camels exhibiting symptoms or those suspected of being infected are isolated and treated to prevent the spread of the disease.

Preventive strategies encompass a comprehensive set of actions, which involve: examining the epizootic conditions in areas with a history of the aforementioned diseases; methodically identifying animals that show a susceptibility to these diseases through established diagnostic techniques; implementing both preventive and therapeutic interventions; and meticulously evaluating the outcomes of these interventions to consolidate the gains achieved.

These preventive actions are structured to unfold over a three-year timeline, with each year dedicated to specific objectives. The first year is focused on laying the groundwork with preparatory activities. The subsequent year is aimed at enhancing the overall conditions and practices within the farm. In the final year, the emphasis shifts to assessing the impact of the measures taken, reinforcing the positive outcomes, and aiming for the complete eradication of the disease within the affected population.

Materials and Methods. Trypanosomes are single-celled organisms with a length of 20-32 micrometers and a width of 1.4-2.8 micrometers. They have a whip-like shape, with their front end narrowing down, the middle part expanding, and the rear part being regular. The outer surface of their body has a covering, and inside the cytoplasm, there are various organelles. In the middle of their body, there's a nucleus that resembles a small bean. At the rear, structures called kinetoplasts are located. Starting from these structures, the organism's flagellum extends, assisting the undulating membrane of the trypanosome, then curving around and forming a tail-like projection. Typically, trypanosomes reproduce by dividing in the blood of vertebrate animals. First, the flagellum divides, followed by the body splitting into two or four parts. Subsequently, they can be found in lymph, lymph nodes, and various tissues and organs [4-6].

Trypanosomes are two-host organisms, and their hosts include cattle, camels, and blood-sucking insects such as tsetse flies, mosquitoes, and biting midges. When trypanosomes enter an animal's system, the animal produces specific antibodies against them, which then attack the trypanosomes. As these organisms are destroyed, they release toxins called trypanotoxins into the body. These toxins circulate throughout the entire body via the bloodstream, disrupting nutrient absorption and the functioning of the nervous system. This causes a decrease in the red blood cells and leads the blood to become watery. The infected animal becomes extremely lethargic, loses its appetite, and can experience a body temperature rise up to 40-41°C. Subsequently, the animal's body starts producing trypanolysins to neutralize the harmful effects of the trypanotoxins. This leads to an alleviation of the clinical symptoms of the disease, improving the animal's condition and increasing its appetite. However, when the level of antibodies and trypanolysins drops in the animal's bloodstream, the remaining trypanosomes start multiplying again.

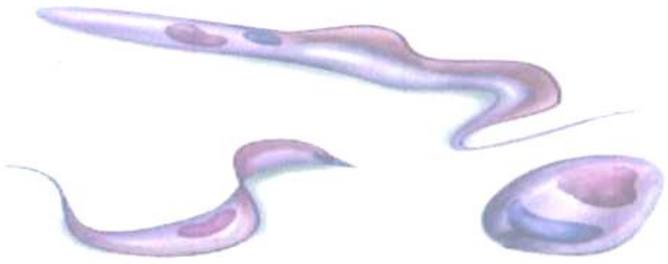

Figure 1 – Trypanasoma protozoa

As a result, the clinical symptoms of the disease in the animal become less pronounced. The animal's vigor improves, and its appetite starts to increase. However, when the levels of antibodies and trypanolysins in the animal's bloodstream decrease, the surviving trypanosomes begin to multiply again. Once their numbers reach a certain level, the body once again produces antibodies to counteract them. This re-initiates the immune response processes against the infection. Due to this immunological cyclical nature, the animal's health deteriorates, and its productivity diminishes [7-9].

The incubation period for this disease is 1-3 weeks. External lymph nodes become swollen, and the animal can't put weight on its hind legs. The underside of the animal and the color of its skin become affected. First, the skin around the neck becomes thin, and then it becomes scarred. The disease causes the animal's meat to become lean. Hence, the public refers to trypanosoma disease as "thin" or "emaciated."

Results and Discussion. To diagnose and monitor trypanosomiasis (a disease caused by trypanosomes), researchers evaluate epidemiological data, identify clinical signs, and conduct microscopic and serological examinations. The clinical symptoms of the disease are not always apparent. Among the signs that can be observed include restlessness, intermittent fever, keratitis, conjunctivitis, and pale spots appearing on various parts of the body. Laboratory tests play a decisive role in diagnosis. A common method is to stain a blood smear using the Romanovsky technique or to examine a blood sample under a microscope in search of trypanosomes. However, microscopic methods are not always definitive. This is because trypanosome parasites are not consistently present in the animal's blood; they mainly thrive inside the animal's internal organs. As a result, it's suggested to test the animal's blood serum through serological reactions.

For cattle, the blood serum is examined using a complement fixation test, while for small livestock, a formalin reaction test is used. To perform the latter test, 2 drops of pure formalin are mixed with 1ml of the animal's blood serum and then placed in a thermostat or chamber for a day. If the livestock is infected with trypanosomiasis, the serum will become gelatinous; if the animal is disease-free, the serum remains unchanged [11-12].

In some cases, to confirm a diagnosis, the bioassay method is used. This involves taking blood from an animal suspected to have the disease, mixing it with sodium citrate, and then injecting it into laboratory animals. If the original animal was infected, parasites can be found in the lab animals' blood within 6-15 days.

In other countries, to treat livestock infected with trypanosomiasis, they administer the drug "cymelarsan" in a liquid form, calculated at a dose of 0.25 mg/kg. Aside from specific medications, symptomatic treatments are used based on the signs of the disease. Improving the nutrition and care of the infected animal is essential. After treating the livestock, they should be monitored for 6 months, and they must undergo clinical, microscopic, and serological tests. Animals confirmed to be free from the disease don't need to be retreated [13,14].

For agricultural operations to stay clear of the trypanosomiasis infection, livestock should be checked every three months in the spring, summer, and fall using at least three diagnostic methods.

To protect livestock from the disease, it's essential to use areas without marshes and to choose elevated locations for water sources. Currently, in the Turkestan region, there's a push to expand livestock farming, leading to an increased demand for products derived from it, such as meat, milk, and wool. To address the rising demand, it's essential to expand the livestock population while ensuring they are not afflicted with various illnesses, such as invasive trypanosomiasis.

The situation poses significant responsibilities for livestock experts and the field of veterinary science. In pursuit of an effective solution to this yet unresolved issue, according to the decree №130 issued on May 17, 2017, by the Akim (governor) of the Turkestan region, it was determined that for the "Development of a list of exogenous diseases in animals that are to be diagnosed and prevented at the expense of the budget," various types of animals, notably livestock including cattle which have been increasingly affected by trypanosomiasis in recent years, should undergo diagnostic tests funded by the regional budget throughout the region.

In accordance with the mentioned decree and as established by the Veterinary Regulations, through the order No. 68 issued on January 24, 2005, by the Ministry of Agriculture of the Republic of Kazakhstan, it is vital to annually examine cattle in areas not free from trypanosomiasis. This examination involves clinical and serological tests, particularly using the complement fixation reaction, to inspect blood samples. Identifying infected cattle and isolating them to prevent the disease from spreading to healthy animals is deemed crucial [15,16].

However, in recent years, cattle in the Turkistan region have been left unchecked against trypanosomiasis. As a result, this disease has become widespread among the cattle population. According to the findings of conducted epizootic monitoring studies, around 350 or more cattle in the region are found to be infected by trypanosomiasis each year. This disease alone leads to a yearly economic loss of about 70 million Tenge for the region. In 2018, out of 100 cattle examined in the Otyrar district, 24 were found to be infected, constituting a 24% infection rate. Therefore, in accordance with the decree No. 130 dated May 17, 2019, issued by the regional governor, it is essential to test the cattle for trypanosomiasis using local budget funds. Scientists from the "Kazakh Scientific-Research Veterinary Institute" and the "South Kazakhstan Scientific-Research Veterinary Station" are collaborating on this. They are using a specially developed trypanosomiasis antigen, derived from native strains known for its high sensitivity. All 19,291 cattle in the region are being tested with this antigen to identify and isolate infected cattle, ensuring the disease doesn't spread further [17].

To determine the sensitivity and diagnostic reliability of the antigen, derived from local strains, used in the complement fixation test to detect trypanosomiasis, blood samples from a total of 50 cattle from the "AgroNurAsia" and "Jana Zher" farms in the Otyrar district were examined. These tests were conducted at the research center of JSC "Kazakh GZVI" and at the "Veterinary Medicine" department's laboratory of the University named after M. Auezov. The results of the study can be seen in Table 1 below.

Table 1 – The results of testing cattle for trypanosomiasis using the serological complement fixation reaction

No.	Farming name	Type of animal tested	Number of animal tested	Complement fixation reaction		
				Positive	Suspect	Negative
1	Otyrar district Shilik rural district “Zhana Zher” farm	Camel	20	5	-	15
2	Otyrar district Koksaray rural district "AgroNurAsia" LLP	Camel	30	1	3	26
Total:			50	6 (12%)	3 (6%)	41(82%)

Based on the results shown in Table 1, out of 30 tested animals from "AgroNurAsia" JSC, 1 tested "positive", 3 were "suspect", and 26 showed a "negative" reaction to trypanosomiasis. Additionally, in the Shilik rural district related to this region, out of 20 tested animals from the "Zhana Zher" farm, 5 tested "positive", while the remaining 15 showed a "negative" reaction. Overall, out of the 50 animals tested, only 6 samples showed a "positive" result in the complement fixation test, accounting for 12% of the total. Thus, it was determined that the trypanosomal antigen derived from local strains, used in the complement fixation reaction, can be useful for identifying infected animals with trypanosomiasis.

The complement fixation reaction (CFR) currently doesn't fully meet the demands of veterinary diagnostic practice. Therefore, scientific work has been conducted to quickly and comprehensively diagnose trypanosomiasis in animals using the direct hemagglutination reaction (DHAR) in laboratory tests.

To isolate strains of trypanosome parasites, blood from cattle and horses was tested multiple times. As a result, trypanosomes were found, and blood samples were taken from all the infected animals and introduced into laboratory white mice, guinea pigs, and several dogs. While white mice and guinea pigs were used to maintain the trypanosome parasite strains, dogs were utilized due to the high volume of their blood, allowing for the collection of a significant mass of parasites.

After 20-25 days from the infection, a large volume of blood was drawn from the infected dogs. Parasitic mass was then separated from the blood composition using established methods. This isolated trypanosome mass was exposed to low-frequency (22 kHz) and high-intensity (50-100 W/cm) ultrasonic waves from a USND-2 device for a duration of 30-40 minutes until disruption. Through this disruption process, the significant antigenic components of the parasite were prepared. Subsequently, the prepared trypanosome antigen was placed on formalin-fixed sheep red blood cells, producing an erythrocytic diagnosticum for trypanosomiasis. To attach trypanosome antigens to the formalin-fixed 2.5% erythrocytes, several chemical conjugates were used, including tannin, rivanol, and amidol, among others. Based on comparative research using the mentioned conjugates, it was determined that a dilution of 1:2000 of tannin was the most effective chemical conjugate for producing an erythrocytic diagnosticum used for detecting trypanosomiasis. For comparison, diagnosticums prepared with amidol and rivanol methods showed sensitivities at titers of 1:400 and 1:600 respectively, while the erythrocytic diagnosticum obtained using the tannin method showed much higher sensitivity, equivalent to titers of 1:800 and 1:1000. The conducted studies confirmed that in the process of preparing the erythrocytic antigenic diagnosticum for detecting trypanosomiasis, attaching the antigen to formalin-fixed erythrocytes using the tannin method yields a diagnosticum with higher sensitivity [18-20].

Several series (5 in total) of trypanosomiasis diagnosticums were prepared using the aforementioned tannin method, and their diagnostic specificity was evaluated. From the conducted research, it was determined that the diagnosticum's titer for detecting the disease in the indirect hemagglutination reaction (IHA) was equivalent to 1:50. In subsequent stages, the effectiveness of this erythrocytic antigenic diagnosticum for trypanosomiasis, when used in the indirect hemagglutination reaction, was studied for its ability to distinguish animals infected with trypanosomiasis.

Table 2 – The results of serological IHA (Indirect Hemagglutination Reaction) studies related to the diagnosis of trypanosomiasis in livestock

No.	Farming name	Type of animal tested	Number of animal tested	IHA (Indirect Hemagglutination Reaction)		
				Positive	Suspect	Negative
1	Ordabasy district, Bogen rural district	Camel	35	8	-	27
2	Otyrar district Koksaray rural district "AgroNurAsia" LLP	Camel	42	3	1	38
Total:			77	11 (14%)	1 (0,01%)	65 (84%)

In Table 2, in the Bogen village district related to the Ordabasy region, out of 35 livestock examined with IHA (Indirect Hemagglutination Assay), 8 tested positive and the remaining 27 showed a negative reaction. Similarly, in the Köksaray village district of the Otrar region, related to the AgroNurAsia LLP, out of 42 livestock examined with IHA, 3 were positive, 38 were negative, and 1 showed ambiguous results. Overall, from the serological examinations conducted, 77 livestock were tested. Of these, 11 showed serologically positive results, and 65 were negative, which correspondingly represent 14% and 84%. The IHA's laboratory procedure was executed according to specific methodologies. Primarily, the IHA method was conducted on clean, specialized polystyrene plates. For the first row of these plates, from wells 1 to 8, they are filled with 0.5 ml of a physiological solution containing 0.7% gelatin.

To enhance the diagnostic efficiency of the IHA (Indirect Hemagglutination Assay), methods of performing the reaction using a micro-method were compared. For this, the IHA procedure is placed on a microtiter plate. From the 2nd to the 8th wells of the plate's first row, 0.05 ml of physiological solution is filled using a special micro-pipette, in 2 drop volumes. Subsequently, 0.05 ml (2 drops) of a 1:50 dilution of the blood sample to be tested is added to the 1st and 2nd wells. Starting from the 2nd well up to the 7th, blood dilution is performed with 0.05 ml of diluter. After that, all wells receive 0.025 ml (1 drop) of the trypanosomal erythrocytic diagnosticum. After gently mixing the reaction, it's left undisturbed at room temperature on a stable surface for 2 hours. After the duration, the reaction's result can be visually observed. If agglutination occurs in at least the first two or three wells, the reaction is considered positive.

As a result of the conducted research studies, for the first time, methods to detect trypanosomiasis in cattle using serological IHA (Indirect Hemagglutination Assay) were clarified and recommended for use in veterinary laboratory practice.

Conclusion. In the Turkestan region, the annual testing of 20,291 head of cattle using the Complement Fixation Test (CFT) and the Indirect Hemagglutination Assay (IHA) has been financially supported by the regional budget for veterinary diagnostic activities. As a result of this support, the early and prompt detection of trypanosomiasis infection in the region's cattle can prevent the widespread spread of the disease. This early detection can contribute to an increase in livestock numbers, enhance the quality of the products derived from them, improve the epidemiological situation, and facilitate the robust development of cattle farming.

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ТҮЙІН

Мақалада Түркістан облысында түйе трипанасомозы бойынша жүргізілген зерттеу қарастырылған. Бұл зерттеу ауруды анықтауды жақсарту үшін жергілікті штаммнан алынған антигендермен серологиялық сынақтарды қолданды. Трипанасомоз тек түйелерге ғана емес, жылқыларға, есектерге және иттер, қояндар, теңіз шошқалары және ақ тышқандар сияқты әртүрлі зертханалық жануарларға да әсер етеді. Су арқылы берілетін бұл ауру Орта Азия республикаларында және Қазақстанда, әсіресе Түркістан, Қызылорда, Атырау облыстарының аудандарында, сондай-ақ Жайық және Сырдария өзендерінің бойында жиі кездеседі. Шыбындар мен масалар сияқты жәндіктер әдетте векторлық жолмен берілетін бұл аурудың белгілі тасымалдаушылары болып табылады. Ауру күзде күрт өседі, жұқтырған жануарлар көбінесе жаңа инфекциялардың негізгі көзіне айналады.

Сонымен қатар малдарды бұл паразитарлық аурудан алдын алу және қорғау түйелерді аурудың негізгі тасымалдаушылары өсетін жерлерден алыс жайылымдарды пайдалану ұсынылады. Осы проблемаларды ескере отырып, Түркістан облысында түйе шаруашылығын дамытуға баса назар аударылады. Бұл зерттеулердің мақсаты түйе өнімдерін, оның ішінде ет пен жүн өндірісін барынша арттыру болып табылады. Сондықтан түйелердің санын арттыру үшін трипанасомоз ауруын болдырмауды және ауруды тез арада анықтау өте маңызды.

РЕЗЮМЕ

В статье рассмотрено исследование, проведенное в Туркестанской области по трипанасомозу верблюдов. В этом исследовании использовались серологические тесты с антигенами, полученными из местного штамма, для улучшения выявления заболеваний. Трипанасомоз поражает не только верблюдов, но и лошадей, ослов и различных лабораторных животных, таких как собаки, кролики, морские свинки и белые мыши. Это заболевание, передающееся через воду, распространено в республиках Средней Азии и Казахстане, особенно в районах Туркестанской, Кызылординской, Атырауской областей, а также вдоль рек Урал и Сырдарья. Насекомые, такие как мухи и комары, являются известными переносчиками этого заболевания, которое обычно передается трансмиссивным путем. Заболеваемость имеет тенденцию к резкому росту осенью, причем скрыто зараженные животные часто становятся основным источником новых инфекций.

Учитывая серьезные последствия этой болезни, крайне важно регулярно следить за популяциями верблюдов в пострадавших районах для раннего выявления и предотвращения широкомасштабных вспышек. Использование антигенных препаратов из местных штаммов паразитов признано критически важным для быстрой диагностики трипаназоматоза. Кроме того, для охраны скота рекомендуется использовать пастбища, удаленные от мест размножения комаров. В свете этих проблем в Туркестанской области уделяется повышенное внимание развитию верблюдоводства. Целью данного развития является максимальное увеличение производства верблюжьей продукции, в том числе мяса и меха. Однако для успешного увеличения численности верблюдов важно обеспечить их здоровье и отсутствие таких заболеваний, как инвазивный трипаназоматоз.

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