



Development of a latex agglutination test with recombinant variant surface glycoprotein for serodiagnosis of surra

S. Rogé^{a,e,*}, R. Baelmans^b, F. Claes^c, V. Lejon^d, Y. Guisez^e,
D. Jacquet^b, P. Büscher^a

^a Department of Biomedical Sciences, Unit of Parasite Diagnostics, Institute of Tropical Medicine, Nationalestraat 155, B-2000 Antwerp, Belgium

^b Production and Applied Technology Unit, Institute of Tropical Medicine, Nationalestraat 155, B-2000 Antwerp, Belgium

^c Animal Health Service, Food and Agriculture Organization of the United Nations (FAO), Viale delle Terme di Caracalla, 10532 Rome, Italy

^d Institut de Recherche pour le Développement, Unite Mixte de Recherche UMR177 – Intertryp, Campus International de Baillarguet A A-17/G, 34398 Montpellier, France

^e Laboratory for Molecular Plant Physiology and Biotechnology, Department of Biology, University of Antwerp, Groenenborgerlaan 171, B-2020 Antwerp, Belgium

ARTICLE INFO

Article history:

Received 27 June 2014

Received in revised form 16 August 2014

Accepted 23 August 2014

Keywords:

Trypanosoma evansi

Surra

Domestic animals

Antibody detection

RoTat 1.2

Serodiagnosis

ABSTRACT

Serodiagnosis of surra is commonly performed with the CATT/*Trypanosoma evansi* direct agglutination test. This antibody detection test is based on lyophilised bloodstream form trypanosomes propagated in rats and presenting the predominant variant surface glycoprotein (VSG) RoTat 1.2 on their surface. Recently, the N-terminal fragment of VSG RoTat 1.2 has been expressed as a recombinant protein in the yeast *Pichia pastoris* and showed diagnostic potential in ELISA. This recombinant antigen has now been incorporated in a latex agglutination test, the rLATEX/*T. evansi*. In this study, we compared the diagnostic accuracy of rLATEX/*T. evansi* and CATT/*T. evansi* with immune trypanolysis (TL) as reference test on a total of 1717 sera from camels, horses, bovines, water buffaloes, dogs and sheep. The rLATEX/*T. evansi* displayed a slightly better agreement with TL than CATT/*T. evansi* (kappa [κ] respectively 0.84 and 0.72). The sensitivities of rLATEX/*T. evansi* (84.2%, 95% CI 80.8–87.1) and CATT/*T. evansi* (84.0%, 95% CI 80.6–87.0) were similar, but rLATEX/*T. evansi* was significantly more specific (97.7%, 95% CI 96.7–98.4) than CATT/*T. evansi* (89.4%; 95% CI 87.6–91.1). We consider the rLATEX/*T. evansi* an alternative for the CATT/*T. evansi*, with the advantage that the use of a purified recombinant antigen leads to a more standardised diagnostic test with an improved specificity. Moreover, it eliminates the use of laboratory animals and can be easily scaled-up, e.g. in biofermentors.

© 2014 Elsevier B.V. All rights reserved.

1. Introduction

Surra, an infectious disease caused by the protozoan parasite *Trypanosoma evansi*, affects a large spectrum of wild and domestic animal species in the northern part of Africa, the Middle East, Asia and Latin America. This large and diverse host range, including carriers with mild or no symptoms, together with the parasite's mechanical transmission through non-specific vectors, such as bloodsucking

* Corresponding author at: Department of Biomedical Sciences, Institute of Tropical Medicine, Nationalestraat 155, B-2000 Antwerp, Belgium. Tel.: +32 32476795; fax: +32 32476373.

E-mail address: sroge@itg.be (S. Rogé).

flies and vampire bats, contribute to its widespread occurrence. The principal host species vary geographically, but camels (both dromedary and Bactrian), horses, buffaloes and cattle are particularly affected, although other animals, including wildlife, are also susceptible. Symptoms include fever, anaemia, loss of appetite, weight and productivity, paralysis and death depending on the host species. Surra can also lead to immunosuppression and can thus aggravate concomitant infections or impede vaccination campaigns (Desquesnes et al., 2013a,b).

As with all bloodstream form African trypanosomes, *T. evansi* parasites are densely coated with dimers of a single type of variant surface glycoprotein (VSG). This VSG coat is highly immunogenic and induces a strong humoral immune response in the host able to destroy trypanosomes that are recognised by VSG-specific antibodies. Periodic VSG switches sustain the infection and lead to varying parasite loads in the blood (Jones and McKinnell, 1985; Pays et al., 2004). Low parasitaemia often renders microscopic parasite detection poorly sensitive. Therefore, serodiagnosis based on the detection of *T. evansi* specific antibodies is recommended by the World Organisation for Animal Health (Organisation Internationale des Epizooties, OIE) (OIE, 2012). Previous studies have shown that one particular VSG, the RoTat 1.2 that was first described in a *T. evansi* strain isolated in 1982 from an Indonesian water buffalo, is expressed early during the infection by almost all *T. evansi* strains throughout their geographical distribution (Bajyana Songa and Hamers, 1988; Verloo et al., 2001; Claes et al., 2004).

Based on earlier studies, the immune trypanolysis test (TL) can be considered as the most specific reference test for the detection of anti-RoTat 1.2 antibodies in *T. evansi* infected animals (Verloo et al., 2000, 2001; Holland et al., 2005). This test makes use of a cloned population of live trypanosomes all expressing the VSG RoTat 1.2 that, in the presence of anti-RoTat 1.2 antibodies and of guinea pig complement, will be killed by antibody-mediated complement lysis (Holland et al., 2002). Clearly, TL is restricted to specialised laboratories and therefore, OIE rather recommends alternative tests such as the CATT/*T. evansi* or ELISA for screening animals for *T. evansi* infection (OIE, 2012). Both CATT/*T. evansi* and TL make use of native antigens produced through infections of laboratory rodents. Replacing the native by recombinant proteins produced by a simpler and more standardised expression system delivers pure and stable antigens that may result in higher specificity. A VSG RoTat 1.2 fragment has been expressed recombinantly in *Spodoptera frugiperda* and showed excellent diagnostic potential but the expression was poorly reproducible (Urakawa et al., 2001; Lejon et al., 2005). Recently, we also expressed the variant N-terminal part of the RoTat 1.2 VSG in the methylotrophic yeast *Pichia pastoris*. In an ELISA format, the affinity purified recombinant RoTat 1.2 (rRoTat 1.2) showed good diagnostic potential when tested with sera from goats experimentally infected with *T. evansi* and with sera from naturally infected and non-infected dromedary camels (Rogé et al., 2013). In the present study, we incorporated the rRoTat 1.2 as *T. evansi* specific antigen in a rapid latex agglutination test format (rLATEX/*T. evansi*). Subsequently, we evaluated the diagnostic

performance of this new test, along with the OIE recommended CATT/*T. evansi*, on a large collection of sera from camel, water buffalo, bovine, horse, dog and sheep. TL was used as reference test for presence of *T. evansi* specific antibodies. The thermostability of the rLATEX/*T. evansi* was evaluated as well.

2. Materials and methods

2.1. Production of rRoTat 1.2

The rRoTat 1.2 was expressed and purified according to Rogé et al. (2013) with a yield of up to 20 mg per litre yeast culture.

2.2. Coupling of rRoTat 1.2 to latex particles

One hundred mg of carboxyl modified green polystyrene latex of 0.799 μm diameter (1 mL of Estapor K1.08, 10% suspension, OEM Diagnostics, Merck Millipore) was mixed with 1 mg rRoTat 1.2 in phosphate buffered saline (PBS; 0.01 M phosphate, 0.14 M sodium chloride, pH 7.4) for 15 min at 4°C on a roller mixer. For covalent coupling of rRoTat 1.2 to the latex particles, 250 mg of EDC (1-Ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride, Thermo Scientific), freshly dissolved in 1 mL of H₂O, was added and the mixture was kept at 4°C for 1 h on a roller mixer. The mixture was washed two times by centrifugation (470 g, 4°C, 1 h) with 8 mL of ice-cold TBSA (Tris buffered saline; 0.02 M, pH 7.4, complemented with 1% bovine serum albumin). The final latex sediment was resuspended in 2.5 mL TBSA supplemented with 10% w/v sucrose, resulting in a 4% latex suspension. The latex reagent was sonicated on ice to obtain a monodisperse suspension (Vibra-Cell, 6 mm probe, amplitude 80, pulse 3 s, 9 W output). Aliquots (0.25 mL) of this suspension were dispensed in 2.5 mL penicillin vials, snap frozen in liquid nitrogen and lyophilised with the following settings: 60 h at -30°C, 10 h at 0°C, 10 h at 20°C, 11 h at 25°C, all at 100 μbar . Finally, the vials were flushed with nitrogen gas, stoppered and stored at -20°C.

2.3. Serum collection

For the evaluation of the diagnostic accuracy of CATT/*T. evansi* and rLATEX/*T. evansi*, a collection of 1717 archived sera were analysed with TL as reference test. Host species, origin, year of collection and status in TL are shown in Table 1. Six hundred and thirty three naturally infected and non-infected camel sera originated from Niger, Mali, Spain (Gran Canaria) and Morocco. Six hundred ninety seven bovine sera were collected in Suriname and Belgium while 25 dog sera originated from Belgium, 88 sheep sera from France and the United Kingdom (Scotland), 50 horse sera from Spain (Gran Canaria) and 224 water buffalo sera from the Philippines and Indonesia. Some sera were collected from experimentally infected animals, i.e. the sheep sera from Scotland and the water buffalo sera from Indonesia.

For testing the thermostability of the rLATEX/*T. evansi*, a panel of nine positive and four negative reference sera

Table 1

Host species, origin, year of collection, number and status in immune trypanolysis of all serum samples used in this study. TL pos: immune trypanolysis positive; TL neg: immune trypanolysis negative.

| Species | Origin | Year | Number | TL pos | TL neg | Reference |
|----------------------------|----------------------|-------------|--------|--------|--------|--------------------------|
| Camel | Niger | 1995 | 257 | 245 | 12 | Verloo et al. (1998) |
| Camel | Mali | 1989–1991 | 245 | 13 | 232 | Diall et al. (1994) |
| Camel | Spain (Gran Canaria) | 1997–1999 | 28 | 13 | 15 | Gutierrez et al. (2000) |
| Camel | Morocco | 1997–1998 | 103 | 64 | 39 | Atarhouch et al. (2003) |
| Water buffalo | Philippines | 1995 | 194 | 163 | 31 | – |
| Water buffalo ^a | Indonesia | Before 1999 | 30 | 9 | 21 | Davison et al. (1999) |
| Bovine | Suriname | 1992–1993 | 634 | 0 | 634 | Van Vlaenderen (1996) |
| Bovine | Belgium | 1989 | 63 | 0 | 63 | – |
| Horse | Spain (Gran Canaria) | 2009 | 50 | 0 | 50 | Gutierrez et al. (2010) |
| Dog | Belgium | 1988 | 25 | 0 | 25 | – |
| Sheep | France | 2007 | 81 | 0 | 81 | Desquesnes et al. (2008) |
| Sheep ^a | UK (Scotland) | 1993–1994 | 7 | 7 | 0 | Onah et al. (1996)) |
| Total | | | 1717 | 514 | 1203 | |

^a Experimental infections.

from different host species (camel, sheep, cattle, goat and rabbit) was used.

2.4. rLATEX/*T. evansi* test protocol

Lyophilised latex reagent was resuspended with 1 mL H₂O to obtain a 1% (w/v) suspension. Two-fold serial dilutions of serum were prepared in phosphate buffered saline (PBS; 0.014 M, pH 7.2, supplemented with 0.1% sodium azide). On a reaction zone of a plastic test card (10 white reaction zones of 18 mm diameter, Immuno-Cell) 20 µL of rLATEX/*T. evansi* reagent were mixed with 20 µL of serum dilution and spread over the reaction zone. The card was rocked on a horizontal rotator (eccentric deviation 12 mm) at 70 rpm. After 5 min, the degree of agglutination was scored macroscopically as follows: invisible (0) or hardly visible (1) = 'negative'; manifest (2) = 'weakly positive'; intense (3) = 'positive'; maximal (4) = 'strongly positive'. The titre of a serum was defined as the reciprocal of the highest serum dilution still giving a positive score of at least 2.

2.5. CATT/*T. evansi* test protocol

The CATT/*T. evansi* was performed with one drop of resuspended reagent and 25 µL volumes of two-fold dilutions of the sera according to the instructions of the manufacturer (Institute of Tropical Medicine, Antwerp, Belgium). Agglutination patterns were scored as for rLATEX/*T. evansi*. The end titre of a serum was defined as the reciprocal of the highest serum dilution still giving a positive score of at least 2. A serum was considered positive when its end titre was ≥ 4 .

2.6. Immune trypanolysis test protocol

Immune trypanolysis was performed according to Van Meirvenne et al. but with *T. evansi* VAT RoTat 1.2 (Van Meirvenne et al., 1995; Verloo et al., 2000). Live trypanosomes were incubated for 90 min with test serum diluted 1:4 with guinea pig serum as the source of complement. If variant specific antibodies are present in the serum, antibody mediated complement lysis of the RoTat

1.2 trypanosomes can be observed under the microscope. Samples were considered positive for the presence of RoTat 1.2 antibodies when 50% or more of the trypanosomes were lysed.

2.7. Stability tests

To analyse the short-term thermostability of the rLATEX/*T. evansi*, the reactivity of the reagent was tested with a panel of positive and negative reference sera, after storage of the reagent under the following conditions: A: lyophilised reagent stored at 4 °C for one week, B: lyophilised reagent stored at 45 °C for one week, C: lyophilised reagent stored at 45 °C for one week, followed by reconstitution with buffer and 20 cycles of freezing at –20 °C and thawing.

2.8. Statistical analysis

Sensitivities and specificities with 95% binomial Wilson confidence intervals were calculated using Stata/IC V10.1 (Stata Corp., College Station, TX, USA). McNemar χ^2 test was used to test differences in sensitivity and specificity of the rLATEX/*T. evansi* compared to the CATT/*T. evansi* using the TL as reference test. The level of agreement between diagnostic tests was determined using Cohen's kappa coefficient interpreted following Landis and Koch (Cohen, 1960; Landis and Koch, 1977).

3. Results

To determine the best cut-off serum dilution for both the rLATEX/*T. evansi* and CATT/*T. evansi*, a subset of the serum sample collection, including all different host species, was tested in dilutions ranging from 1:4 to 1:32. The specificity of both tests increased with the serum dilution ranging from 89.3 to 99.9% for CATT/*T. evansi* and from 97.6 to 100% for rLATEX/*T. evansi* while the sensitivity dropped with increasing serum dilution, from 83.8 to 28.9% for CATT/*T. evansi* and from 83.8 to 19.4% for rLATEX/*T. evansi*. Given that the Youden index ($J = \text{sensitivity} + \text{specificity} - 1$) was highest at serum dilution 1:4 for both agglutination tests, the 1:4 dilution, the same dilution that is used in TL, was

Table 2

Contingency table with results of all sera tested at 1:4 serum dilution with TL, CATT/*T. evansi* and rLATEX/*T. evansi*.

| TL | CATT/ <i>T. evansi</i> | | rLATEX/ <i>T. evansi</i> | |
|-------|------------------------|------|--------------------------|------|
| | Pos | Neg | Pos | Neg |
| Pos | 432 | 82 | 433 | 81 |
| Neg | 127 | 1076 | 28 | 1175 |
| Total | 559 | 1158 | 461 | 1256 |

retained as cut-off dilution for further analysis (Youden, 1950).

Thus, the full collection of test sera ($n = 1717$) was tested with rLATEX/*T. evansi* and CATT/*T. evansi* at serum dilution 1:4. The results are represented in Table 2.

Considering TL as reference test, the sensitivity of the CATT/*T. evansi* is 84.0% (95% CI 80.6–87.0) and the specificity is 89.4% (95% CI 87.6–91.1). The agreement between CATT/*T. evansi* and TL is substantial ($\kappa = 0.72$, 95% CI 0.66–0.78). For the rLATEX/*T. evansi* the sensitivity is 84.2% (95% CI 80.8–87.1) and is not significantly different from the sensitivity of CATT ($p = 0.91$). The specificity of rLATEX/*T. evansi* is 97.7% (95% CI 96.7–98.4) and is significantly different from that of CATT ($p < 0.001$). Agreement between rLATEX/*T. evansi* and TL is almost perfect ($\kappa = 0.84$, 95% CI 0.78–0.90). The agreement between CATT/*T. evansi* and rLATEX/*T. evansi* was substantial ($\kappa = 0.69$, 95% CI 0.64–0.75).

For each host species, sensitivities and specificities of CATT/*T. evansi* and rLATEX/*T. evansi* were not significantly different except for the water buffalo and bovine sera (Table 3). rLATEX/*T. evansi* was significantly more specific than CATT/*T. evansi* with water buffalo sera ($p < 0.001$) and with bovine sera ($p < 0.001$). It appeared that CATT/*T. evansi* was positive in 11 of 21 experimental water buffalo sera collected prior to infection while all these sera were negative in rLATEX/*T. evansi*. Also, CATT/*T. evansi* was false positive in 103 of the 634 bovine sera collected in Suriname, compared to 13 false positives in rLATEX/*T. evansi*.

The short-term thermostability of the rLATEX/*T. evansi* was tested with nine positive and four negative control sera after storage of the latex reagent under three conditions (Table 4). Compared to storage for one week at 4 °C (condition A), storage for one week at 45 °C (condition B) resulted in an initial drop in end titre for three positive sera. Twenty cycles of freeze-thawing of the reagent stored first for 1 week at 45 °C resulted in the further drop in end titre of only 1 positive serum. All negative sera remained negative

Table 3

Percent sensitivity (Se) and specificity (Sp) with 95% confidence interval (CI) of CATT/*T. evansi* and rLATEX/*T. evansi* with TL as reference test for each host species ($n = 1717$). na = not applicable.

| Host species | CATT/ <i>T. evansi</i> | | rLATEX/ <i>T. evansi</i> | |
|---------------|------------------------|-------------------------------|--------------------------|--------------------|
| | Se (95% CI) | Sp (95% CI) | Se (95% CI) | Sp (95% CI) |
| Camel | 88.9 (85.1–91.9) | 99.7 (98.1–99.9) | 92.2 (88.9–94.6) | 98.0 (95.7–99.1) |
| Water buffalo | 73.8 (66.8–79.8) | 76.9 ^a (63.9–86.3) | 68.0 (60.7–74.5) | 100.0 (93.1–100.0) |
| Bovine | na | 84.1 ^a (81.2–86.6) | na | 96.8 (95.3–97.9) |
| Horse | na | 100.0 (92.9–100.0) | na | 100.0 (92.9–100.0) |
| Dog | na | 100.0 (86.7–100.0) | na | 100.0 (86.7–100.0) |
| Sheep | 100.0 (64.6–100.0) | 96.3 (89.7–98.7) | 100.0 (64.6–100.0) | 100.0 (95.5–100.0) |

^a Significantly different from specificity of rLATEX/*T. evansi* ($p < 0.001$).

Table 4

End titres of nine positive (1–9) and four negative (10–13) reference sera obtained with rLATEX/*T. evansi* stored at different conditions. (A) Lyophilised reagent, 4 °C, 1 week; (B) lyophilised reagent, 45 °C, 1 week; (C) lyophilised reagent, 45 °C, 1 week, reconstitution, 20 cycles of freeze-thawing.

| Serum | Species | Storage condition | | |
|-------|---------|-------------------|-----|-----|
| | | A | B | C |
| 1 | Rabbit | 64 | 64 | 64 |
| 2 | Camel | 32 | 32 | 16 |
| 3 | Camel | 16 | 8 | 8 |
| 4 | Camel | 16 | 8 | 8 |
| 5 | Camel | 16 | 8 | 8 |
| 6 | Camel | 4 | 4 | 4 |
| 7 | Sheep | 8 | 8 | 8 |
| 8 | Sheep | 4 | 4 | 4 |
| 9 | Goat | >16 | >16 | >16 |
| 10 | Camel | <4 | <4 | <4 |
| 11 | Sheep | <4 | <4 | <4 |
| 12 | Bovine | <4 | <4 | <4 |
| 13 | Rabbit | <4 | <4 | <4 |

(end titre < 4) with the latex reagent stored at the different conditions.

4. Discussion

T. evansi causes important economic losses in many countries in Africa, Latin America and Asia. Serodiagnosis of the infection, e.g. with CATT/*T. evansi*, is based on detection of antibodies against specific antigens that are prepared through massive infection of laboratory rodents. In order to avoid unnecessary sacrifice of laboratory rodents and to standardise antigen production, we have developed a recombinant VSG RoTat 1.2 fragment with proven diagnostic potential (Rogé et al., 2013). In this study, we used this His tag affinity purified recombinant antigen to develop a latex agglutination test and we evaluated the diagnostic performance of this rLATEX/*T. evansi* on a collection of 1717 animal sera. rLATEX/*T. evansi* proved to be 84.2% sensitive and 97.7% specific and agreement with the TL as reference test for RoTat 1.2 specific antibodies was almost perfect ($\kappa = 0.84$). While the sensitivity of rLATEX/*T. evansi* was similar to CATT/*T. evansi*, its specificity was significantly higher, particularly in buffalo and bovine. The lower specificity of CATT/*T. evansi* (89.4%) is probably due to the fact that this reagent consists of whole trypomastigote cells of *T. evansi* RoTat 1.2 that are formaldehyde-fixed and stained. In contrast with rLATEX/*T. evansi* particles that

are coated exclusively with the N-terminal domain of VSG RoTat 1.2, thus exposing only specific epitopes, the particles in CATT/*T. evansi* expose on their surface not only RoTat 1.2 specific epitopes but also less specific epitopes from other proteins present on the surface of trypanosomes, like invariant surface glycoproteins (ISGs). This may lead to cross-reaction with antibodies against other infectious agents. For example, a low specificity of CATT/*T. evansi* was observed by Van Vlaenderen in bovines from Suriname where the presence of *T. evansi* could not be confirmed but where *T. vivax* was suspected to circulate (Van Vlaenderen, 1996). In our study, we tested a subset of the same bovine serum collection and observed a similar low specificity of 84.1% for CATT/*T. evansi* but a high specificity for rLATEX/*T. evansi* (96.8%). On the other hand, the 11 false positives observed among the 21 Indonesian experimental buffalo sera collected prior to infection cannot be explained by the presence of other pathogenic trypanosomes since in Indonesia only *T. evansi* has been described (Payne et al., 1991; Reid, 2002; Desquesnes et al., 2013b). Most probably, in these buffalo sera, the CATT/*T. evansi* cross-reacts with non-trypanosome specific antibodies or with other serum components while the rLATEX/*T. evansi* does not show any false positive.

Preliminary stability tests showed that in its lyophilised form, rLATEX/*T. evansi* remained stable for at least one week at high ambient temperature (45 °C) but once resuspended the reagent could not be stored unless it is frozen. Repeated thawing and freezing did not seem to affect the reactivity of the reagent.

Reports on the use of recombinant proteins for sero-diagnosis of *T. evansi* are rare. Sengupta et al. (2012) expressed a very similar N-terminal fragment of RoTat 1.2 VSG in *Escherichia coli* and showed its diagnostic potential in ELISA but only in experimentally infected rabbits, bovine and water buffalo, not yet in naturally infected animals. Other researchers screened the *T. brucei* genome for tandem repeat coding genes, expressed several tandem repeat sequences in *E. coli* and showed the diagnostic potential of GM6 (Thuy et al., 2012). GM6 is a flagellum-associated protein of which the diagnostic value was described much earlier (Müller et al., 1992). A recombinant fragment of *T. evansi* GM6, TeGM6-4r, expressed in *E. coli*, was evaluated in ELISA during a survey on water buffaloes in Vietnam with CATT/*T. evansi* as reference test. The low specificity of TeGM6-4r (58.3%) compared to CATT was ascribed to cross-reactions with the non-pathogenic *T. theileri* (Nguyen et al., 2014). In previous studies, we reported on ISG 75, recombinantly expressed in *E. coli* and in *Pichia pastoris* (Tran et al., 2009; Rogé et al., 2013). Both were evaluated in ELISA on a subset of the dromedary camel sera from Niger that were also tested in this study. Only the recombinant ISG 75 fragment expressed in *E. coli* proved to be sensitive (94.6%) and specific (100%) (Tran et al., 2009). For a yet unexplained reason, recombinant ISG 75 expressed in *Pichia pastoris* did hardly react with the *T. evansi* infected camel sera (Rogé et al., 2013).

Although the rLATEX/*T. evansi* showed high sensitivity when tested on different host species in the current study, it will probably not detect infections with *T. evansi* type B that is known not to express RoTat 1.2 VSG (Ngaira et al.,

2003, 2004). *T. evansi* type B is a rare trypanosome occurring in camels which has only been isolated in Kenya but is suspected to circulate in Ethiopia as well (Hagos et al., 2009). Therefore, it might be of interest to consider combining different recombinant antigens for the preparation of rLATEX/*T. evansi* thus broadening the spectrum of antibodies that can react with the reagent. This strategy has been used with native VSGs in a latex agglutination test and recently also in a chromatographic lateral flow test for human African trypanosomiasis (Büscher et al., 1991, 1999, 2013, 2014).

As is the case for CATT/*T. evansi*, rLATEX/*T. evansi* is conditioned for a minimum of 50 tests per vial. Although it can be frozen repeatedly after reconstitution, it cannot be considered an individual rapid diagnostic test and it still needs a rotator to perform the test. For applications where an individual format is more appropriate, we are currently developing an immunochromatographic test making use of the same recombinant RoTat 1.2 antigen.

We conclude that the rLATEX/*T. evansi* can become an alternative for the CATT/*T. evansi*, with the advantage that the use of a recombinant antigen not only improves the stability and specificity of the test but also precludes the sacrifice of laboratory animals. The production of this recombinant antigen can furthermore be easily scaled-up, e.g. in biofermentors. Yet, the combination of several recombinant antigens in the reagent as well as the development of an individual test format deserves further investigation.

Conflict of interest statement

None of the authors declare a conflict of interest.

Acknowledgments

This study received financial support from the Research Foundation Flanders (FWO) Krediet aan Navorsers (1516907N), the Belgian Directorate General for Development Cooperation and the Secondary Research Funding of the Institute of Tropical Medicine (SOFI-B) and the Institute of Tropical Medicine (RATT project). We thank Epcó Hasker (Institute of Tropical Medicine) for his advice on statistical analysis.

References

- Atarhouch, T., Rami, M., Bendahman, M.N., Dakkak, A., 2003. Camel trypanosomiasis in Morocco 1: results of a first epidemiological survey. *Vet. Parasitol.* 111, 277–286.
- Bajjana Songa, E., Hamers, R., 1988. A card agglutination test (CATT) for veterinary use based on an early VAT RoTat 1/2 of *Trypanosoma evansi*. *Ann. Soc. Belg. Méd. Trop.* 68, 233–240.
- Büscher, P., Draelants, E., Magnus, E., Vervoort, T., Van Meirvenne, N., 1991. An experimental latex agglutination test for antibody detection in human African trypanosomiasis. *Ann. Soc. Belg. Méd. Trop.* 71, 267–273.
- Büscher, P., Lejon, V., Magnus, E., Van Meirvenne, N., 1999. Improved latex agglutination test for detection of antibodies in serum and cerebrospinal fluid of *Trypanosoma brucei gambiense* infected patients. *Acta Trop.* 73, 11–20.
- Büscher, P., Gillemann, Q., Lejon, V., 2013. Novel rapid diagnostic test for sleeping sickness. *N. Engl. J. Med.* 368, 1069–1070.
- Büscher, P., Mertens, P., Leclipteux, T., Gillemann, Q., Jacquet, D., Mumba Ngoyi, D., Pyana, P.P., Boelaert, M., Lejon, V., 2014.

- Sensitivity and specificity of HAT Sero-K-SeT, a rapid diagnostic test for serodiagnosis of sleeping sickness caused by *Trypanosoma brucei gambiense*: a case-control study. *Lancet Glob. Health*, [http://dx.doi.org/10.1016/S2214-109X\(14\)70203-7](http://dx.doi.org/10.1016/S2214-109X(14)70203-7).
- Claes, F., Radwanska, M., Urakawa, T., Majiwa, P., Goddeeris, B., Büscher, P., 2004. Variable surface glycoprotein RoTat 1.2 PCR as a specific diagnostic tool for the detection of *Trypanosoma evansi* infections. *Kinetoplastid Biol. Dis.* 3, 1–6.
- Cohen, J., 1960. A coefficient of agreement for nominal scales. *Educ. Psychol. Meas.* 20, 37–46.
- Davison, H.C., Thrusfield, M.V., Muharsini, S., Husein, A., Partoutomo, S., Rae, P.F., Masake, R., Luckins, A.G., 1999. Evaluation of antigen detection and antibody detection tests for *Trypanosoma evansi* infections of buffaloes in Indonesia. *Epidemiol. Infect.* 123, 149–155.
- Desquesnes, M., Bossard, G., Patrel, D., Herder, S., Patout, O., Lepetitcolin, E., Thevenon, S., Berthier, D., Pavlovic, D., Brugidou, R., Jacquiet, P., Schelcher, F., Faye, B., Touratier, L., Cuny, G., 2008. First outbreak of *Trypanosoma evansi* in camels in metropolitan France. *Vet. Rec.* 162, 750–752.
- Desquesnes, M., Dargantes, A., Lai, D.H., Lun, Z.R., Holzmüller, P., Jittapalpong, S., 2013a. *Trypanosoma evansi* and surra: a review and perspectives on transmission, epidemiology and control, impact, and zoonotic aspects. *Biomed. Res. Int.*, <http://dx.doi.org/10.1155/2013/321237>, 20 pp.
- Desquesnes, M., Holzmüller, P., Lai, D.H., Dargantes, A., Lun, Z.R., Jittapalpong, S., 2013b. *Trypanosoma evansi* and surra: a review and perspectives on origin, history, distribution, taxonomy, morphology, hosts, and pathogenic effects. *Biomed. Res. Int.*, <http://dx.doi.org/10.1155/2013/194176>, 22 pp.
- Diall, O., Bajjana Songa, E., Magnus, E., Kouyate, B., Diallo, B., Van Meirvenne, N., Hamers, R., 1994. Evaluation d'un test sérologique d'agglutination directe sur carte dans le diagnostic de la trypanosome caméline à *Trypanosoma evansi*. *Rev. Sci. Tech. Off. Int. Epizoot.* 13, 793–800.
- Gutierrez, C., Juste, M.C., Corbera, J.A., Magnus, E., Verloo, D., Montoya, J.A., 2000. Camel trypanosomiasis in the Canary Islands: assessment of seroprevalence and infection rates using the card agglutination test (CATT/T. *evansi*) and parasite detection tests. *Vet. Parasitol.* 90, 155–159.
- Gutierrez, C., Desquesnes, M., Touratier, L., Büscher, P., 2010. *Trypanosoma evansi*: recent outbreaks in Europe. *Vet. Parasitol.* 174, 26–29.
- Hagos, A., Yilkal, A., Esayass, T., Alemu, T., Fikru, R., Feseha, G., Goddeeris, B.M., Claes, F., 2009. Parasitological and serological survey on trypanosomiasis (surra) in camels in dry and wet areas of Bale Zone, Oromyia Region, Ethiopia. *Rev. Méd. Vét.* 12, 569–573.
- Holland, W.G., Thanh, N.G., My, L.N., Magnus, E., Verloo, D., Büscher, P., Goddeeris, B., Vercruyse, J., 2002. Evaluation of whole fresh blood and dried blood on filter paper discs in serological tests for *Trypanosoma evansi* in experimentally infected water buffaloes. *Acta Trop.* 81, 159–165.
- Holland, W.G., Thanh, N.G., Do, T.T., Sangmaneedet, S., Goddeeris, B., Vercruyse, J., 2005. Evaluation of diagnostic tests for *Trypanosoma evansi* in experimentally infected pigs and subsequent use in field surveys in north Vietnam and Thailand. *Trop. Anim. Health Prod.* 37, 457–467.
- Jones, T.W., McKinnell, C.D., 1985. Antigenic variation in *Trypanosoma evansi*: variable antigen type development in mice, sheep and goats. *Trop. Med. Parasitol.* 36, 53–57.
- Landis, J.R., Koch, G.G., 1977. The measurement of observer agreement for categorical data. *Biometrics* 33, 159–174.
- Lejon, V., Claes, F., Verloo, D., Maina, M., Urakawa, T., Majiwa, P., Büscher, P., 2005. Recombinant RoTat 1.2 variable surface glycoprotein for diagnosis of *Trypanosoma evansi* in dromedary camel. *Int. J. Parasitol.* 35, 455–460.
- Müller, N., Hemphill, A., Imboden, M., Duvallet, G., Dwinger, R.H., Seebeck, T., 1992. Identification and characterization of two repetitive non-variable antigens from African trypanosomes which are recognized early during infection. *Parasitology* 104, 111–120.
- Ngaira, J.M., Bett, B., Karanja, S.M., Njagi, E.N.M., 2003. Evaluation of antigen and antibody rapid detection tests for *Trypanosoma evansi* infection in camels in Kenya. *Vet. Parasitol.* 114, 131–141.
- Ngaira, J.M., Njagi, E.N.M., Ngeranwa, J.J.N., Olembo, N.K., 2004. PCR amplification of RoTat 1.2 VSG gene in *Trypanosoma evansi* isolates in Kenya. *Vet. Parasitol.* 120, 23–33.
- Nguyen, T.T., Zhou, M., Ruttayaporn, N., Nguyen, Q.D., Nguyen, V.K., Goto, Y., Suzuki, Y., Kawazu, S., Inoue, N., 2014. Diagnostic value of the recombinant tandem repeat antigen TeCM6-4r for surra in water buffaloes. *Vet. Parasitol.* 201, 18–23.
- OIE, 2012. Manual of Diagnostic Tests and Vaccines for Terrestrial Animals, <http://www.oie.int/manual-of-diagnostic-tests-and-vaccines-for-terrestrial-animals/>
- Onah, D.N., Hopkins, J., Luckins, A.G., 1996. Haematological changes in sheep experimentally infected with *Trypanosoma evansi*. *Parasitol. Res.* 82, 659–663.
- Payne, R.C., Sukanto, I.P., Djauhari, D., Partoutomo, S., Wilson, A.J., Jones, T.W., Boid, R., Luckins, A.G., 1991. *Trypanosoma evansi* infection in cattle, buffaloes and horses in Indonesia. *Vet. Parasitol.* 38, 109–120.
- Pays, E., Vanhamme, L., Pérez-Morga, D., 2004. Antigenic variation in *Trypanosoma brucei*: facts, challenges and mysteries. *Curr. Opin. Microbiol.* 7, 369–374.
- Reid, S.A., 2002. *Trypanosoma evansi* control and containment in Australia. *Trends Parasitol.* 18, 219–224.
- Rogé, S., Van Reet, N., Odiwuor, S., Tran, T., Schildermans, K., Vandamme, S., Vandenberghe, I., Vervecken, W., Gillingwater, K., Claes, F., Devreese, B., Guisez, Y., Büscher, P., 2013. Recombinant expression of trypanosome surface glycoproteins in *Pichia pastoris* for the diagnosis of *Trypanosoma evansi* infection. *Vet. Parasitol.* 197, 571–579.
- Sengupta, P.P., Balumahendiran, M., Balamurugan, V., Rudramurthy, G.R., Prabhudas, K., 2012. Expressed truncated N-terminal variable surface glycoprotein (VSG) of *Trypanosoma evansi* in *E. coli* exhibits immunoreactivity. *Vet. Parasitol.* 187, 1–8.
- Thuy, N.T., Goto, Y., Lun, Z.R., Kawazu, S., Inoue, N., 2012. Tandem repeat protein as potential diagnostic antigen for *Trypanosoma evansi* infection. *Parasitol. Res.* 110, 733–739.
- Tran, T., Claes, F., Verloo, D., De Greve, H., Büscher, P., 2009. Towards a new reference test for surra in camels. *Clin. Vaccine Immunol.* 16, 999–1002.
- Urakawa, T., Verloo, D., Moens, L., Büscher, P., Majiwa, P.A.O., 2001. *Trypanosoma evansi*: cloning and expression in *Spodoptera fugiperda* insect cells of the diagnostic antigen RoTat 1.2. *Exp. Parasitol.* 99, 181–189.
- Van Meirvenne, N., Magnus, E., Büscher, P., 1995. Evaluation of variant specific trypanolysis tests for serodiagnosis of human infections with *Trypanosoma brucei gambiense*. *Acta Trop.* 60, 189–199.
- Van Vlaenderen, G., 1996. In Search of Cattle Trypanosomiasis in Suriname. Prince Leopold Institute of Tropical Medicine, Antwerp, pp. 3–79.
- Verloo, D., Tibayrenc, R., Magnus, E., Büscher, P., Van Meirvenne, N., 1998. Performance of serological tests for *Trypanosoma evansi* infections in camels from Niger. *J. Protozool. Res.* 8, 190–193.
- Verloo, D., Holland, W., My, L.N., Thanh, N.G., Tam, P.T., Goddeeris, B., Vercruyse, J., 2000. Comparison of serological tests for *Trypanosoma evansi* natural infections in water buffaloes from north Vietnam. *Vet. Parasitol.* 92, 87–96.
- Verloo, D., Magnus, E., Büscher, P., 2001. General expression of RoTat 1.2 variable antigen type in *Trypanosoma evansi* isolates from different origin. *Vet. Parasitol.* 97, 183–189.
- Youden, W.J., 1950. An index for rating diagnostic tests. *Cancer* 3, 32–35.