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The expression of RoTat 1.2 variable surface glycoprotein (VSG) in *Trypanosoma evansi* and *T. equiperdum*

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Abstract

In order to define whether the variable antigenic type RoTat 1.2 is restricted to *Trypansoma evansi* and could be used as antigen in serological tests to differentiate *T. evansi* from *Trypansoma equiperdum*, the appearance of RoTat 1.2-specific antibodies in rabbits, experimentally infected with *T. evansi* and *T. equiperdum*, respectively, was analyzed. Ten strains of *T. evansi* and 11 strains of *T. equiperdum* originating from Asia, Europe, Africa and Latin America were tested. Rabbit pre-infection sera and sera of days 7, 14, 25, 35 post-infection (p.i.) were analyzed for the presence of antibodies reactive with RoTat 1.2 in immune trypanolysis, ELISA/*T. evansi* and CATT/*T. evansi*. Within the duration of the infection (maximum 35 days), all *T. evansi* as well as 9 out of 11 *T. equiperdum* infected rabbits became positive in all these tests. The rabbits infected with *T. equiperdum* OVI (South Africa) and BoTat 1.1 (Morocco) remained negative in the immune trypanolysis test although the latter rabbit became positive in the CATT/*T. evansi* and ELISA/*T. evansi*. On the contrary, both rabbits were positive in immune trypanolysis when tested against their respective infecting population.

From these data, we conclude that most *T. equiperdum* strains express isoVATs of RoTat 1.2. This explains, in part, why antibody tests based on *T. evansi* RoTat 1.2 cannot reliably distinguish

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between infections caused by *T. evansi* and those caused by *T. equiperdum* unless it can be proven that most described *T. equiperdum* are actually misclassified *T. evansi*.
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Keywords: Trypanosoma equiperdum; *Trypanosoma evansi*; Variable surface glycoprotein (VSG); Characterization; Dourine; Surra

1. Introduction

The sexually transmitted trypanosome parasite *Trypanosoma equiperdum* causes the disease dourine in horses. It is morphologically identical to the insect vector transmitted *Trypanosoma evansi*, which causes surra in multiple animal species including horses. In many regions of the world, both parasites occur together and current diagnostic tests are unable to differentiate between them (Brun et al., 1998; Claes et al., submitted).

Within the mammalian host, the cell membrane of a trypanosome is covered with a monolayer of variable surface glycoprotein (VSG) (Pays, 1999). This VSG, which determines the variable antigenic type (VAT) of an individual trypanosome, is highly immunogenic and elicits VAT specific antibodies with agglutinating and lytic activities (Van Meirvenne et al., 1995). The variable antigen type (VAT) RoTat 1.2 has been cloned from a *T. evansi* strain, isolated in 1982 from a water buffalo in Indonesia. All *T. evansi* strains hitherto tested express this VAT while *T. brucei* strains do not (Büscher, personal communication). Based on the RoTat 1.2 VAT, different diagnostic antibody detection tests for *T. evansi* have been developed, namely CATT/*T. evansi*, a direct agglutination test (Bajyana Songa and Hamers, 1988), LATEX/*T. evansi* (Verloo et al., 2001), an indirect agglutination test, ELISA/*T. evansi* (Verloo et al., 2001) and immune trypanolysis (Van Meirvenne et al., 1995). However, based on anecdotal evidence, it appears that *T. equiperdum* infected laboratory animals and horses suspected of dourine also react positively in the CATT/*T. evansi* prepared with fixed whole trypanosomes of the RoTat 1.2 VAT. In order to define whether this VAT is restricted to *T. evansi*, we studied the appearance of RoTat 1.2 specific antibodies in rabbits, experimentally infected with 10 *T. evansi* and 11 *T. equiperdum* strains for a maximum period of 35 days.

2. Materials and methods

2.1. Trypanosome populations

A collection of 10 *T. evansi* and 11 *T. equiperdum* populations derived from strains isolated all over the world was used in this experiment (Table 1). All populations were kept as cryostabilates in liquid nitrogen, and expanded by growth in OF1 mice or Whistar rats when needed.

2.2. Rabbit infections

Cryopreserved trypanosomes were thawed and inoculated intra-peritoneally in OF1 mice. Three days after infection, the mice were anaesthetized and exsanguinated by cardiac

Table 1
Data on the *T. evansi* and *T. equiperdum* populations used in this study

ITMAS cryo-stabilate	Code	Species	Origin	Host	Year of isolation	References
RoTat 1.2	060297	*T. evansi*	Indonesia	Buffalo	1982	Bajyana Songa and Hamers, 1988
AnTat 3.1	270274C	*T. evansi*	South America	Capybara	1969	Van Meirvenne et al., 1977
Stock Philippines	060297	*T. evansi*	Philippines	Water buffalo	1996	Verloo et al., 2001
Stock Colombia	100297	*T. evansi*	Colombia	Horse	1973	Gibson et al., 1980
Stock Kenya	110297	*T. evansi*	Kenya	Camel	1980	Gibson et al., 1983
Stock Kazakstan	060297	*T. evansi*	Kazakstan	Camel	1995	Touratier, personal communication
Stock Br E18	020297	*T. evansi*	Brazil	Capybara	1986	Stevens et al., 1989
Stock CAN 86 K	170297	*T. evansi*	Brazil	Dog	1986	Stevens et al., 1989
Stock STIB 816	020297	*T. evansi*	P.R. China	Camel	1978	Lun et al., 1992b
Stock Vietnam WH	101298	*T. evansi*	Vietnam	Water buffalo	1998	Verloo et al., 2000; Holland et al., 2001
ATCC 30019	020301	*T. equiperdum*	France	Horse	Unknown	Tobie, 1951; Hajduk, 1976; Shu and Stuart, 1994
ATCC 30023	280201	*T. equiperdum*	France	Horse	Unknown	Hajduk, 1976; Van Der Ploeg et al., 1984
American stabilate	220101	*T. equiperdum*	America	Horse	Unknown	Hagebock et al., 1993
Canadian stabilate	290101	*T. equiperdum*	Canada	Horse	Unknown	Hagebock et al., 1993
Alfort	241199A	*T. equiperdum*	Unknown	Unknown	Unknown	Clausen, personal communication
SVP	241199B	*T. equiperdum*	Unknown	Unknown	Unknown	Clausen, personal communication
Hamburg	251199A	*T. equiperdum*	Unknown	Unknown	Unknown	Clausen, personal communication
AnTat 4.1	210983A	*T. equiperdum*	Unknown	Unknown	Unknown	Van Meirvenne, 1976, PhD thesis, University of Antwerp
STIB 818	010999	*T. equiperdum*	P.R. China	Horse	1979	Lun et al., 1992a; Lun et al., 1992b; Zhang and Baltz, 1994; Brun and Lun, 1994
OVI	241199C	*T. equiperdum*	South Africa	Horse	1977	Barrowman, 1976; Williamson et al., 1988
BoTat 1.1	240982A	*T. equiperdum*	Morocco	Horse	1923	Capbern et al., 1977; Baltz, 1982, PhD thesis, University of Bordeaux II

puncture with a heparinized syringe. From this blood, a suspension in phosphate buffered saline glucose (PBSG) was prepared to contain five trypanosomes per microscopic field (400× magnification) according to the matching method (Herbert and Lumsden, 1976). One milliliter of this suspension was injected intravenously in the ear vein of adult New Zealand white rabbits. Parasitaemia was monitored weekly by the mini-haematocrit centrifugation technique (MHCT) according to Woo (1969). Five milliliters of blood were taken from an ear vein at days 0, 7, 14, 25 and 35 post-infection (p.i.) for the preparation of serum. All sera were stored at −20 °C. Rabbits were euthanized after the last blood collection.

2.3. 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). The test was executed on the rabbit sera, diluted 1:8 in phosphate buffered saline (PBS).

2.4. ELISA/*T. evansi*

The antibody detection ELISA using purified RoTat 1.2 VSG was performed as described by Verloo et al. (2001). Percent positivity (PP) was calculated relative to a monovalent polyclonal antiserum obtained from a rabbit infected for 7 days with *T. evansi* RoTat 1.2. The cut-off was set at 60% PP. All serum samples were tested in duplo and the mean PP was calculated.

2.5. Immune trypanolysis

Immune trypanolysis was performed with *T. evansi* VAT RoTat 1.2, with *T. equiperdum* VAT BoTat 1.1 and the *T. equiperdum* OVI strain, according to Van Meirvenne et al. (1995). Rabbit sera were tested at a 1:4 dilution in PBS. In the absence of lysis with the negative control, i.e. without addition of infection serum, test samples were considered positive when 50% or more of the trypanosomes were lysed. The lysis of the trypanosomes is observed under the microscope (400× magnification).

3. Results

An overview of the results is given in Table 2. All rabbits became parasitologically positive in MHCT from day 7 p.i. onwards. All 10 *T. evansi* infected rabbits became positive in CATT/*T. evansi*, ELISA/*T. evansi* and RoTat 1.2 immune trypanolysis within the course of the 35 days infection. Nine out of the 11 *T. equiperdum* strains also induced antibodies reacting with the *T. evansi* RoTat 1.2 VSG in all these tests. Only the *T. equiperdum* OVI infected rabbit remained negative in all the RoTat 1.2 based tests, while the *T. equiperdum* BoTat 1.1 infected rabbit was negative in the RoTat 1.2 immune trypanolysis test but positive in CATT/*T. evansi* and ELISA/*T. evansi*. Immune trypanolysis with the BoTat 1.1 clone and the OVI strain was only positive with the corresponding BoTat 1.1 and OVI infected rabbits.

Table 2
Results in CATT/*T. evansi*, ELISA/*T. evansi* and immune trypanolysis RoTat 1.2, BoTat 1.1 and OVI tests obtained with day 35 p.i. sera from rabbits infected with the different trypanosome populations

Code	Species	CATT/*T. evansi*	ELISA/*T. evansi* (PP)	Immune trypanolysis RoTat 1.2 (%)	Immune trypanolysis BoTat 1.1 (%)	Immune trypanolysis OVI (%)
RoTat 1.2	*T. evansi*	+	243	100	0	0
AnTat 3.1	*T. evansi*	+	226	100	0	0
Stock Philippines	*T. evansi*	+	246	100	0	0
Stock Colombia	*T. evansi*	+	268	100	0	0
Stock Kenya	*T. evansi*	+	221	100	0	0
Stock Kazakstan	*T. evansi*	+	250	100	0	0
Stock Br E18	*T. evansi*	+	234	100	0	0
Stock CAN 86K	*T. evansi*	+	219	100	0	0
Stock STIB 816	*T. evansi*	+	267	100	0	0
Stock Vietnam	*T. evansi*	+	236	90	0	0
ATCC 30019	*T. equiperdum*	+	241	100	0	0
ATCC 30023	*T. equiperdum*	+	252	100	0	0
American stabilate	*T. equiperdum*	+	237	80	0	0
Canadian stabilate	*T. equiperdum*	+	290	100	0	0
Alfort	*T. equiperdum*	+	267	100	0	0
SVP	*T. equiperdum*	+	219	100	0	0
Hamburg	*T. equiperdum*	+	213	80	0	0
AnTat 4.1	*T. equiperdum*	+	235	100	0	0
STIB 818	*T. equiperdum*	+	277	100	0	0
OVI	*T. equiperdum*	−	21	0	0	100
BoTat 1.1	*T. equiperdum*	+	257	0	100	0

4. Discussion

The diagnostic sensitivity and specificity of the RoTat 1.2 immune trypanolysis test, CATT/*T. evansi* and ELISA/*T. evansi* for *T. evansi* infections have been evaluated previously in water buffaloes (Verloo et al., 2000) and camel (Gutierrez et al., 2000). From these studies, it appeared that the RoTat 1.2 VSG is a good antigen in antibody detection diagnosis for *T. evansi* infections. This was already stated by Verloo et al. (2001) and is confirmed in this present study since all newly tested *T. evansi* infected rabbits became positive shortly after infection.

However, we cannot exclude that some strains of *T. evansi* may not express this particular VSG, as has been observed in human sleeping sickness for the LiTat 1.3 VSG in *T. brucei gambiense* (Dukes et al., 1992; Cross et al., 1998). Therefore, to justify the ongoing use of RoTat 1.2 VSG as a diagnostic antigen for *T. evansi*, it will be necessary to evaluate the expression of this VSG in every newly isolated *T. evansi* strain by immune trypanolysis.

From the 11 *T. equiperdum* strains tested here, only the BoTat 1.1 and the OVI did not induce VAT-specific antibodies reacting with RoTat 1.2 in the immune trypanolysis test during the infection period. The positive reactions in CATT/*T. evansi* and ELISA/*T. evansi* observed with the BoTat 1.1 infected rabbit can be explained by the fact that in these tests,

other epitopes than strictly VAT specific epitopes are exposed on the purified VSG in ELISA or on the trypanosome surface in CATT (Van Meirvenne, 1999).

All nine other *T. equiperdum* strains induced antibodies against RoTat 1.2 which specifically reacted in immune trypanolysis, thus making them indistinguishable from *T. evansi* in this test. Hitherto, the RoTat 1.2 VAT was proven only to appear in *T. evansi* infections and not in other *Trypanozoon* taxa (Büscher, personal communication).

Previous differentiation attempts based on kinetoplast DNA (Lun et al., 1992a), isoenzyme analysis (Lun et al., 1992b) and Southern blot analysis (Zhang and Baltz, 1994) revealed small differences among some *T. evansi* strains. However, some *T. equiperdum* (STIB 818, BoTat 1.1 and the OVI strain) could not be distinguished from *T. evansi* by the criteria used. In a recent study of microsattellite markers (Biteau et al., 2000), no consistent differences were found between *T. equiperdum* (STIB 818, BoTat 1.1 and the OVI strain) and a number of *T. evansi* isolates. Additionally, the ribosomal RNA gene internal transcribed spacer 1 (ITS-1) analysis by Desquesnes et al. (2001) and by our laboratory (Claes et al., Proceedings of the 26th ISCTRC Congress, Ouagadougou, Burkina Faso, 1–5 October 2001) did not allow to distinguish between *T. equiperdum* and *T. evansi*.

Moreover, the clinical picture of dourine (*T. equiperdum*) appears to be very similar to chronic *T. evansi* and *T. brucei brucei* infection in horses (Stephen, 1986). Finally, experimental infections in horses with different strains of *T. equiperdum*, do not always result in the appearance of dourine specific clinical signs (skin plaques, nervous symptoms); only 50% of the horses infected with the OVI strain developed clinical sign of dourine (Williamson et al., 1988), while none of the horses infected with the American and Canadian *T. equiperdum* strains, showed pathognomic signs of dourine (Hagebock et al., 1993).

Given the results obtained in this study, two hypotheses may be postulated; RoTat 1.2 is not strictly *T. evansi* specific or RoTat 1.2 expressing putative *T. equiperdum* strains have been misclassified as *T. evansi* and BoTat 1.1 and OVI are the only genuine *T. equiperdum* strains in the tested collection. Although we cannot exclude that BoTat 1.1 and OVI might have expressed the RoTat 1.2 VAT later than 35 days after infection, the observed results are strongly suggestive for the absence of the RoTat 1.2 gene in their VAT repertoire. Based on the recently disclosed DNA sequence of the RoTat 1.2 gene (Urakawa et al., 2001), a RoTat 1.2 specific PCR has been developed (Claes et al., submitted). The results obtained so far with this PCR confirm the above-mentioned hypothesis.

Moreover, investigating the genome of a large collection of *T. equiperdum* and *T. evansi*, as in the present study, with molecular techniques, such as random amplified polymorphic DNA (RAPD), amplified fragment length polypmorphism (AFLP) and DNA sequencing, could help to reveal the relationship between the two parasites. These investigations should be followed by experimental horse infections with the putative *T. equiperdum* or *T. evansi* to confirm their pathogenicity leading to dourine or surra, respectively.

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