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ACTIVITY OF TRIPANOSOMAL ANTIGEN IN VARIOUS SEROLOGICAL REACTIONS

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Abstract: dourine is diagnosed using serological tests such as the classical complement fixation test (CFT), the horse complement fixation test (HCFT). We have recently developed a long-term variant of HCFT – horse complement long fixation (HCLFT). The main component that directly affects the sensitivity of the above tests is the trypanosomal antigen. From the correct definition of the working titer of the latter depends on the clarity of setting and the course of reactions. The aim of our work was to determine the working titer of the trypanosomal antigen in CFT, HCFT and HCLFT. The working antigen titer for each serological reaction remains stable after its determination. This indicator was determined by the checkerboard method of titration of the antigen and positive serum. We have established the working titer of the antigen in the antigenic unit (AU). At the same time, we took 1,5 AU for the working titer of the trypanosome antigen. It was 1:20 in CFT, 1:100 in HCFT and 1:150 in HCLFT. It is these dilutions of the antigen that we will use when setting up the above tests.

Keywords: dourine, antigen, antibody, titer, horse complement fixation test.

Introduction

Dourine is a contagious disease with a chronic or acute course of breeding animals belonging to the equine family, which is transmitted by direct contact from animal to animal during coitus. The causative agent of the accidental disease is *Trypanosoma* (Trypanozoon) *equiperdum* (Doflein, 1901) [1-3].

Dourine is the only trypanosomiasis that is transmitted without the participation of invertebrates. *T. equiperdum* differs from other trypanosomes in that it is mainly a tissue parasite and is rarely found in the blood.

The final diagnosis is determined by the recognition of clinical signs and identification of the parasite. Since this is rarely possible, the diagnosis is usually based on clinical signs and serological evidence obtained during complement fixation test (CFT).

In infected animals, serum antibodies are present even in the absence of clinical signs. To confirm infection in clinical cases and in latent carriers CFT can be used. In uninfected animals, especially donkeys, the result of CFT is often uncertain. An indirect reaction of fluorescent antibodies can be used to confirm the infection or to make a final decision in the case of an uncertain CFT result. Solid-phase enzyme immunoassay is also used [1, 4, 5].

We have previously developed a horse complement fixation test (HCFT) [6], where the limiting factor of reactions is horse complement. In experimental horse infections, trypanosomal antibodies began to be detected 21 days after infection in the CFT, 7-10 days later – in the HCFT. At the same time, positive results were maintained in the CFT for two months, in the HCFT – for nine months (follow-up period) [7].We have worked out a technique

for staging a long–term variant of HCFT horse complement long fixation (HCLFT) [8]. It should be noted that the specificity of serodiagnostics of accidental disease can be improved by standardizing critical reagents, including antigens, and by developing an international standard for *T. equiperdum*-positive serum [9].

To be used in serological reactions, the antigen must be active, specific, and not have anti-complementary and pro-complementary (hemolytic or conglutinating) properties [10]. Antigenic activity is a broad concept. One of its features is that the activity of the antigen depends on its dilution, i.e. the correct choice of the working titer. The aim of our research was to determine the working titer of trypanosomal antigen in CFT, HCFT and HCLFT.

Material and methods

The lyophilized trypanosome antigen for CFT was obtained from the limited liability company of the scientific and production company "Biocenter" (Omsk, Russia), series No. 9, manufactured on 09.2020 (STO 11889413-0003-2008). The antigen was titrated by the checkerboard method by using positive trypanosomal serum. As a positive serum, the blood serum of horses that reacted positively in the CFT was taken [11].

As a control, a saline solution was added to a row with a positive serum, and to a row with an antigen. The results were taken into account according to the degree of hemolysis delay in CFT, and conglutinations in HCFT and HCLFT.

Results

As can be seen from Table 1, in CFT trypanosomal antigen in dilutions of 1:10, 1:20 and 1:30 showed a positive serum titer of 1:40, whereas in subsequent dilutions the latter began to decrease, i.e. in dilutions of the antigen 1:40 it was 1:20, in dilutions of the antigen 1:50 – 1:10, etc.

Table 1 – Determination of antigen titer in the CFT by the checkerboard method

Positive serum	Dilution of trypanosomal antigen						
	1:10	1:20	1:30	1:40	1:50	1:60	SS
1:5	4+	4+	4+	4+	2+	+	-
1.10	4+	4+	4+	3+	-	-	-
1:20	4+	4+	4+	2+	-	-	-
1:40	2+	2+	2+	-	-	-	-
1:80	-	-	-	-	-	-	-
SS	-	-	-	-	-	-	-
Note: -, +, 2+, 3+, 4+ – degrees of hemolysis; SS – saline solution (control)							

Trypanosomal antigen with saline solution and positive serum with saline solution (controls) gave complete hemolysis of erythrocytes.

When titrating trypanosomal antigen and positive serum in HCFT (Table 2) in the dilution of the antigen at 1:30, the titer of the positive serum was 1:320, and with the dilution of the antigen 1:50, it was 1:160. Dilutions of the antigen 1:100 and 1:150 showed a serum titer of 1:80 and with dilutions of the antigen 1:200 and 1:250, a serum titer of 1:5 was established.

Table 2 – Results of titration of trypanosomal antigen and positive serum in HCFT

Antigen dilution	Dilution of positive trypanosome serum							
	1:5	1:10	1:20	1:40	1:80	1:160	1:320	1:640
1:30	4+	4+	4+	4+	4+	4+	2+	-
1:50	4+	4+	4+	4+	4+	4+	-	-
1:100	4+	4+	4+	4+	2+	+	-	-
1:150	4+	4+	4+	4+	2+	-	-	-
1:200	3+	-	-	-	-	-	-	-
1:250	2+	-	-	-	-	-	-	-
1:300	+	-	-	-	-	-	-	-
SS	-	-	-	-	-	-	-	-
Note: -, +, 2+, 3+, 4+ – degrees of conglutination; SS – saline solution (control)								

Trypanosomal antigen with saline solution and positive serum with saline solution (controls) gave complete conglutination of erythrocytes.

When titrating trypanosomal antigen and positive serum in HCLFT (Table 3) in the dilution of the antigen at 1:30, the titer of the positive serum was 1:320, and with dilutions of the antigen 1:50 and 1:100, it was 1:160. Dilution of the antigen 1:150 and 1:200 showed a serum titer of 1:80 and when diluting the antigen 1:250, a serum titer of 1:40 was established.

Table 3 – Titration results of trypanosomal antigen and positive serum in HCLFT

Antigen dilution	Dilution of positive trypanosome serum								
	1:5	1:10	1:20	1:40	1:80	1:160	1:320	1:640	SS
1:30	4+	4+	4+	4+	4+	4+	3+	-	-
1:50	4+	4+	4+	4+	4+	3+	+	-	-
1:100	4+	4+	4+	4+	4+	3+	-	-	-
1:150	4+	4+	4+	4+	4+	+	-	-	-
1:200	4+	4+	4+	4+	3+	-	-	-	-
1:250	4+	4+	4+	3+	+	-	-	-	-
1:300	3+	+	-	-	-	-	-	-	-
SS	-	-	-	-	-	-	-	-	-
Note: -, +, 2+, 3+, 4+ – degrees of conglutination; SS – saline solution (control)									

Trypanosomal antigen with saline solution and positive serum with saline solution (controls) gave complete conglutination of erythrocytes.

Discussion

Trypanosomal antigen for CFT had the greatest activity in CFT in dilutions of 1:10-1:30, which cause the highest titer of positive serum (1:40).

Trypanosomal antigen showed the greatest activity in HCFT in dilutions of 1:100-1:150, which caused the highest titer of positive serum (1:80).

The greatest activity of the antigen in HCLFT was in the dilutions of the trypanosomal antigen 1:200, the titer of the positive serum was 1:80.

In further studies, when setting up the above serological tests for the diagnosis of dourine, we took the dilution of the antigen, which showed the highest titer of positive serum, for the antigenic unit (AU). We found that 1 AU in the CFT is the dilution of the antigen 1:30, 1.5 AU - 1:20 (30:1,5), in HCFT 1 AU is the dilution of the antigen 1:150, 1.5 AU is 1:100, in HCLFT 1 AU is the dilution of the antigen 1:200, 1.5 AU – 1:150 gives a good result.

Conclusion

The working titer of trypanosomal antigen in CFT, HCFT and HCLFT was determined. It was 1:20 in CFT, 1:100 in HCFT and 1:150 in HCLFT. It can be noted that the higher the working titer of the antigen, we get a high titer of positive serum, which shows the sensitivity of the test.

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ТРИПАНОСОМДЫҚ АНТИГЕННІҢ ӘРТҮРЛІ СЕРОЛОГИЯЛЫҚ РЕАКЦИЯЛАРДА БЕЛСЕНДІЛІГІ

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Аннотация: жылқы киеңкісін балау үшін комплемент байланыстыру реакциясы (КБР), жылқы комплементін байланыстыру реакциясы (ЖКБР) сияқты серологиялық тесттер қолданылады. Жақын арада біз ЖКБР-дің ұзақ нұсқасы жылқы комплементін ұзақ байланыстыру реакциясы (ЖКҰБР) жасадық. Осы тесттердің сезімталдығына тікелей әсер ететін негізгі компонент – ол трипаносомдық антиген. Реакцияның дұрыс қойылуы және жүруі антигеннің жұмыс титрін дұрыс анықтауға байланысты. Біздің зерттеулеріміздің мақсаты КБР, ЖКБР және ЖКҰБР-да трипаносомдық антигеннің жұмыс титрін анықтау болды. Әрбір серологиялық реакция үшін антигеннің жұмыс титрі анықталғаннан кейін тұрақты болып қалады. Бұл көрсеткішті антиген мен позитивті қан сарысуын шахмат әдісімен титрлеу арқылы анықтадық. Біз антигеннің жұмыс титрін антигендік бірлікпен (АБ) анықтадық. Сонда, трипаносомды антигеннің жұмыс титрі ретінде 1,5 АБ алдық. Ол КБР-да 1:20, ЖКБР-да 1:100 және ЖКҰБР-да 1:150 болды. Антигеннің дәл осындай ерітінділерін осы аталған реакцияларды қойған кезде қолданатын боламыз

Түйін сөздер: киеңкі, антиген, антидене, титр, жылқы комплементін байланыстыру реакциясы.

АКТИВНОСТЬ ТРИПАНОСОМНОГО АНТИГЕНА В РАЗЛИЧНЫХ СЕРОЛОГИЧЕСКИХ РЕАКЦИЯ

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Аннотация: случная болезнь лошадей диагностируется при помощи серологических тестов, таких как классическая реакция связывания комплемента (РСК), реакция связывания комплемента лошади (РСКЛ). Нами в последнее время разработана длительный вариант РСКЛ – реакция длительного связывания комплемента лошади (РДСКЛ). Основным компонентом, который непосредственно влияет на чувствительность вышеуказанных тестов, является трипаносомный антиген. От правильного определения рабочего титра последнего зависит четкость постановки и ход реакций. Целью нашей работы явилось определить рабочий титр трипаносомного антигена в РСК, РСКЛ и РДСКЛ. Рабочий титр антигена для каждой серологической реакций остается стабильным после его определения. Данный показатель определяли шахматным методом титрования антигена и позитивной сыворотки. Нами установлено рабочий титр антигена в антигенной единице (АЕ). При этом мы брали 1,5 АЕ за рабочий титр трипаносомного антигена. Она была 1:20 в РСК, 1:100 в РСКЛ и 1:150 в РДСКЛ. Именно эти разведения антигена мы будем использовать при постановке вышеуказанных тестов.

Ключевые слова: случная болезнь, антиген, антитело, титр, реакция связывания комплемента лошади.