Expression of RoTat 1.2 Cross-reactive Variable Antigen Type in *Trypanosoma evansi* and *T. equiperdum*

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ABSTRACT: **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* isolates hitherto tested express this VAT. In a study on the differential diagnosis of *T. equiperdum* and *T. evansi* in horses, we investigated serological evidence for the expression of RoTat 1.2 in 11 *T. evansi* and six *T. equiperdum* populations originating from Asia, Europe, Africa, and the Americas. Preinfection sera and sera of days 7, 14, 25, and 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 experiment, all rabbits infected with *T. evansi* became positive in the three serological tests. Five out of six rabbits infected with *T. equiperdum* also became positive in the three tests. Only one *T. equiperdum* strain (the OVI strain from South Africa) did not induce the production of antibodies reactive with RoTat 1.2 and thus might not contain or express a VSG that shares epitopes similar to those on the RoTat 1.2 VSG. The data lead to the conclusion that *T. equiperdum* can express VSGs containing epitopes serologically similar to those in the *T. evansi* RoTat 1.2 VAT. This explains, in part, why the antibody detection tests based on RoTat 1.2 VSG cannot reliably distinguish between the infections caused by *T. evansi* and those caused by *T. equiperdum*. There are no data that contradict the possibility that the putative *T. equiperdum* strains, which express VSGs with epitopes similar to those on RoTat 1.2, are actually *T. evansi*.**

KEYWORDS: ***Trypanosoma evansi*; *Trypanosoma equiperdum*; RoTat 1.2; surra; dourine; variable antigen type**

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INTRODUCTION

The sexually transferred parasite *Trypanosoma equiperdum*, which causes the disease dourine in horses, is morphologically identical to *Trypanosoma evansi*, which causes surra in multiple species including horses. In many regions of the world, both parasites occur together, and current diagnostic tests are unable to distinguish between them.

Within the mammalian host, the cell membrane of a trypanosome is covered with a monolayer of variant surface glycoproteins (VSG).[1] This VSG, which determines the variable antigen type (VAT) of the individual trypanosome, is highly immunogenic and elicits VAT-specific antibodies with agglutinating and lytic activity.[2] Based on the RoTat 1.2 VAT, different diagnostic antibody detection tests for *T. evansi* have been developed, namely CATT/*T. evansi*,[3] LATEX/*T. evansi*, ELISA/*T. evansi*,[4] and immune trypanolysis.[2] On the basis of anecdotal evidence, however, it appeared that *T. equiperdum*–infected laboratory animals and horses also reacted positively in the CATT/*T. evansi* test based on the RoTat 1.2 VSG. In order to define whether or not the RoTat 1.2 VSG is restricted to *T. evansi*, we studied the appearance of RoTat 1.2–specific antibodies in rabbits experimentally infected with 11 *T. evansi* and six *T. equiperdum* strains.

MATERIALS AND METHODS

Trypanosome Populations

A collection of 11 *T. evansi* and six *T. equiperdum* populations derived from strains isolated all over the world was used in this experiment (TABLE 1).

Rabbit Infections

Cryostabilates, stored in liquid nitrogen, were inoculated intraperitoneally in OF1 mice. Three days after infection, the mice were anesthetized and exsanguinated by cardiac puncture with a heparinized syringe. From this blood, a suspension in phosphate-buffered saline glucose (PSG) was prepared containing five trypanosomes per microscopic field (400× magnification) according to the matching method.[5] One milliliter of this suspension was injected intravenously into the ear vein of adult New Zealand white rabbits; parasitemia was monitored weekly by the mini-hematocrit centrifugation technique (MHCT) according to Woo.[6] Five milliliters of blood were taken from an ear vein at days 0, 7, 14, 25, and 35 post-infection for the preparation of serum. All sera were stored at –20°C.

Immune Trypanolysis with T. evansi *RoTat 1.2*

Immune trypanolysis was performed with *T. evansi* VAT RoTat 1.2 according to Van Meirvenne and others.[2] Rabbit sera were tested at a 1:4 dilution in phosphate-buffered saline (PBS). In the absence of lysis of the negative control, samples were considered positive when 50% or more of the trypanosomes were lysed.

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

ITMAS cryo-stabilate code		Origin	Host	Year of isolation
Stock Philippines	060297	The Philippines	Water buffalo	1996
Stock Colombia	100297	Colombia	Horse	1973
Stock Kenya	110297	Kenya	Camel	1980
Stock Kazakhstan	060297	Kazakhstan	Camel	1995
Stock Br E18	020297	Brazil	Capybara	1986
Stock CAN 86K	170297	Brazil	Dog	1986
Stock STIB 816	020297	P.R. China	Camel	1978
RoTat 1.2	060297	Indonesia	Buffalo	1982
AnTat 3.1	270274C	South America	Capybara	1969
Stock Vietnam WH	101298	Vietnam	Water buffalo	1998
AnTat 3.2	190874A	South America	Capybara	1969
Alfort	241199A	Unknown	Unknown	Unknown
SVP	241199B	Unknown	Unknown	Unknown
OVI	241199C	South Africa	Horse	1977
Hamburg	251199A	Unknown	Unknown	Unknown
AnTat 4.1	210983A	Unknown	Unknown	Unknown
STIB 818	010999	P.R. China	Horse	1979

CATT/T. evansi

The direct agglutination test CATT/*T. evansi* is a direct card agglutination test that uses formaldehyde-fixed, freeze-dried trypanosomes of *T. evansi* VAT RoTat 1.2 stained with Coomassie blue.[3] The test was conducted on rabbit sera, diluted 1:8 in PBS.

ELISA/T. evansi

The antibody detection ELISA using RoTat 1.2 VSG was used as described by Verloo and others.[4] Percent positivity was calculated relative to a monovalent polyclonal serum obtained from a rabbit infected for 7 days with *T. evansi* RoTat 1.2. The cutoff was set at 60% positivity.

RESULTS

An overview of the results is summarized in TABLE 2. All rabbits were detected parasitologically positive in MHCT from day 7 post-infection onwards. In all 11 rabbits infected with different *T. evansi*, antibodies cross-reacting with the RoTat 1.2 VSG were detected in all tests used, within 30 days post-infection. Furthermore, five out of six *T. equiperdum* strains also induced antibodies cross-reacting with the

TABLE 2. Results of CATT/*T. evansi*, ELISA/*T. evansi* and immune trypanolysis RoTat 1.2 tests on day 35 post-infection sera from rabbits infected with different trypanosome populations

Code	Species	CATT/*T. evansi*	ELISA/*T. evansi*	Immune trypanolysis RoTat 1.2
RoTat 1.2	*T. evansi*	+	+	+
AnTat 3.1	*T. evansi*	+	+	+
AnTat 3.2	*T. evansi*	+	+	+
Stock Philippines	*T. evansi*	+	+	+
Stock Colombia	*T. evansi*	+	+	+
Stock Kenya	*T. evansi*	+	+	+
Stock Kazakstan	*T. evansi*	+	+	+
Stock Br E18	*T. evansi*	+	+	+
Stock CAN 86K	*T. evansi*	+	+	+
Stock STIB 816	*T. evansi*	+	+	+
Stock Vietnam	*T. evansi*	+	+	+
Alfort	*T. equiperdum*	+	+	+
SVP	*T. equiperdum*	+	+	+
OVI	*T. equiperdum*	–	–	–
Hamburg	*T. equiperdum*	+	+	+
AnTat 4.1	*T. equiperdum*	+	+	+
STIB 818	*T. equiperdum*	+	+	+

T. evansi RoTat 1.2 VSG. Only the South African *T. equiperdum* strain (OVI) did not react in any of the RoTat 1.2–based tests. A perfect concordance among these three serological tests was observed.

DISCUSSION

All RoTat 1.2 VSG-based diagnostic tests seem to have a good analytical sensitivity since all *T. evansi* strains tested positive consistently in all tests. Diagnostic sensitivity from the tests mentioned above was evaluated for *T. evansi* infections in water buffaloes[7] and camels.[8,9] Judging from the data obtained and from practical use in the field, the RoTat 1.2 VSG seemed to be a useful antigen in screening tests. Because genetic losses of the VSG genes can occur (as observed in the loss of the LiTat 1.3 VSG of *T. brucei gambiense*[11]), we cannot exclude the possibility that some strains of *T. evansi* might not express the predominant RoTat 1.2 VSG. Consequently, it is still necessary to evaluate the serological tests based on RoTat 1.2 in newly isolated *T evansi* populations.

Within six *T. equiperdum* isolates tested here, only the OVI strain did not generate antibodies reacting with RoTat 1.2 VSG during the test period. On the basis of their

serological reactivity with the RoTat 1.2 VSG, all other *T. equiperdum* strains are indistinguishable from *T. evansi*. Although the OVI strain might express the RoTat 1.2 VAT in infections longer than 30 days, the results obtained might be explained by the absence of the RoTat 1.2 gene in the OVI repertoire.

Hitherto, the Rotat 1.2 VSG was only proven to appear in *T. evansi* and not in other trypanosomes from the *Trypanozoon* subspecies, including *T. b. brucei*, *T. b. gambiense* and *T. b. rhodesiense*.[4] Given the data obtained in this study, we cannot exclude the possibility that RoTat 1.2 VSG is restricted to *T. evansi*, that positive *T. equiperdum* stocks are actually misclassified as *T. evansi*, and that the OVI strain is the only real *T. equiperdum* in the studied collection. As in the beginning of the previous century,[12] antigens derived from *T. evansi* populations have been used in the complement fixation test (CFT) to eradicate dourine in the United States and Western Europe, it is possible that later on some of these diagnostic *T. evansi* strains might have been mistaken for *T. equiperdum*.

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