Research Article

Development and Testing of Latex Antigen for Serological Diagnosis of Emphysematous Carbuncle in Animals

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Abstract | The annual increase in the incidence of emphysematous carbuncle in animals in Kazakhstan forces a constant search for methods aimed at its elimination. One such approach is the timely and rapid diagnosis of the disease, which will allow the timely implementation of preventive measures. The purpose of the study was to develop an express test to identify cases of animal morbidity for emphysematous carbuncle in the field. For this purpose, a serological method was used using the latex agglutination test (LAT). When preparing the diagnostic, antibodies to surface proteins associated with the flagellar antigen *Clostridium chauvoei* were used, which sensitised latex microparticles with a size of 0.8-1.1 microns from Thermo Fisher Scientific Corporation. In the process of testing the prepared diagnostic system with vaccine strains of the causative agent of emphysematous carbuncle used for active immunisation of animals in Kazakhstan and field experiments with biological material from sick and deceased animals, results were obtained that indicate that the developed test system can be used to diagnose animal diseases. Testing the system on vaccine strains showed a highly specific immunological reaction of the test drug to the causative agents of emphysematous carbuncle, regardless of their biological properties. The sensitivity of the developed test, even with a limited number of studies conducted, was 80%. There was almost always confirmation of the initial diagnosis made after agglutination results in a reaction medium from two crosses or more. This was done by isolating the pathogen during microbiological cultivation. Therefore, the high correlation (r=0.76, P<0.05) between the results of express testing and the detection of the pathogen by bacteriological cultivation, as the main diagnostic method of emphysematous carbuncle, allows recommending its use if it is necessary to diagnose the disease in pasture conditions quickly.

Keywords | Express test, Polyclonal antibodies, Vaccine strain, Correlation analysis, C*lostridium chauvoei*

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INTRODUCTION

Emphysematous carbuncle (emcar) is an acute infectious grazing disease of ruminants caused by the spore-forming anaerobic microorganism *Clostridium chauvoei*. The disease is widespread among young ruminants in the world, including in Central Asian countries such as Kazakhstan, Uzbekistan, Tajikistan, and other post-Soviet countries. Five toxins from C. chauvoei are known so far: oxygen-labile hemolysin D, deoxyribunoclease (DNAse), hyaluronidase, and neuraminidase. They destroy muscle tissue and the formation of characteristic clinical signs.

Economic impacts

Due to the possibility of remaining in the soil for a long time, the infection has a stationary character and, according

to Abutalip *et al.* (2020), causes substantial damage to the livestock industry of the country. Therefore, the mortality of animals is quite high and reaches, according to Salimova *et al.* (2022), up to 100%. The main reason for the death of animals, according to Okafor *et al.* (2023), is a heart problem that is registered in more than 70% of cases of emphysematous carbuncle. According to the authors, the main pathological effect on the body is caused by soluble toxins.

Kazakhstan epidemiology

In Kazakhstan, emphysematous carbuncle are registered almost every year for a long period of time. Perhaps this is due to the substantial territories used as natural pastures and suitable climatic conditions for developing this disease. According to Kayirbolat (2020), in recent years, an increase in animal cases of emphysematous carbuncle has been observed in the Republic of Kazakhstan. It is an annual occurrence throughout the territory of the republic; the highest incidence was reported in the West Kazakhstan region in 2015 and 2019, while the lowest incidence was in Atyrau.

Objective of the study

The objective of this work was to create a quick diagnostic test that could identify *Clostridium chauvoei* antigen in clinical samples from animals that were affected, based on the facts mentioned above. The study aimed to directly address existing deficiencies in veterinary capabilities that support the pathogen's circulation and obstruct appropriate mitigation efforts by developing a rapid diagnosis technique. This objective is in line with more general requirements to limit the disease's financial effects and lessen the severity of emphysematous carbuncle transmission cycles throughout Kazakhstan's livestock industries. To safeguard food-producing animal assets that are essential to the country's agricultural output and rural lives, the research offers a technical solution that advances emphysematous carbuncle management and control efforts.

Literature review

The diagnosis of emphysematous carbuncle requires a thorough approach that, according to Okafor *et al.* (2023) and Hussain *et al.* (2021), should take into account both clinical and pathoanatomical signs as well as lab test results. However, this approach to the diagnosis of emphysematous carbuncle is quite difficult. Thus, according to Espíndola *et al.* (2021), to obtain reliable results, pathological material must be delivered to the laboratory very quickly, which is not always possible. Henrich (2021) associates this with the active development of saprophytic microflora in the sample. Because of this, the main methods could be express tests that can be done in the field or polymerase chain reaction (PCR) methods that find the pathogen's nucleotide sequence in pathological material.

According to Rychener *et al.* (2017), tests based on PCR methods are mostly used to diagnose Clostridium chauvoei species specifically. Heckler *et al.* (2018) indicate that these tests do not need any further laboratory confirmation. In addition, a substantial number of researchers, in particular, Guiselini *et al.* (2020), Nasir *et al.* (2020), and Hussain *et al.* (2019) give an advantage in the diagnosis of emphysematous carbuncle to the newest and most reliable molecular methods. Whereas serological methods are used in very rare cases and are used to identify common clostridium antigens. Thus, in the report of Morrell *et al.* (2022), it is indicated that the test with labelled fluorescent antibodies (FAT) is not specific for *C. chauvoei* and will be positive in the presence of *C. septicum*, *C. novyi*, or *C. sordelii*. Therefore, microbiological confirmation of the pathogen is a prerequisite in cases where the serological method is used. Thomas (2018) also suggests the same. In other studies, in particular, in the paper of Saadat *et al.* (2017), it is indicated that in the case of using PCR and the enzyme immunoassay (ELISA) in the diagnosis of brucellosis, the second method was identified as being more informative since it had a higher sensitivity and specificity. Similar results from using the enzyme immunoassay (ELISA) were obtained only in the case of *Clostridium chauvoei*. The effectiveness of this approach is also confirmed in the study by Sousa *et al.* (2023), indicating that the serological method is the main one in laboratory diagnostics in Brazil.

However, the most effective, even in cases of only a preliminary diagnosis, are express diagnostic methods based on antigen-antibody interaction. Among such methods, indirect hemagglutination, lamellar hemagglutination, and enzyme immunoassay are used (Claus and Macheak, 1972; Tamura *et al.*, 1985; Hansford, 2020). They do not require specific, expensive equipment, and reagents for conducting examinations can be prepared independently. Heller (1920) reports that the agglutination reaction is the simplest method of detecting and differentiating anaerobic clostridium. Recently, many authors, such as Shahrabadi *et al.* (1984) and Martin and Naylor (1994), have considered the latex agglutination test (LAT) method the fastest method of detecting clostridial antigens and toxins in pathological material. According to data from Fedorova *et al.* (2008), the diagnostic process doesn't take more than 10 minutes. Unlike the traditional agglutination reaction, using latex particles instead of erythrocytes lowers the chance of cross-reactions of antigens, making it more specific. Based on such characteristics, this method was chosen for conducting studies.

MATERIALS AND METHODS

Polyclonal antibodies to the flagellated antigen *Clostridium chauvoei* (*Clostridium chauvoei* Flagella-specific Rabbit Polyclonal Antibody) of the German company Biotrend

Chemikalien GmbH and commercial chloromethyl latex beads with a diameter of 0.8-1.1 microns from Thermo Fisher Scientific Corporation were used to create the diagnosticum.

A glycine-salt buffer was used to help the antibodies stick to the surface of latex beads, which was how the diagnostic was made. 1 ml of a 4% suspension of latex beads was introduced into 1 ml of glycine-salt buffer (0.1 M glycine; 0.15 M NaCl; pH 8), and 1 ml of a solution of polyclonal antibodies was added to do this. The mixture was incubated in a shaker incubator (IKA KS4000i control) for 2 hours at 37°C to sensitise latex particles. After the end of the incubation period of sensitisation, the suspension was additionally kept for 8 hours at 4°C in the refrigerator to increase the level of antibody adsorption, after which the latex beads were washed from the remnants of unrelated antibodies. To do this, the suspension was spun at 5000 RPM for 10 minutes. Then, the precipitate was washed three times for 15 minutes each time with a glycine-salt buffer solution that had 1% bovine serum albumin added to block empty spots on the surface of the latex particles. The resulting precipitate was mixed back into a glycine-protein buffer solution with 0.01% sodium azide in a volume of 2 ml to fixate and preserve the antibodies at the same time since this is the preservative used by the manufacturer of polyclonal antibodies. There were no titrations or quantitative tests of the diagnostic's antigen-binding ability because it was only going to be used for qualitatively determining the presence of microorganisms with *Clostridium chauvoei*.

When testing the prepared diagnostic, a suspension of a microbiological culture of *C. chauvoei* clostridium grown in laboratory conditions in the blood serum of non-immunised calves was used as a positive control, and pure blood serum of calves was used as a negative control. The latex agglutination test was conducted on clean slides by mixing a drop of diagnostic and 1-2 drops of the tested suspension at room temperature. The reaction was observed visually in crosses for 10 minutes with constant rocking. Crosses are the visual markers that are used to indicate the amount of agglutination that takes place during the reaction. The number of crosses observed corresponds to the degree of agglutination. The positive result was considered agglutination by 2-4 crosses, doubtful by 1 cross, and negative by the absence of signs of agglutination within 10 minutes after mixing the components. The diagnostic that was made could be used again if it showed a reaction of three to four crosses with a positive control and no agglutination with a negative control.

The commonly used latex agglutination test (LAT) uses the visual observation of agglutination to identify the presence of particular antigens or antibodies in a sample. Depending on the target of interest, latex particles in LAT are coated with either antigens or antibodies. If the target is present, it binds to the right antibodies or antigens on the latex surface when the sample carrying the antibody or antigen is mixed with the latex particles. This makes the particles stick together, which is also known as agglutinate. The presence of the target material in the sample can be qualitatively determined by looking for this agglutination, which is evident to the naked eye. The rationale behind choosing latex agglutination as the diagnostic method lies in its simplicity, cost-effectiveness, and reliability in detecting *Clostridium chauvoei* antigens. The production of crosses, which is a visual indicator of agglutination and the presence of the target antigen, is the anticipated result. The diagnostic hopes that this method will help quickly find and treat emphysematous carbuncle infections in cattle by quickly and accurately identifying *C. chauvoei* in microbiological cultures, blood serum samples, and oedematous fluid from animals that have the infection.

In the future, the performance of the latex diagnostic will be tested using vaccine strains of clostridium, which are used to immunise animals on the territory of the Republic of Kazakhstan and in the field to detect cases of animal infection with the causative agent of emphysematous carbuncle. Therewith, oedematous fluid from the affected areas of the animal was used as pathological material during express testing. The effectiveness of the diagnostic, when tested in the field, was further confirmed by conducting microbiological cultivation of pathological material from the examined animals. The results of the study were processed using variational statistics methods in the Statistica v 14.0.1 software product from Tibco Software Inc.

RESULTS AND DISCUSSION

The epizootic situation with the incidence of animals with emphysematous carbuncle in Kazakhstan is quite tense. As a result of the long distances between pastures making it hard to store and transport pathological material to the lab, the first step towards getting rid of emphysematous carbuncle is to create an easy test for diagnosing it. When choosing a diagnostic method, the main conditions were the following: During its implementation, stationary equipment (which is used only in laboratory conditions) should not be used, and the possibility of setting the test in the field. The latex agglutination test was determined to be the most acceptable option since only a slide and one test reagent was sufficient for its implementation. One known problem with this method is that it could lead to immune system interactions in the reaction mixture. To lower the risk of nonspecific reactivity caused by possible reactions to erythrocyte antigens, immunoneutral latex beads were used to carry the antibodies.

Table 1: Comparative evaluation of the results of the latex agglutination rapid test when tested with vaccine strains of *Clostridium chauvoei,* which are used in the Republic of Kazakhstan.

Immunological preparation	Manufacturer of the drug	Vaccine strain	Results of latex agglutination reaction	
			After 10 minutes	After 30 minutes
Formolvaccine concentrated hydroxyaluminium against emphysematous carbuncle of cattle and sheep	Almaty Trading LLP, Kazakhstan	Culture of the R15 strain of *C. chauvoei*	+++	+++
Anthrax and Emphysematous Carbuncle Associated Live vaccine	Oryol biofactory, Russia	Vaccine strain 2/14 *C. chauvoei*	++	++
Formolvaccine against emphysematous carbuncle of cattle and sheep concentrated aluminium hydroxide	Biotron Group, Kazakhstan	Culture of the R15 strain of *C. chauvoei*	+++	+++
Vaccine against emphysematous carbuncle of cattle and sheep	Antigen LLP, Kazakhstan	Not specified by the manufacturer	+++	++++
Multiclos complex vaccine against animal clostridiosis	Laboratorios Oveyero, Spain	Clostridium complex *C. septicom, C. novyi* type B, *C. sordellii, C. perfringens* type C D, *C. chauvoei, C. haemolyticum*	++++	++++

Source: compiled by the authors.

Considering that the causative agent of emphysematous carbuncle belongs to clostridiosis, which is quite widespread in Kazakhstan and also causes ruminant diseases such as bradzot, malignant edema, and anaerobic enterotoxemia. Therefore, for the preparation of the diagnostic, it was decided to use commercial antibodies specific to the surface protein of the flagella of the causative agent of emphysematous carbuncle (*C. chauvoei*), whereas another class of antibodies sensitive to toxins do not have such specificity and may cross-react with toxins of other clostridium pathogens, which could reduce the diagnostic value of the developed test. After the diagnostic preparation procedure described in the section Materials and methods, it was tested for specificity using a suspension of a microbiological culture of *Clostridium chauvoei* obtained by cultivation as a positive control and immune-negative embryonic serum of calves as a negative control. When receiving a reaction of 3-4 crosses with positive control and in the absence of signs of agglutination with negative control, the diagnostic was used for further studies.

Another part of the test was to see if the diagnostic could work with vaccine strains of *Clostridium chauvoei.* In Kazakhstan, these strains are used to specifically prevent emphysematous carbuncle. The results of the testing are shown in Table 1.

Most monovaccines against emphysematous carbuncle, which are used to immunise animals in the Republic of Kazakhstan, contain microorganisms from the strain R15 *Clostridium chauvoei,* which has a high reactivity and can protect animals from infection in 100% of cases 14 days after vaccination (Kapustin, 2016; Dushayeva *et al*., 2013). When the new diagnostic test was tested with vaccine antigens from the R15 strain, a three-cross reaction was seen no matter who made the biopreparation. This shows that the new express test has a strong species-specific reaction. The reaction was checked twice, 10 and 30 minutes after the introduction of the test antibodies. This was due to the information that during the agglutination reaction, a specific immune response is observed during the first 5-10 minutes, and further processes occurring in the reaction medium in later lines should not be considered (Brovkina, 2011; Kulibaba *et al*., 2023). In the case of the developed express test, no substantial changes were observed during the evaluation of the reaction for a considerable time, which allows conclusions about the stability of the resulting test system and the possibility of its use in field tests.

A substantial difference was identified between manufacturers when testing associated vaccines for the reactivity of the resulting serological test system. It makes sense to think that a drop in the concentration of antigen in the reaction mixture would cause the hemagglutination system to work less effectively. This is what happened with the live vaccine against anthrax and the emphysematous carbuncle of the Oryol biofactory. As a result of a decrease in the antigen concentration, the assessment of the reaction of latex agglutination with the vaccine of the Oryol biofactory, including strain 2/14 *C. chauvoei*, was estimated at two crosses, which is lower than the reaction activity in monovaccines. The exact opposite situation was observed in the case of the Multiclos vaccine from a Spanish manufacturer. The agglutination reaction was most active when 4 crosses were present, even though there were a lot of different infectious antigens in this biopreparation. The

vaccine manufacturer does not indicate the concentration of different microorganisms included in the associated biological product, so it was impossible to determine what caused the increase in the activity of the test system. Either it was due to the amount of microbial antigen *Clostridium chauvoei* or the result of a cross-reaction with surface antigens of other clostridium included in the vaccine Multiclos. Therefore, in future studies, it is planned to conduct several experiments to identify the cross-reactivity of the resulting test system with the antigens of pathogens from other clostridioses.

The next stage of testing the diagnostic effectiveness of the developed express test was its use in the field on biological material obtained from dead or live animals with clinical signs corresponding to emphysematous carbuncle. The latex agglutination test was done at the same time that the material was chosen to be cultured in a bacteriological laboratory to find out what kind of pathogen it was. Sampling of pathological material was conducted in accordance with the Order of the Minister of Agriculture of the Republic of Kazakhstan No. 7-1/393, "On approval of the rules for sampling of transported (transportable) objects and biological material (2015). Bacteriological studies were conducted in accordance with GOST 26503-85, Agricultural animals. Methods for laboratory diagnostics of clostridium (2022). Crops for isolation and accumulation of pathogens were conducted on Kitta-Tarozzi medium for 24-48 hours at 37°C under anaerobic conditions. When turbidity was detected, they were transplanted to meat-peptone agar with the addition of sheep's blood. Morphological, biochemical, and microbiological characteristics served to distinguish the grown colonies. The results obtained are presented in Table 2.

Table 2: Comparative characteristics of the results of diagnostic studies of a number of animal cases with clinical signs of emphysematous carbuncle in 2022-2023.

No.	Type and condition of the animal	The result of the examination by the reaction of latex agglutination	The result of a microbiological examination
1	Cattle, corpse	+++	positive
2	Cattle, corpse	+++	positive
3	Sheep, clinical signs	+	negative
4	Cattle, clinical signs	++	positive
5	Cattle, corpse	+++	positive
6	Sheep, corpse	++++	positive
7	Cattle, clinical signs	-	negative
8	Cattle, corpse	+++	positive
9	Cattle, corpse	++	positive
10	Cattle, clinical signs	+	negative
11	Cattle, corpse	+++	positive

Source: compiled by the authors.

Field studies of latex diagnostics were conducted in the Almaty and Zhambyl regions in 2022-2023. Oedematous fluid obtained by inspiration with a disposable syringe from the affected areas of the animal's body was used to test the diagnostic drug. Testing was mainly conducted with biological material from deceased animals, although some were alive at the time of the diagnosis. All deceased animals succumbed to testing, and only animals with clinical signs of the disease at the time of the study were among the patients. As a result of express testing, higher antigen titers were observed in biological material taken from dead animals than from alive ones with clinical signs. So, the test study of pathological material from dead bodies had an average effectiveness level of three crosses. However, the level of agglutination from living animals did not go above two or three crosses, which is a questionable result. Perhaps this was due to the low concentration of microorganisms in the tissue of living animals due to the activity of antimicrobial mechanisms in the body, while in deceased animals, the number of bacteria increases due to the lack of a protective effect, and therefore, the effectiveness of the test was higher. In connection with this effect, several experiments were conducted using an increased volume of a test suspension of sensitised latex beads when testing oedematous infiltrate from alive animals. 1 to 5 drops of the test reagent were mixed with 1-2 drops of oedematous fluid. An increase in the amount of reagent used during the testing of biological material from sick animals did not affect the results of the agglutination reaction in the tests, so these studies were not reflected in the materials of the paper. As a result of test results from live animals, the diagnostic tool is less useful. To fix this, it is planned to find other biological materials with a higher concentration of pathogens. This will be the focus of future research so that the proposed method can work just as well on both living and dead animals.

During diagnostic studies, the test system was tested on the biological material of two animal species such as cattle and sheep. In both cases, comparable results were obtained with a bacteriological study, which confirms the possibility of using the developed test for different animal species. The sensitivity of the test, despite the small number of studies conducted, was 80%. When comparing the results of the express test with the results of the microbiological study, almost 100% of the result were obtained with the intensity of agglutination in the reaction medium of 2 crosses and higher, while the reaction with the intensity of agglutination of 1 cross turned out to be doubtful, and with microbiological cultivation, this result was not confirmed. One of the reasons for this discrepancy in the results may be a subjective approach to evaluating testing, especially with a slight reaction to the diagnosis. As a result, a positive test with latex antigen at a level of 2 to 4 crosses makes

the diagnosis of emphysematous carbuncle very likely. The results of a correlation analysis between the positive results of the latex agglutination test and the release of the pathogen as a result of microbiological cultivation as the primary diagnostic method support this. The obtained correlation coefficient was 0.76 and had a confidence level of $P<0.05$. A graphical representation of the correlation analysis is shown in Figure 1.

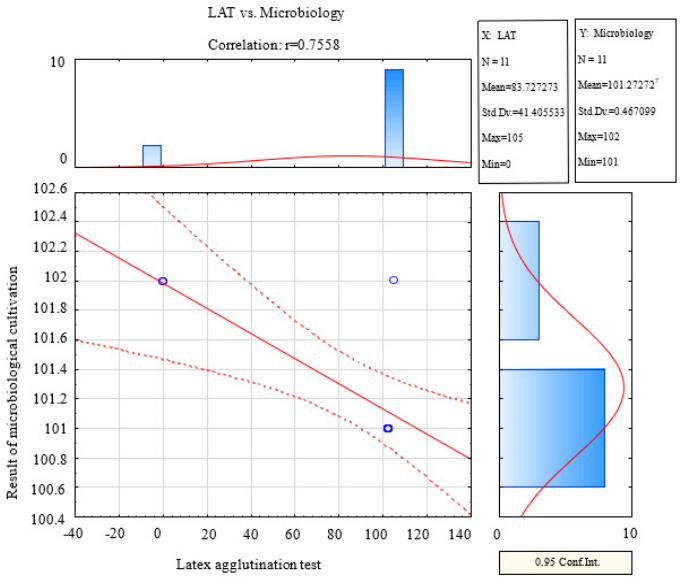

Figure 1: Correlation matrix between the results of the latex agglutination test and microbiological examination of pathological material in the determination of *Clostridium chauvoei*.

Source: compiled by the authors based on the results of analysis conducted in the Statistica 14.0.1 software product.

The correlation analysis between the results of the two methods allowed for the statement that the proposed serological method of latex agglutination can be successfully used for rapid diagnosis of emphysematous carbuncle in the field.

Animal husbandry is very common in Kazakhstan since natural pastures occupy a substantial part of its territory, the peculiarity of which is the uncontrolled situation with most infectious diseases. Infections are especially dangerous, the causative agent of which is capable of forming spores and thus will persist for a long time in the soil of pastures. This situation contributes to the development of the focal nature of pasture diseases and the sporadic incidence among animals. The increase in the dynamics of the incidence of animals for clostridiosis indicates their wide spread in Kazakhstan; therefore, foci of morbidity are found in all regions of the country. In recent years, there has been a substantial increase in the incidence of animals on pastures, mainly for emphysematous carbuncle, which is the most common of all pasture diseases (Karbivska *et al.*, 2020). The research by Abutalip *et al.* (2020) showed that the disease spread very quickly in three areas: West Kazakhstan, East Kazakhstan, and Zhambyl. It spread at a medium rate in Almaty, Aktobe, and Pavlodar. And it spread very slowly in four areas: Kostanay, Atyrau, Karaganda, and Akmola. Based on epizootological indicators, it was determined that in 71.4% of the territory of the Republic of Kazakhstan (10 regions for the period 2010-2020), the intensity of the infectious situation in the emcar of animals was determined to be threatening. The territory of the remaining four regions (28.6%) of the republic (Kyzylorda, North Kazakhstan, Mangystau, and Turkestan) is safe from this disease (Lyubchyk *et al.*, 2019; Bulegenova *et al.*, 2019).

The danger of emphysematous carbuncle, like other similar diseases (for example, malignant edema, blackleg, and tetanus), according to the Helicon Company (2022), lies in the acute and ultra-acute course of the disease when animals die even before diagnosis. Epidemiological data claim that these illnesses can harm a variety of animal species, including livestock and wildlife, and they can cause large financial losses in agricultural environments. These illnesses are severe and frequently fatal; they are characterised by increasing tissue necrosis, a rapid onset, and systemic symptoms like septicemia and toxemia. Due to a lack of veterinary infrastructure in the semi-desert and steppe areas of the country, clostridious diseases are not diagnosed in time, which means that sources of infection can't be eliminated through sanitation measures. As a result, sick animals and their bodies continue to infect the soil, creating new foci (Ayanbayev *et al.*, 2015). This could have been prevented if a diagnosis had been made promptly. Therefore, the development of a diagnostic test that could be used in the field will substantially change the situation with the prevention of emphysematous carbuncle. This assumption is confirmed in the study by Compiani *et al.* (2021), which indicates that the lack of diagnosis contributes to further contamination of pasture soil and an increase in the likelihood of further cases of the disease.

As the test was being made, the main antigenic proteins that could be used to identify *Clostridium chauvoei* in the pathological material were looked at Considering that the causative agent of emphysematous carbuncle belongs to a large group of microorganisms called clostridioses, which, depending on the type of pathogen, are capable of causing such common diseases in Kazakhstan as botulism, tetanus, necrotic enteritis, gangrenous dermatitis, ulcerative enteritis of quails, bradzot, malignant edema, anaerobic enterotoxemia, and emphysematous carbuncle itself. Based on this, several antigenic proteins were identified to be typical for most clostridia and cause cross-reactions during diagnosis. When antigens or antibodies interact

with substances other than those, they were intended to particularly target, a phenomenon known as cross-reaction takes place. Cross-reactions can cause false-positive test findings and lower the test's specificity when used in diagnostic contexts. On the one hand, cross-reactive reactions are great when making vaccines because they let you make a drug that can protect against multiple types of clostridiosis pathogens at the same time. On the other hand, they should never be used when making diagnostics because they make them less specific.

Wensel *et al.* (2020) came to similar conclusions when researching *Clostridium perfringens* and Zhao *et al.* (2022) when working with *Clostridium tetani.* Many species of both pathogenic and saprophytic clostridium are closely related in antigenic terms. Mussayeva *et al.* (2022) assert that in addition to somatic and flagellar antigens, these microorganisms also produce antigens of toxins and enzymes that are responsible for the presence of cross-serological reactions between different species of Clostridium. Cross-serological reactions happen when antibodies made against one antigen react with related antigens from other species or strains. Here, it shows that cross-reactivity can occur when antibodies produced against one species of Clostridium react with antigens from other species. Antigenic complexes connected to clostridium toxins were the main cause of cross-reactivity. Therefore, commercial antibodies obtained before the flagellar protein antigens of *Clostridium chauvoei* were used in these studies. The study by Guyassa (2022), indicates that the concentration and state of vegetative cells are the main factors in the immunogenicity (ability of a substance to elicit an immune response in the body) of the causative agent of emphysematous carbuncle, while antigens associated with toxins are insignificant and have nonspecific serological reactivity, confirmed this strategy. Therefore, even for the production of immunological preparations for the specific prevention of this disease, elements of flagellar antigens in the vegetative cells of *Clostridium chauvoei* are used. With these almost identical testing results, the developed diagnostic is associated with the vaccine strain R15 *C. chauvoei*, which formed the basis of most biologics used in Kazakhstan. Emphysematous carbuncle's species-specific feature is the presence of these protein antigens that help microorganisms stick to the surface. This species, like *Clostridioides difficile*, has flagella and can move around (Razim *et al.*, 2021; Akhmetov and Chidunchi, 2015; Issimov *et al.*, 2022). Therefore, the testing of different vaccine strains with a diagnostic showed high specificity at the level of 3-4 crosses and confirmed the possibility of its use in the field to detect surface antigens of the emphysematous carbuncle pathogen.

Field research with pathological material obtained from sick and fallen animals was the next and more important stage of testing the developed serological preparation. For the preliminary exclusion of negative results, biomaterial was used only from animals with clinical signs of emphysematous carbuncle and all deceased animals without exception. In only one case, a negative result was obtained from a sick animal with clinical signs similar to an emphysematous carbuncle. However, the results of a microbiological analysis also supported the test's efficacy. In this case, it was also negative. In almost all examinations, the titer of antigens was higher in the biomaterial from corpses than from sick animals with clinical signs. Perhaps this is due to the low concentration of bacterial agents in the biological material of sick animals, and after their deaths, the number of microorganisms increases due to a decrease in the action of the body's defences (local and humoral). This assumption is the most rational since antibodies to the surface proteins of microbial cells were used to determine the antigens. If antibodies that match the antigens of toxins were used to diagnose, them it would be reasonable to think that the production of toxins would go up a lot while the number of microbes stayed the same. This means that the results of the serological tests show that the disease-causing agent for emphysematous carbuncle has gone up a lot in the bodies of animals that have it. Scientific publications confirming this assumption have not been found, but the paper of Abreu *et al.* (2017) also suggested that septicaemia is the basis of the pathological process of the development of emphysematous carbuncle disease. Septicaemia is a pathological process underlying the development of emphysematous carbuncle disease. It implies that the microbial cells' active reproduction and dissemination within the body have an impact on the disease's clinical manifestations. That is, the active reproduction and spread of microbial cells in the animal's body leads to the dynamics of clinical changes in the body. In the same way, this can explain why the agglutination reaction of the newly created antigen with biological material from animals only got stronger when the animals were sick or had died.

The effectiveness of the test system for express testing of emphysematous carbuncle was confirmed as a result of parallel analysis of pathological material by bacteriological methods with the isolation of the pathogen as the main diagnostic feature. As a result, the presence of the pathogen was confirmed in 100% of cases when assessing the serological reaction in 2 crosses and higher. A correlation coefficient is a statistical metric that quantifies the degree of relationship between two variables. The degree of agreement between these two diagnostic techniques for identifying emphysematous carbuncle is shown in this instance by the correlation coefficient between the findings of the bacteriological and serological investigations (Danilenko *et al.*, 2021).

Therefore, the correlation coefficient between the results of the serological and bacteriological studies was very high, measuring 0.76 at P<0.05. This means that this method can be used as a diagnostic tool to quickly find sick and dead animals on pasture and take veterinary and sanitary steps to control the outbreak of emphysematous carbuncle in Kazakhstan. Given that bacteriological methods are expensive, time-, and labour-consuming examinations, the developed test system can become an effective diagnostic method (Qureshi *et al*., 2020; Coutinho *et al*., 2016).

After conducting a series of experiments to determine the effectiveness of the developed test system for diagnosing animal diseases for emphysematous carbuncle in the field, some nuances were noticed, the investigation of which will increase the effectiveness of the developed diagnostic system. First, it will be necessary to test the reagent to identify possible cross-reactive reactions with the antigens of flagella from other clostridiums, particularly *Clostridioides difficile*. In addition, a promising area of future research will be the search for biological material to increase the effectiveness of the method when testing sick animals when the titer of the pathogen in the body is low. These areas will be the goals of future studies.

CONCLUSIONS AND RECOMMENDATIONS

Based on the results obtained during test analyses of the developed diagnostic latex reagent for serological determination of antigens of the causative agent of the emphysematous carbuncle of animals, the following conclusions and suggestions can be made for future research.

The use as a target of protein molecules of flagellar antigens and complementary polyclonal antibodies of the Flagella-specific rabbit polyclonal antibody of the German company biotrend chemikalien GmbH sensitised on latex beads with a diameter of microparticles 0.8-1.1 microns from Thermo Fisher Scientific Corporation allowed creating a specific diagnostic reagent for the detection of clostridium type *C. chauvoei,* causing emphysematous carbuncle in ruminants. The latex diagnostic drug used in the agglutination reaction does not need special equipment and devices when reproducing the reaction, which allows it to be used in the field and a quick evaluation result to recommend it as an express test. Test analyses of the developed diagnostic with vaccine strains of *Clostridium chauvoei* used for animal immunisation in the territory of the Republic of Kazakhstan showed high specificity and sensitivity regardless of the manufacturer of the biological product. Testing the effectiveness of the diagnostic on biological material obtained from sick and deceased animals showed the sensitivity of the test in the field at the level of 80%. Therewith, higher results of the agglutination reaction were obtained when using material from deceased animals a reaction at the level of 3-4 crosses, while from live animals with clinical signs of the disease, the reaction level did not exceed 2 crosses.

The results obtained by the agglutination reaction were confirmed by the isolation of *Clostridium chauvoei* from pathological material by bacteriological seeding. The correlation coefficient between the agglutination test results and bacteriological seeding was 0.76 at P<0.05, which allows recommending this method for detecting animal diseases for emphysematous carbuncle in the field. It is planned to conduct a test for the cross-reaction of the diagnostic with surface antigens of other clostridia to increase the reproducibility of the results and search for a biomaterial to increase the sensitivity of the test system in sick animals to improve the effectiveness of the test system in future studies.

This study also emphasizes the need for ongoing research into quick diagnoses for infectious animal illnesses like emphysematous carbuncle. In comparison to gold standard techniques like PCR, future research should carefully measure diagnostic performance criteria like sensitivity, specificity, and positive/negative predictive values. Multi-centre trials testing the latex assay on other clinical sample types would also be helpful in determining its true clinical value. It will be helpful to determine the best sample matrices that produce consistently good diagnostic sensitivity outside of oedematous fluid. Investing in efficient surveillance techniques is still essential for animal health, considering the consequences that uncontrolled clostridial illnesses have on the economy and welfare. Fast on-site testing capabilities could support better disease management tactics, prompt therapies, and early identification.

NOVELTY STATEMENT

The novelty of this article lies in the development of a specific latex antigen-based express test for the serological diagnosis of emphysematous carbuncle in animals, demonstrating high specificity and sensitivity without the need for special equipment, enabling rapid field application.

AUTHOR'S CONTRIBUTION

Each author has participated in the concept and design; analysis and interpretation of data; drafting or revising of the manuscript and each author has approved the manuscript as submitted. All authors agree to be accountable for all aspects of the work.

Conflict of interest

The authors have declared no conflict of interest.

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