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

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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.

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

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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 H_2O, 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