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Establishment of a panel of reference *Trypanosoma evansi* and *Trypanosoma equiperdum* strains for drug screening

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Abstract

The animal pathogenic protozoan, *Trypanosoma evansi*, leads to a wasting disease in equines, cattle and camels, commonly known as Surra. It is extensively distributed geographically with a wide range of mammalian hosts and causes great economical loss. *Trypanosoma equiperdum* causes a venereal disease called Dourine in horses and donkeys. Chemotherapy appears to be the most effective form of control for *T. evansi*, whereas infections caused by *T. equiperdum* are considered incurable. Due to emerging drug resistance, efficient control of *T. evansi* is severely threatened, emphasising the urgent need to find new alternative drugs.

A drug profile for a panel of *T. evansi* and *T. equiperdum* strains has been established for the four standard drugs currently used in treatment. The ^{3}H-hypoxanthine incorporation assay was used to obtain 50% inhibitory concentration (IC_{50}) values for each standard drug against the various strains. The results indicate the presence (and in some cases, the emergence) of drug resistance in several strains. This panel of characterised strains with known drug sensitivities and resistances will be of great value for the screening of new active compounds, in comparison with the four standard drugs currently available.

Keywords: *Trypanosoma evansi*; *Trypanosoma equiperdum*; Chemotherapy; Drug resistance; Drug screening

1. Introduction

Trypanosoma evansi is a flagellated animal pathogenic protozoan parasite, where infection leads to a wasting disease called Surra. This disease has a wide geographical distribution and range of mammalian hosts, causing great economical loss in areas of Africa, Asia and South America as thousands of animals die each year resulting from infection (Giardina et al., 2003). *T. evansi* is pathogenic in most domestic animals and some wild animals (Lun et al., 2004; Zhou et al., 2004). However, the main host species varies according to geographical location: camels are most often affected in the Middle East and Africa, horses in South America and horses, mules, buffalo and deer in China. The severity of the disease varies according to the strain of *T. evansi* in question and host factors like general health and stress of the animal (Brun et al., 1998).

Unlike other trypanosome species (such as *Trypanosoma brucei brucei* and *Trypanosoma congolense*), *T. evansi* is not restricted to Africa alone, since transmission is mechanically performed by biting flies of the genera *Tabanus*, *Stomoxys* and *Lyperosia*. Successful transmission depends on the time taken for a contaminated vector to find a new host, as the viable trypanosomes remain within the mouthparts of the flies only and are therefore susceptible to rapid

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dessication. No intermediate hosts are present and there is no insect developmental cycle available in *T. evansi* (Foil, 1989).

Although *T. evansi* and *Trypanosoma equiperdum* are closely related, they remain classified as two distinct species (Hoare, 1972; Claes et al., 2003, 2006). *T. equiperdum* causes a venereal disease called Dourine in horses and donkeys and is morphologically indistinguishable to other *Trypanozoon* species (Verducci et al., 1989; Brun et al., 1998). Disease transmission is thought to occur during coitus as no arthropod vector has been discovered for this species. Dourine is found in Africa, Asia, Southern and Eastern Europe, Russia and Mexico (Davila and Silva, 2000).

Both *T. evansi* and *T. equiperdum* infections are fatal if left untreated. *T. equiperdum* infections are considered incurable in terms of chemotherapy, where administered drugs can reach parasites within the blood, yet cannot necessarily access parasites hidden in certain tissues, (OIE, Surra fact-sheet, 2004 (http://www.oie.int/eng.htm); OIE homepage, 2005 (http://www.oie/int/eng/en_index.htm); Claes et al., 2005), while treatment for *T. evansi* infection is dependent on four drugs, namely suramin, diminazene aceturate, quinapyramine and cymelarsan (Zhang et al., 1992; Brun and Lun, 1994). While the first three have been utilised for more than 50 years, cymelarsan, belonging to the family of melaminophenyl arsenicals, was pharmaceutically developed less than 20 years ago (Williamson, 1970; Zelleke et al., 1989; Raynaud et al., 1989; Tuntasuvan et al., 2003). As drug resistance is emerging, severe drawbacks in controlling *T. evansi* are occurring, hence emphasising the urgency and necessity for finding new alternative drugs (Kaminsky et al., 1997; De Koning, 2001; Suswam et al., 2001). Inappropriate use of these drugs can easily lead to resistance in the field, as shown by Zhang et al. (1993), who induced resistance in laboratory mice resulting in *T. evansi* resistance to suramin, diminazene and cymelarsan. Zhou et al. (2004) tested various *T. evansi* isolates and found differences in their levels of sensitivity to suramin and quinapyramine, with some being highly sensitive and others showing complete drug resistance. Similarly, El Rayah et al. (1999) demonstrated suramin and quinapyramine resistance in 16 *T. evansi* isolates from Sudan.

Since many of the hosts affected in these disease endemic countries represent expensive and prestigious animals, a new drug against these trypanosome infections would have a potentially high market value amongst the other anti-trypanosomal agents. In order to aid drug discovery within this field, a panel of reference *T. evansi* and *T. equiperdum* strains was established and their sensitivity profiles in relation to existing drugs were determined.

2. Materials and methods

2.1. Parasite strains

In total, 11 *T. evansi* strains were included in this study (Table 1). STIB 806K (kinetoplastic) strain was isolated by Z.R. Lun, from a water buffalo in China and was used as a reference strain in all experiments performed. Two African strains, STIB 780 and STIB 781, were received from the International Livestock Research Institute (ILRI) in Nairobi, Kenya. The remaining eight *T. evansi* strains were obtained from the cryobank of the Institute of Tropical Medicine (ITM) in Antwerp, Belgium.

Likewise, 11 *T. equiperdum* strains were also used for this study (Table 1). All *T. equiperdum* strains were obtained from the ITM cryobank in Antwerp, Belgium, with the exception of STIB 818, which was collected by Z.R. Lun from China. The BoTat 1 (Bordeaux *Trypanosoma* antigen type 1) clone was derived from a *T. equiperdum* strain from the Institut Pasteur in Paris, France. The strain, originally isolated in 1924 from a horse in Morocco, was transferred to Bordeaux in 1961 and maintained in serial passages in mice until 1971, whereupon it was cloned and stored in liquid nitrogen. The BoTat 1 strain was designated as a reference strain in all experiments performed.

Unfortunately, the precise history for some of the strains is unknown, especially in the case of the AnTat 4.1 (Antwerp *Trypanosoma* antigen type 4) strain, the Hamburg strain and the SVP (Staatliches Veterinärmedizinisches Prüfungsinstitut) strain. Nevertheless, the Hamburg, SVP and Alfort strains are currently used as reference *T. equiperdum* strains for the diagnosis of Dourine in Germany. Even though the majority of data for these strains are unknown, this remains the most globally updated list of *T. equiperdum* strains (Claes et al., 2005).

2.2. Mice

All mice used were female NMRI, aged between 3 and 4 weeks and were maintained under standard animal housing facilities in air conditioned rooms (22–23 °C), with a relative humidity of 60–70%. The mice were specific pathogen free (SPF) and were kept in standard Macrolon type II cages, with pelleted food and water *ad libitum*.

Table 1
T. evansi and *T. equiperdum* strain information detailing the originating country, host and year isolated including the average survival days for NMRI mice infected separately with these study strains

Strain	Country	Host	Year isolated	Average survival (days)
T. evansi strains				
CAN 86/Brazil	Brazil	Dog	1986	8
Colombia	Colombia	Horse	1973	8
Kazakhstan	Kazakhstan	Bactrian camel	1995	8
Merzouga 56	Morocco	romedary camel	1998	–
MHRYD/Brazil	Brazil	Capybara	1986	–
Philippines	Philippines	Water buffalo	1996	6
RoTat 1.2	Indonesia	Water buffalo	1982	7
STIB 780	Kenya	Camel	1982	5
STIB 781	Kenya	Camel	1984	5
STIB 806K	China	Water buffalo	1983	7.5
Vietnam	Vietnam	Water buffalo	1998	7
T. equiperdum strains				
Alfort	Unknown	Horse	1949	7
American	America	Horse	Unknown	6.5
AnTat 4.1	Unknown	Unknown	Unknown	5.5
ATCC30019	France	Horse	1903	5
ATCC30023	France	Horse	1903	5
BoTat 1.1	Morocco	Horse	1924	4
Canadian	Canada	Horse	Unknown	9
Hamburg	Unknown	Unknown	Unknown	7.5
OVI	South Africa	Horse	1977	5
STIB 818	China	Horse	1979	2
SVP	Unknown	Unknown	Unknown	5

–, denotes unsuccessful *in vivo* propagation.

2.3. Standard trypanocidal drugs

Suramin (Germanin® from Bayer, Leverkusen, Germany), diminazene aceturate (D-7770, Sigma, St Louis, MO, USA), cymelarsan (MelCy®, Rhône Mérieux, Toulouse, France) and quinapyramine sulphate (Trypacide®, May & Baker, Lagos, Nigeria) were used as the standard drugs in this study.

2.4. Stock solutions and dilutions

A 10 mg amount of each drug was weighed out in powder form and dissolved in 1 ml of sterile distilled water, to provide a 10 mg/ml stock solution. These stock solutions were then stored frozen at −20 °C. From these stock solutions, further drug dilutions were made for use in the *in vitro* drug sensitivity assays, using culture medium as a solvent. Drug dilutions were made fresh on the day of each experimental assay procedure.

2.5. Culture medium

Bloodstream form trypanosomes were cultivated in Minimum Essential Medium (MEM) (powder, GIBCO/BRL, No: 11400–033) with Earle's salts, supplemented with 25 mM HEPES, 1 g/l additional glucose, 2.2 g/l $NaHCO_3$ and 10 ml/l MEM non-essential amino acids (50× concentration). The medium was then further supplemented by adding 1% of a 2-mercaptoethanol stock (14 μl of 12 mM 2-mercaptoethanol was diluted in 10 ml of distilled water), 1% of a stock consisting of 100 mM sodium pyruvate and 50 mM hypoxanthine and 15% heat inactivated horse serum, according to Baltz et al., 1985. The complete medium is called Baltz MEM (BMEM). For the *in vitro* drug sensitivity assays, the 50 mM hypoxanthine was not present in the complete BMEM medium used.

2.6. Radioactive hypoxanthine

Radioactively labelled (8-^{3}H) hypoxanthine (TRK74, Amersham Biosciences UK Limited, Buckinghamshire, UK) was used for the drug sensitivity assays.

2.7. Average survival in mice

Female NMRI mice (two mice per strain) were infected with the different *T. evansi* and *T. equiperdum* strains. As a control, the average survival day for each

strain was calculated after infecting the mice with 1×10^4 parasites and then allowing the parasitaemia to establish itself. The development of parasitaemia was observed daily by using a tail blood examination technique. Once these average survival days were known, experimental trypanosome populations could be propagated successfully, through infecting two new NMRI mice (per strain) with a 1×10^4 parasite concentration and then collecting the trypanosomes at peak parasitaemia.

2.8. In vivo trypanosome propagation

Trypanosomes were collected from donor mice using a 1 ml syringe with a 25 gauge sterile needle containing 20 μl of heparin. Once the blood had been collected, it was placed into a 5 ml Bijou bottle and immediately placed on ice. Thereafter, the mouse blood was passed through a DEAE (diethylaminoethyl)-cellulose column, in order to separate the trypanosomes from the blood. The filtrate was collected in a 15 ml Falcon tube and left on ice.

2.9. In vitro drug sensitivity assay

Once the trypanosomes had been passed through the DEAE-cellulose column, the resulting suspension could be concentrated by centrifugation, resuspended in fresh culture medium and counted using a cell analyser system (CASY, Schaerfe System). The ^{3}H-hypoxanthine incorporation assay, was used to determine the drug sensitivity for all four standard drugs for each of the trypanosome strains tested. The exact assay procedure is detailed in Brun and Kunz (1989) and has been modified slightly for use in this study.

Briefly, 50 μl of BMEM containing no hypoxanthine, were added to each well of a 96-well microtitre plate, except for the last four wells on the top row (where 100 μl were added instead to act as a negative control) and all the wells on the second row. The drugs were applied at 75 μl volumes into the empty wells of this second row, according to the required starting concentration of each drug being tested. Thereafter, 25 μl were removed from this second row using a multichannel pipette and mixed with the wells in the row beneath. Again 25 μl were removed from this third row, placed into the next row down and mixed several times. This step was repeated until the last row had been reached. The final 25 μl from the last row were discarded. This process created a threefold serial drug dilution down the microtitre plate, which enabled a drug range of 1000–1.37 ng/ml to be tested for suramin, diminazene and quinapyramine. The drug range tested for cymelarsan included 30–0.04 ng/ml. In the case of suramin, an additional drug range, starting at 30 μg/ml was applied. The trypanosome density was then adjusted to provide a 2×10^6 ml^{-1} starting concentration. A volume of 50 μl of this trypanosome suspension was then added to all 96 wells, with the exception of the four negative controls in the top row, and the plates were then incubated in a humidified atmosphere at 37 °C and 5% CO_2. After 24 h incubation, the plates were removed and 20 μl of a 1 μCi solution of radioactive hypoxanthine mixed with BMEM (containing no hypoxanthine) were placed into each well. The plates were then returned to the incubator for a further 16 h incubation under the same conditions. After a complete incubation time of 40 h, the plates were removed from the incubator and the wells harvested using a 96-well harvester (1290–004 Betaplate™, Berthold Technologies (Schweiz) GmbH, Regensdorf, Switzerland), followed by a liquid scintillation counter (1205 Betaplate™, Berthold Technologies (Schweiz) GmbH, Regensdorf, Switzerland) to measure the radioactivity. All experiments were performed three to four times and in duplicate. The data obtained were further analysed by transferring them into an SOP template in a graphics programme (Microsoft Excel) for determination of IC_{50} values.

3. Results

In this study, a total of 11 *T. evansi* and 11 *T. equiperdum* strains were available for drug profiling. Out of these 22 strains, 20 were successfully propagated in mice and then tested with the ^{3}H-hypoxanthine incorporation assay to give IC_{50} values against the four standard drugs currently used for treatment.

The average survival days for NMRI mice, when infected separately with either the *T. evansi* or *T. equiperdum* study strains is shown in Table 1.

The drug sensitivities established for the nine *T. evansi* strains against the four current standard drugs are shown in Table 2. The STIB 806K *T. evansi* reference strain typified IC_{50} values considered to be sensitive to the drugs suramin (70.4), diminazene (4.5) and cymelarsan (1.4). All other strains, except the two Kenyan strains and the Colombian strain, showed similar sensitivity to suramin. These two Kenyan strains isolated from camels (STIB 780 and STIB 781), produced IC_{50} values of 14,500 and 11,000 ng/ml, respectively (around 200 times higher than the reference strain), demonstrating resistance to suramin, whilst a reduced sensitivity (four times greater than the

Table 2
Mean drug sensitivities (given as IC_{50} in ng/ml) obtained for the four standard drugs tested against *T. evansi* and *T. equiperdum* study strains

Strain	Standard drugs (inhibitory concentration (IC_{50}) values in ng/ml)			
	Suramin	Diminazene	Cymelarsan	Quinapyramine
T. evansi strains				
CAN86/Brazil	76.5 (±4.21)	2.7 (±0.28)	0.8 (±0.00)	15.8 (±0.35)
Colombia	278.9 (±5.84)	2.2 (±0.14)	0.5 (±0.07)	84.5 (±0.00)
Kazakhstan	97.8 (±1.48)	4.1 (±0.07)	1.1 (±0.14)	12.8 (±0.00)
Philippines	81.5 (±3.42)	20.2 (±0.35)	2.8 (±0.28)	7.4 (±4.24)
RoTat 1.2	69.5 (±6.99)	15.9 (±0.07)	2.2 (±0.00)	14.4 (±1.70)
STIB 780	14500.0 (±0.00)	1.9 (±0.22)	0.2 (±0.07)	<0.1 (±0.00)
STIB 781	11000.0 (±0.00)	5.4 (±0.42)	<0.1 (±0.00)	3.4 (±0.28)
STIB 806K	70.4 (±4.05)	4.5 (±0.07)	1.4 (±0.07)	13.3 (±0.57)
Vietnam	91.1 (±5.58)	8.2 (±0.71)	2.1 (±0.07)	3.0 (±0.28)
T. equiperdum strains				
Alfort	76.9 (±5.91)	6.1 (±0.35)	1.3 (±0.09)	<0.1 (±0.00)
American	22.1 (±6.92)	13.5 (±0.07)	1.4 (±0.21)	<0.1 (±0.00)
AnTat 4.1	46.7 (±7.91)	<0.1 (±0.00)	<0.1 (±0.00)	2.2 (±2.83)
ATCC30019	31.4 (±2.84)	14.3 (±0.92)	0.9 (±0.07)	<0.1 (±0.00)
ATCC30023	75.6 (±4.17)	3.6 (±0.57)	1.8 (±0.28)	<0.1 (±0.00)
BoTat 1.1	87.5 (±3.30)	4.3 (±0.21)	0.7 (±0.00)	3.3 (±1.34)
Canadian	68.8 (±6.50)	19.0 (±0.57)	1.6 (±0.14)	47.6 (±0.57)
Hamburg	61.2 (±4.04)	5.5 (±0.42)	0.6 (±0.00)	43.8 (±1.49)
OVI	92.7 (±6.84)	302.5 (±12.1)	17.6 (±0.78)	76.4 (±2.47)
STIB 818	86.6 (±4.60)	19.2 (±0.00)	<0.1 (±0.00)	1.6 (±0.23)
SVP	71.1 (±10.6)	6.5 (±0.42)	2.0 (±0.00)	<0.1 (±0.00)

(±) denotes standard deviations of at least three to four experiments, each performed in duplicate.

reference strain) was observed in the Colombian strain with an IC_{50} value of 278.9 ng/ml.

Diminazene sensitivity is demonstrated in the reference strain, in the Kenyan strains (STIB 780 and STIB 781), the Kazakhstan strain and the South American strains (CAN86/Brazil and Colombia). A reduced sensitivity was seen in strains originating from the South East of Asia, in the Indonesian RoTat 1.2 and the Philippines strain, being approximately four times and five times, respectively, less sensitive than the reference strain. All *T. evansi* strains tested against cymelarsan produced IC_{50} values within the range of >0.1 to 2.8 ng/ml. The Colombian strain showed the least sensitivity to quinapyramine (greater than six times that of the reference strain), followed by the CAN86/Brazil strain, the RoTat 1.2 strain, the Kazakhstan strain and the Philippines strain. The more sensitive *T. evansi* strains were STIB 780, STIB 781 and the Vietnam strain.

Also displayed in Table 2 are the IC_{50} values for the 11 *T. equiperdum* strains tested. All strains demonstrated similar sensitivities for suramin as that of the reference strain, BoTat 1.1 (87.5 ng/ml), with the increasing exceptions of the strains, Hamburg, AnTat 4.1, ATCC30023 and American, which demonstrated approximately 1.5, 2, 3 and fourfold differences, respectively. The AnTat 4.1 strain revealed the highest sensitivity against diminazene, with five *T. equiperdum* strains showing similar diminazene sensitivity as those displayed by the majority of the *T. evansi* strains (Alfort, ATCC30023, Botat 1.1 (reference strain), Hamburg and SVP strains). Both the American and the ATCC30019 strains revealed decreased sensitivity to diminazene, compared to the reference strain, by a factor of three, whilst the Canadian and STIB 818 strains revealed decreased sensitivities through a factor of four. The South African OVI strain was 70 times less sensitive to diminazene as the BoTat 1.1 reference strain, signifying resistance to this drug.

Similar to the *T. evansi* strains, all the *T. equiperdum* strains tested, showed drug sensitivities of >0.1 to 2.0 ng/ml against cymelarsan, apart from the OVI strain, which was 25 times higher than the reference value. The majority of *T. equiperdum* strains demonstrated quinapyramine sensitivities within the range of <0.1 to 2.2 ng/ml. The reference strain (3.3 ng/ml) was 13 times more sensitive than the Hamburg strain (43.8 ng/ml), 14 times more sensitive than the Canadian strain (47.6 ng/ml) and 23 times more sensitive than the OVI strain (76.4 ng/ml) to quinapyramine.

4. Discussion

The aim of this study was to produce a drug profile for a panel of *T. evansi* and *T. equiperdum* strains, against the four standard drugs currently used for treatment of Surra (suramin, diminazene, cymelarsan and quinapyramine). With this collection, it was possible to demonstrate the presence of resistance and reduced drug sensitivity occurring in various locations around the world.

A major advantage of using the ^{3}H-hypoxanthine assay is that it allows original isolates, which have not yet been adapted to *in vitro* cultivation, to be tested under laboratory conditions to determine sensitivities against certain drugs. The IC_{50} values produced in this way tend to reflect a more accurate sensitivity of a specific field isolate to a certain drug, than if the isolate had been culture adapted and kept under *in vitro* conditions for a long period of time, before it is then tested. However, in order to produce such drug sensitivity values, a high parasite starting concentration is required (in this study, 2×10^{6} ml^{-1} parasites were required for each isolate). Whilst many of the field isolates do not contain such high numbers of parasites, it is possible to pass them once through mice and hence establish higher parasitaemias, which in turn can be harvested and utilised in the ^{3}H-hypoxanthine assay. By means of an *in vivo* trypanosome propagation technique in mice, the majority of *T. evansi* and *T. equiperdum* study isolates were primarily propagated, then harvested, by passing the mouse blood through a DEAE-cellulose column, to finally achieve the high parasite concentrations needed to perform the drug sensitivity assays. Two *T. evansi* strains could not be successfully propagated in NMRI mice (Merzouga 56 and MHRYD/Brazil). Although parasites were detected after infection with these strains, no suitable trypanosome propagation could be carried out even after several mouse subpassages. These two strains were thus removed from the study and no further tests were performed with them.

The results produced by the *T. evansi* study strains (seen in Table 2) demonstrate that variations in drug sensitivities for the four standard drugs can occur between the geographically different strains. This is best seen in the two Kenyan strains isolated from camels (STIB 780 and STIB 781), both of which clearly display resistance to suramin with IC_{50} values of 14,500 and 11,000 ng/ml, respectively. In terms of field application, ineffective treatment of these isolates with suramin suggests that either cymelarsan or quinapyramine could be used as an alternative. To add to this dilemma, not only are cymelarsan and quinapyramine not readily available in locations such as Africa, but they are also rather expensive. In addition, reduced suramin sensitivity was also shown in the Colombian strain (278.9 ng/ml), originally isolated from a horse in 1973.

The Indonesian RoTat 1.2 and the Philippines strains demonstrated the least diminazene sensitivity of all the *T. evansi* study strains. Since diminazene is used in these two Southeast Asian countries as the standard drug for treating *T. evansi* infections in cattle and buffalo and as resistance in the field tends to occur primarily through inadequate or erroneous application of subcurative doses of a drug over a period of time (Bacchi, 1993), the reduced sensitivities found in the RoTat 1.2 and the Philippines strains could be due to an overexposure of diminazene treatment in water buffaloes and thus less sensitive strains against this drug.

A substantial reduction in diminazene sensitivity can be seen in the South African *T. equiperdum* OVI strain (302.5 ng/ml). Since *T. equiperdum* infections are not considered curable on a clinical basis and there are currently no officially approved drugs, it is uncertain as to whether the OVI strain has developed diminazene resistance through the misuse of chemotherapeutic regimens in the field. However, this high IC_{50} value seen with diminazene helps to explain the equally high IC_{50} value seen for cymelarsan (17.6 ng/ml). Previous reports have described a TbAT1 gene found in trypanosomes, responsible for encoding a nucleoside P2 transporter (Mäser et al., 1999). This transporter is vital in the uptake of adenosine and adenine within trypanosomes, allowing them to salvage purines and nucleoside bases. Moreover, the P2-transporter is involved in the active drug uptake of diamidine compounds and melaminophenyl arsenicals. Furthermore, loss of this P2-transporter renders that organism resistant to such compounds (Carter and Fairlamb, 1993; Carter et al., 1995; Barrett et al., 1995). Therefore, if the OVI strain is missing a functional P2-transporter, it would be prevented from adequately taking up the drugs, diminazene and cymelarsan. Several studies have already shown the possible cross resistance between diamidine drugs, such as diminazene, and melaminophenyl arsenical compounds, such as cymelarsan (Zhang et al., 1993; Ross and Barns, 1996). Although cymelarsan is officially registered for chemotherapeutic use in horses, as well as in other animals, such as camels and goats, whether this drug is readily available on the veterinary market in South Africa is another issue. Due to the limited availability and high costs of cymelarsan, it is doubtful as to whether the OVI strain has lost part of its cymelarsan sensitivity

through drug overexposure in the field, whereas cross resistance to another drug provides a more probable explanation.

The high IC_{50} value seen with quinapyramine for the South African OVI strain (76.4 ng/ml) could indicate additional cross resistance between quinapyramine, cymelarsan and diminazene. Ndoutamia et al. (1993) were able to induce quinapyramine resistance in *T. congolense in vivo*, which was associated with cross resistance to isometamidium, homidium and diminazene. As cross resistance is frequently seen in drugs belonging to similar chemical families, this could be an explanation why the OVI strain demonstrates such reduced sensitivity against all three standard drugs (diminazene, cymelarsan and quinapyramine).

The use of quinapyramine as the drug of choice in Colombia to treat *T. evansi* infections in horses, could explain the reduced sensitivity to this drug. As the Colombian strain was itself isolated from an equine host, the overuse and mismanagement of quinapyramine therapy in the field could be causing the emergence of potential resistance, which consequentially would lead to less sensitive *T. evansi* strains in South America. This could also be the case for the standard drug suramin, which was previously used to treat infected horses and cattle throughout South America, hence leading to the reduced sensitivity shown by the Colombian strain against suramin.

With this range of *T. evansi* and *T. equiperdum* isolates from around the world, a reference panel of isolates with known drug sensitivity could be established. The study has demonstrated drug resistance and reduced drug sensitivity in certain strains, a development which has to be further monitored.

Moreover, this leads to the suggestion that this drug profile could also be used as a reference when screening and testing new compounds against these two infectious agents. Since chemotherapy remains the most efficient and effective way to control Surra, it is vital that new, effective and preferably cheaper drugs are discovered to combat infection as soon as possible.

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