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Comparison of serological tests for equine trypanosomosis in naturally infected horses from Kazakhstan

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Abstract

In this study, we compared the complement fixation test (CFT), the horse complement fixation test (HCFT) and a card agglutination test for trypanosomosis (CATT/*T. evansi*) for the diagnosis of equine trypanosomosis in the Republic of Kazakhstan. Cohen's kappa test was used to evaluate the concordance between the three tests. Kappa scores for CFT versus HCFT and CATT are both 0.6165 (95% Confidence Interval CI 0.414–0.819) indicating a ''substantial'' agreement between CFT and HCFT or CATT, respectively. Kappa for HCFT versus CATT is 0.395 (CI 0.142–0.648) indicating a ''fair'' agreement between the two tests. In the absence of a golden standard, seroprevalence and sensitivity and specificity of the three tests were estimated using maximum likelihood estimation. CFT has a sensitivity of 57.2% (CI 31.5–79.5%) and a specificity of 95.8% (CI 89.2–98.5%), HCFT has a sensitivity of 80.6% (CI 44.1–95.6%) and a specificity of 99.5% (CI 90.7–100%), CATT has a sensitivity of 80.2% (CI 44.5–95.2%) and a specificity of 98.5% (CI 79.5–99.9%). The seroprevalence of equine trypanosomosis in Kazakhstan was estimated at 16.4% (CI 9.4–27.0%). The data suggest that for epidemiological studies and the control of equine trypanosomosis serological tests prove useful since they have a high specificity and a satisfactory sensitivity. Field applicable tests, such as CATT/*T. evansi* may be used to replace laboratory-based tests, such as CFT and HCFT.

Keywords: Equine trypanosomosis; *T. equiperdum*; *T. evansi*; Diagnosis; Republic of Kazakhstan

1. Introduction

Among the widespread protozoan illnesses bringing significant economic damage to the development of horse- and camel-breeding in the Republic of

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Kazakhstan, trypanosomosis is believed to occupy an important place (Ilgekbayeva et al., 1999). For mass screening of the horse population for equine trypanosomosis, serological methods have been the method of choice since the early 20th century (Luckins, 1994). The only diagnostic test for equine trypanosomosis at this moment, recommended by the World Animal Health Organisation (OIE), is the complement fixation test (CFT) (Watson, 1915). CFT detects antibodies against *T. equiperdum* in the serum of the host. Using this test, *T. equiperdum* has been eradicated in the USA, Canada and the EU. More recently, a horse complement fixation test (HCFT) has been developed for the diagnosis of dourine (Ilgekbayeva, 1994). Ilgekbayeva et al. (1999) have demonstrated that in HCFT antibodies are found earlier, in high titres and during a longer time in comparison to CFT. However, both these diagnostics tools are laboratory tests and thus samples need to be transported and processed in a serological laboratory. On the other hand, a diagnostic antibody detection test for *T. evansi* based on the RoTat 1.2 VAT has been developed, namely CATT/*T. evansi*, a direct card agglutination test (Bajyana Songa and Hamers, 1988). This test is fast, uses a standardised antigen and can be performed in situ, i.e. without the need of a fully equipped laboratory. Recently, it has been proven that most so-called *T. equiperdum* strains also express isoVATs of *T. evansi* RoTat 1.2. Therefore, the CATT/*T. evansi* may prove to be a good test for equine trypanosomosis, regardless whether the causative agent is *T. evansi* (surra) or *T. equiperdum* (dourine) (Claes et al., 2003a).

While serological tests can be the method of choice for mass screening of populations, their main limitation will remain the failure to demonstrate the parasite. Unfortunately, parasitological techniques are known to lack sensitivity, especially for the detection of *T. equiperdum*, which is considered to be a tissue parasite rather than a blood parasite (Brun et al., 1998). As a result, serological tests, such as CFT are used as a reference test for equine trypanosomosis since there are no adequate parasitological tests available.

In this study, we compared the classical CFT, HCFT and CATT/*T. evansi* for the diagnosis of equine trypanosomosis in Kazakhstan. Cohen's kappa test was used to measure the level of agreement between the laboratory tests (CFT and HCFT) and the field test (CATT).

Next to this we used the package ''Tests in the Absence of a Gold Standard (TAGS)'' (Pouillot et al., 2002) to estimate, under assumption of conditional independence, the seroprevalence and both sensitivity and specificity of the three tests relative to the ''seropositive status'' of the animal.

2. Material and methods

2.1. Sampling information

Serum samples from 132 horses in the Almaty region (in the south-eastern part of the country) were taken between January 2004 and March 2004. Serum samples from 89 imported (naïve) horses were used as a negative population. The serological screening of the sera was carried out in Almaty at the regional branch of the Republican Veterinary Laboratory of the Republic of Kazakhstan.

2.2. Complement fixation test (CFT)

CFT was performed according to the Manual of Standards for Diagnostic Tests and Vaccines (edition 2000) from the OIE (www.oie.int, 2004). The *Trypanosoma* antigen was manufactured by LTD SME ''Biocentre'' (Russia). Test sera were screened in serial dilutions in phosphate buffered saline (PBS). A sample is considered positive when the titre is 5 or higher.

2.3. Horse complement fixation test (HCFT)

HCFT was performed according to Ilgekbayeva (1994). The *Trypanosoma* antigen used in this test was the same as was used in CFT. Sera were screened in serial dilutions in PBS. A sample was considered positive when the end titre was 5 or higher.

2.4. Card agglutination test for trypanosomiasis/ T. evansi (CATT/T. evansi)

The CATT/*T. evansi* is a direct card agglutination test which uses formaldehyde fixed, freeze-dried trypanosomes of *T. evansi* VAT RoTat 1.2 stained

with Coomassie blue (Bajyana Songa and Hamers, 1988). Test sera were screened in serial dilutions in phosphate buffered saline (PBS). A sample was considered positive when the end titre was 4 or higher.

2.5. Statistical analysis

The concordance between the different diagnostic tests was determined using Cohen's kappa test (Cohen, 1960). Estimations for the diagnostic sensitivity and specificity as well as for prevalence under the assumption of the absence of a ''golden standard'' were obtained using TAGS (Pouillot et al., 2002). This *R* based package uses maximum likelihood estimation to determine the, in this set up, seven unknown parameters. The basic assumption to use TAGS is that that the test results are independent conditional on the latent status. This latent status is in general assumed to be the disease status but we extended this assumption by saying that due to the fact that all tests are antibody detection tests the conditional independence assumption holds as independence conditional on the ''seropositive status''. This implicates that prevalence estimates should be interpreted as seroprevalence, sensitivity as the probability of observing a positive test result given seropositive and specificity as the probability of a negative test result given seronegative (Verloo et al., 2004).

3. Results

All sera from the naïve population ($n = 89$) tested negative for all three tests. Cross comparisons from the three different tests for the field samples ($n = 132$) and the naïve population ($n = 89$), are shown in Tables 1 and 2. Table 1 compares CFT versus HCFT and CATT, respectively; Table 2 compares the HCFT versus CATT. Kappa scores for CFT versus HCFT and CATT are both 0.6165 (95% CI 0.414–0.819). According to the criteria by Landis and Koch (1977), this indicates a ''substantial'' agreement between CFT and HCFT or CATT, respectively. Kappa for HCFT versus CATT is 0.395 (95% CI 0.142–0.648) indicating a ''fair'' agreement between the two tests.

Table 1
Comparison of CFT results versus HCFT and CATT/*T. evansi* for the tested population, including the ''diseased free'' population ($n = 221$)

		HCFT		CATT/*T. evansi*	
		+	−	+	−
CFT	+	12	7	12	7
	−	6	107 + 89	6	107 + 89

Table 2
Comparison of HCFT results versus CATT/*T. evansi* for the tested population, including the ''diseased free'' population ($n = 221$)

		CATT/*T. evansi*	
		+	−
HCFT	+	8	10
	−	10	104 + 89

Table 3
Distribution of the individual horse data within the ''diseased'' population ($n = 132$) over the outcome of the three different tests

+++	−−−	+−+	+−−	++−	−++	−+−	−−+
8	104	2	5	2	6	2	3

First symbol for CFT result, second symbol for HCFT result, third symbol for result of CATT.

Individual data from the 132 field samples show that 104 animals score negative in all three tests, while eight animals are positive in all three tests. Five horses were positive in CFT only, two in HCFT only and three in CATT only. Two samples were positive in both CFT and HCFT while CATT was negative. Two were positive in CFT and CATT while their HCFT remained negative. Finally six samples tested positive in HCFT and CATT while their CFT result was negative (Table 3). TAGS results of these data results in the following estimations for sensitivity and specificity: CFT has a sensitivity of 57.2% (CI 31.5–79.5%) and a specificity of 95.8% (CI 89.2–98.5%), HCFT has a sensitivity of 80.6% (CI 44.1–95.6%) and a specificity of 99.5% (CI 90.7–100%), CATT has a sensitivity of 80.2% (CI 44.5–95.2%) and a specificity of 98.5% (CI 79.5–99.9%). The seroprevalence of equine trypanosomosis in the studied population was estimated at 16.4% (CI 9.4–27.0%).

4. Discussion

According to the TAGS analysis the seroprevalence of equine trypanosomosis in the studied area of

Kazakhstan is estimated at about 16.4% (CI 9.4–27.0%). Because we used a model that stated that the results of the three antibody detection tests are conditional independent given the latent variable the estimated prevalence of the TAGS model can assumed to be the seroprevalence. This in the philosophy that the different antibody detection tests all give information regarding the ''seropositive status'', but, as no test is perfect they make errors conditional on this status (e.g. sensitivity and specificity). Hence, sensitivity is here an estimate of the proportion of test positives in the seropositive population and specificity the proportion of test negatives in a seronegative population. For a more general discussion on the properties of latent variables in no gold standard models we refer to Verloo et al. (2004).

The estimated 16.4% (CI 9.4–27.0%) seroprevalence is in agreement with previous (unpublished) data, which showed an estimated seroprevalence of 15% (CI 11.98–18.44) using only CFT on 500 horses in the same region (Ilgekbayeva, personal communication). This high level of seroprevalence and the absolute absence of positive test results in imported horses confirms that equine trypanosomosis is endemic in Kazakhstan. A comparable situation can be found in other countries in this region, e.g. Mongolia were a seroprevalence of 7.5% was found using CFT (Clausen et al., 2003). The actual true prevalence may be different but has to be determined using a gold standard. Parasitological techniques, though highly specific unfortunately lack sensitivity, especially for the detection of *T. equiperdum* (Brun et al., 1998).

The kappa test was used to measure the concordance of the data generated by the three different serological tests. The results indicate a fair to substantial concordance between all three tests. A previous study by Williamson et al. (1988) already examined the CATT as an alternative for CFT in naturally infected horses in South Africa. When we perform a kappa test on their data, we obtain similar results as in our study: on a test population of 43 horses a kappa of 0.8583 is found, indicating an ''almost perfect'' agreement between tests. Interestingly all the tested horses showing clinical signs of dourine scored positive in CATT. The authors state that ''CATT could be usefully employed especially as a field test in outlying districts, and should be further investigated''.

Thus, from our observations as well as the results of Williamson et al. (1988), it can be suggested that CATT may be proposed to replace complement fixation tests (CFT and HCFT) as serological mass screening test. The CATT test is faster and easier to perform than complement fixation tests. Moreover, anti-complement factors have no effect on the result. This may be particularly interesting in the testing of donkey sera where anti-complement effects are often observed (Williamson and Herr, 1986). Finally, different reference centres usually use different antigens in CFT, often generated from trypanosome strains with an unknown history (Zablotskij et al., 2003). This makes comparison of results difficult. CATT can overcome this problem, since this test uses a standardised antigen, a RoTat 1.2 cloned population. One important thing to bear in mind is that certain parasite populations may have lost the RoTat 1.2 VSG gene, like was observed with the LiTat 1.3 VSG gene for *T. b. gambiense* in Cameroon (Bromidge et al., 1993). However, until now no such parasite strains were isolated in Asia: strains originating from Kazakhstan, China, Vietnam, Indonesia and Taiwan all tested positive in CATT (Verloo et al., 2001; Claes et al., 2003a). Only antibodies raised against one strain (OVI) isolated from a South African horse, tested negative in CATT. Another strain, BoTat 1.1 appeared to have lost the RoTat 1.2 VSG gene but nevertheless reacted positive in CATT, possibly due to VSG cross-reactions. However, it is hypothesized that these two strains may possibly be *T. b. brucei* populations (Claes et al., 2003b).

The estimations by the TAGS model for conditional independent tests show a quite high specificity for all three test, but a relatively lower sensitivity, ranking from 57.2% over 80.2 to 80.6% for CFT, CATT and HCFT, respectively. The general trend is that all tests show a high specificity. Thus, the card agglutination test and HCFT are scoring significantly better than the CFT for diagnostic sensitivity. Ideally these estimations should be further evaluated by including test which are based on other biological principles (e.g. PCR, antigen detection, ...) into the model. This would drive the latent variable more and more into the direction of disease (hence giving an estimate for true prevalence) while the estimates for diagnostic sensitivity and specificity would be less biased according to their respective epidemiological defini-

tion. In addition the inclusions of more tests in the model would create the opportunity to correct for conditional dependence as more degrees of freedom become available (Goetghebeur et al., 2000).

As serological tests can be the method of choice for mass screening of populations, their main limitation will remain the failure to demonstrate the parasite. The only way to tackle this problem will be the improvement of parasitological tests or the development of molecular tests. However, applicability of molecular tests in the field remains till now impossible and therefore may proof not to be the magic solution in the near future.

In conclusion, this is the first study to address the problem of equine trypanosomosis in the Republic of Kazakhstan. The data suggest that for epidemiological studies and the control of equine trypanosomosis, serological tests prove useful since they have a high specificity and a satisfactory sensitivity. Field applicable tests, such as CATT/*T. evansi* may be used to replace laboratory-based tests, such as CFT and HCFT. Subsequently improved diagnosis possibly followed by treatment will help to control this disease in endemic areas, such as the Republic of Kazakhstan, Southern Africa, Ethiopia and Mongolia.

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