Ticks, ivermectin, and experimental Chagas disease

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Following an infestation of dogticks in kennels housing dogs used for long-term studies of the pathogenesis of Chagas disease, we examined the effect of ivermectin treatment on the dogs, ticks, trypanosome parasites, and also on triatomine vectors of Chagas disease. Ivermectin treatment was highly effective in eliminating the ticks, but showed no apparent effect on the dogs nor on their trypanosome infection. Triatominae fed on the dogs soon after ivermectin treatment showed high mortality, but this effect quickly declined for bugs fed at successive intervals after treatment. In conclusion, although ivermectin treatment may have a transient effect on peridomestic populations of Triatominae, it is not the treatment of choice for this situation. The study also showed that although the dogticks could become infected with Trypanosoma cruzi, *this only occurred when feeding on dogs in the acute phase of infection, and there was no evidence of subsequent parasite development in the ticks.*

For Chagas disease (American trypanosomiasis) vector control, residual spraying of SC or WP formulations of pyrethroid insecticides has proved highly effective in eliminating domestic triatomine populations, but is less effective against peridomestic vector populations (Dias et al. 2002). An alternative approach currently under discussion would involve application of insecticides directly to peridomestic animals, either as pour-on or spray-on formulations or as insecticidal dusts, or by means of systemic products such as ivermectin and fipronil. Such an approach, termed xenointoxication, is predicated on the idea that the treated animals would then carry the insecticide directly to their resting sites where peridomestic Triatominae would be expected to concentrate, being tried by the first time by Romaña and Abalos (1948).

Our opportunity to study the effects of systemic ivermectin on Triatominae and Chagas disease, arose during an infestation of dogticks (*Rhipicephalus sanguineus*) in kennels housing dogs used for long-term studies of the pathogenesis of *Trypanosoma cruzi*, causative agent of Chagas disease (Machado et al. 2001). We were able to examine the possible role of these ticks in the transmission of *T. cruzi*, and also to examine the effects of ivermectin treatment used to eliminate the tick infestations. All experimental procedures were fully cleared by the ethical committee of the Fundação Oswaldo Cruz, which is part of the Ministry of Health of Brazil, in accordance with the ethical code of the Brazilian College of Animal Experimentation (Machado et al. 2001).

MATERIALS AND METHODS

All experimental work was carried out at the Bambui Research Centre of the Oswaldo Cruz Foundation, Brazil, where a research concerning the effects of reinfection on the pathology of *T. cruzi* infection in dogs was conducted. The dogs were young mongrels, maintained in the Centre kennels since birth. They were infected and re-infected with two different Brazilian strains of *T. cruzi,* being all the details pertaining to the experimental design and parasite behaviour and strain characteristics presented and discussed formerly (Machado et al. 2001). During a sudden infestation of *R. sanguineus* in the kennels, affecting all the animals, it became necessary to treat them in order to avoid anaemia and skin infections, as well as to prevent possible interference of tick infestation on the course of the experiments. For this, all the dogs were treated with subcutaneous injections of ivermectin (Detomax ®) at 20 mg a.i./kg. Ivermectin is the 22,23-dihydro derivative of avermectin B_1, a macrocyclic lactone produced by the actinomycete *Streptomyces avermitilis*, which is active against a wide variety of nematode and arthropod parasites (Campbell et al. 1983, Azambuja et al. 1985, Campbell 1985, Rey 1991, Cimermann & Cimermann 1999, Barbosa & Campos 2001, PAHO 2001). The present data are a product of a casual situation, in which some dogs experimentally infected with *T. cruzi* became infested by *R. sanguineus*, so providing the opportunity to observe the possibility of a natural infection of ticks by that flagellate, as well as the eventual action of ivermectin against triatomines and trypanosomes. For this reason the experimental design of the work involves natural limitations, certainly being possible and desirable further investigations.

Fourteen young dogs with a high degree of *R. sanguineus* infestation were included in the present observations. Group I involved six dogs in the chronic phase of infection with both *T. cruzi* strains; Group II involved six dogs in the acute phase of infection; Group III involved two dogs uninfected with *T. cruzi.*

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R. sanguineus was determined by the authors and confirmed by staff of the Zoology Department of the Federal University of Minas Gerais. To measure the infection rate of *R. sanguineus* by *T. cruzi* before the treatment with ivermectin, five fully engorged ticks were collected from each dog. Their digestive tract content was examined both by direct microscopy at 400 times (100 examined fields) and after cultivation in LIT/NNN medium (30 and 60 days of culture at 28°C, considering two tubes for each examined tick). Pre- and post-treatment parasitaemia in the dogs was assessed by daily examination of fresh blood films (Brener 1961, Machado et al. 2001) for a period of 30 days post ivermectin treatment. Xenodiagnosis of each dog was also carried out at 13 days post-treatment using 20 laboratory-reared 4th instar nymphs of *Triatoma infestans*. The faecal material of these bugs was microscopically examined after 30, 60, and 90 days, after which negative bugs were dissected for examination of their entire digestive tract content.

To assess the effect of the ivermectin treatment on feeding triatomine bugs, a xenodiagnosis box containing five 4th instar nymphs of *T. infestans* was applied for 30 min to each of the dogs at intervals of 24, 72, 144, and 312 h after ivermectin treatment. These bugs were then maintained individually and offered a weekly feed on white mice, in order to assess their subsequent survivorship over the following 35 days. To compare the effects on different triatomine species, ten 4th instar nymphs of *T. infestans* and ten 4th instar nymphs of *Rhodnius neglectus* were allowed to feed from the same dog 24h after ivermectin treatment. These bugs were then maintained for 35 days, with a bloodfeed on chicken offered after 15days, in order to assess subsequent survivorship.

RESULTS

Action of ivermectin against tick infestation - The ivermectin treatment appeared to be highly effective against *R. sanguineus*, leading to complete elimination of all signs of tick infestation of the dogs within one week of treatment. Two months later, however, some tick reinfestation was noted – presumably due to ticks hiding within the structure of the kennels. Accordingly, ivermectin treatment was repeated, but accompanied also by residual spraying of the kennels themselves with deltamethrin SC (25 mg a.i./m^2). No ticks have been found since then, either on the dogs or within the kennel structure. There were no discernable side-effects of ivermectin treatment

on the dogs, confirming previously reported studies (Barbosa & Campos 2001, Campbell 1985).

Parasitism of ticks by T. cruzi - No evidence of *T. cruzi* infection was found amongst ticks collected from chronically infected dogs (Group I). However, one of 37 ticks collected from dogs in the acute stage of *T. cruzi* infection (Group II) showed by microscopy a sparse infection of flagellates. LIT/NNN cultures from these ticks showed no flagellates at 30 days, but one culture from 27 of 37 ticks (73%) showed positive at 60 days (Table I).

Influence of ivermectin on T. cruzi infection in dogs - Considering the opportunistic character of this observation, in spite of the natural limitations in terms of technical approach, no apparent influence of ivermectin treatment was discernible on *T. cruzi* parasitaemia in the infected dogs. All treated dogs in the acute phase of infection (Group II) presented detectable parasitaemia by direct blood film examination during the first three weeks of infection, with similar levels of parasitaemia to those observed in untreated dogs during other periods of the long-term study (see Machado et al. 2001). In our experience, dogs in the chronic phase of *T. cruzi* infection never present detectable parasitaemia by direct examination, and this was unchanged by the ivermectin treatment. Similarly, xenodiagnosis of chronically infected dogs did not reveal *T. cruzi* infection, either in ivermectin treated animals or in previously studied untreated animals (Table II).

Action of ivermectin on triatomine bugs - *T. infestans* nymphs fed on dogs 24 h after treatment with ivermectin showed high mortality over the subsequent 35 days, although mortality was greatly reduced for nymphs fed later after ivermectin treatment (Table III). Overall mortality rates after 35 days were similar for *T. infestans* and *R. neglectus*, although with much faster impact on the latter species (Table IV).

TABLE II

Xenodiagnosis with triatomines fed on dogs experimentally infected with *Trypanosoma cruzi* (chronic and acute phase of the infection), 13 days after treatment with ivermectin

Animals	Number of examined triatomines	Positivity for *T. cruzi* (%)
Group I (chronic)	114	0.0
Group II (acute)	111	62.2 [a]

a: corresponding to six positive dogs (100% of positivity).

TABLE I

Direct examination and culture in LIT/NNN medium of the intestinal content of ticks (*Rhipicephalus sanguineus*) fed on dogs experimentally infected with *Trypanosoma cruzi*

Animals [a]	Direct examination		Cultivation in LIT/NNN medium		
	Examined no. of ticks	Positivity for *T. cruzi* (%)	Examined tubes	Positive ticks for *T. cruzi* (%)	
				30 days	60 days
Group I (chronic)	27	0.0	54	0.0	0.0
Group II (acute)	37	2.7	74	0.0	21.6 [b]

a: six dogs in the chronic and six in the acute phase of experimental *T. cruzi* infection; b: corresponding to eight positive ticks fed on two acute dogs (33.3% of dogs).

DISCUSSION

T. cruzi – causative agent of American trypanosomiasis – is generally transmitted to humans through the faecal droplets of infected triatomine bugs (Hemiptera, Reduviidae, Triatominae). In the case of vector-borne transmission, *T. cruzi* does undergo a developmental cycle within the gut of infected bugs, producing infective metacyclic forms in the bug's rectum, with these infective forms passed out in the bug's faecal droplets at the time of its subsequent bloodmeals (Brener & Alvarenga 1976). It is over-simplistic to suggest that there is little interaction between the parasite and its insect vector, although the interaction is by no means as complex as that between, say, forms of *T. brucei* and tsetse (Welburn & Maudlin 2002). Moreover, there is evidence to show that *T. cruzi* can precariously survive in other types of haematophagous insects, and – experimentally at least – can also survive in lepidopteran caterpillars and in leeches (Marsden & Pettitt 1969).

Even since the pioneering work of Carlos Chagas (Chagas 1909) there has been concern that *T. cruzi* might be transmitted by invertebrates other than Triatominae. Various species of flea, fly, bedbug, mosquito, ticks, and lice known from the endemic regions of Chagas disease, have been suggested as possible candidates (Dias 1935, Brumpt 1949, Dias 1979, Rey 1991, Amato Neto et al. 2000). As early as 1913, Arthur Neiva suspected that *T. cruzi* transmission could occur between dogs infected with *R. sanguineus*, although he also considered that such event would probably be unusual. Since then, transient experimental infections were obtained by various authors in species of *Cimex, Ornithodorus, Melophagus, Ixodidae*, and *Argasidae*. Some of these, including *Amblyoma longirostrum* and *Cimex lectularius* were detected naturally infected (Blacklock 1914, Mayer & Rocha Lima 1914, Dias 1935, Pifano 1941 Brumpt 1949, Rey 1991). Considering this matter, Brumpt (1949) suggested that ixodid ticks (*R. sanguineus* and *A. cayennense*) could mechanically transmit *T. cruzi* by means of anterior route (regurgitation) after the ingestion of a great quantity of flagellates, although again this would be a very exceptional occurrence. Storino and Jörg (1994), observed experimental transmission to mice through the biting of infected *C. lectularius*, noting long persistence of the parasite in the digestive tract of this insect (up to 320 days) with apparent developmental forms – spheromastigotes in the proventriculum, and metacyclics in the rectum. These authors also described a possible case of *T. cruzi* transmission by *C. lectularius* in the suburb of Belgrano, Buenos Aires, noting that triatomine bugs are generally absent from this city.

In general, however, the current consensus is that non-triatomine arthropods do not play an epidemiologically significant role in the transmission of *T. cruzi*, although the possibility of rare transmission events must be considered (Amato Neto et al. 2000, WHO 2002). Our results with *R. sanguineus* endorse such a view, showing no evidence of tick-borne transmission, and only sparse tick infection after feeding on dogs in the acute phase of *T. cruzi* infection (i.e. with patent parasitaemias). Ticks fed on dogs in the chronic phase of infection (i.e. without patent parasitaemias) showed no evidence of infection even after lengthy culture of their gut contents. It would appear that although *R. sanguineus* is able to ingest bloodstream forms of *T. cruzi*, parasite survival in the ticks is reduced and infective forms are not developed in the terminal digestive tract. Thus, tick-borne transmission could only be expected due to anterior route regurgitation (or by consumption of an infected tick) (Neiva 1913, Pinto 1920, Brumpt 1949, Pessôa & Martins 1977).

R. sanguineus is a widespread parasite of dogs, recorded from all regions endemic for Chagas disease. It has been associated with anaemia and secondary skin infections in dogs, and is also known to bite humans and transmit important diseases such as Mediterranean and Rocky Mountain rickettsiosis (Rey 1991, PAHO 2001). Our

TABLE III

Mortality rates (%) for *Triatoma infestans* fed in dogs in different hour times after treatment with ivermectin

Group of dogs [a]	Mortality rate of nymphs (%) in different periods after treatment (hours)			
	24	72	144	312
Treated with ivermectin	83.3	53.3	13.3	0.0
Non treated	0.0	0.0	3.3	0.0

a: six dogs of each group, with five 4th instar nymphs of *T. infestans* fed in each one.

TABLE IV

Cumulative daily mortality of *Triatoma infestans* and *Rhodnius neglectus* fed in the same dog treated 24 hours before with ivermectin

Triatomine species [a]	Cumulative mortality rate (%) up to 35 days of observation							
	Days after treatment with ivermectin							
	1	2	5	8	12	15	21	35
T. infestans	0	10	10	10	20	20	30	100
R. neglectus	70	80	90	90	90	90	90	100

a: ten 4th instar nymphs for each species.

results endorse those of previously published studies (Campbell et al. 1983, Campbell 1985, Rey 1991) showing that subcutaneous injection of ivermectin is highly effective in eliminating the ticks, however, ivermectin injection showed no effective action against *T. cruzi* in acute or chronically infected dogs, as expected from previous in vitro studies (Coura & Castro 2002). Ivermectin seems to be active against helminths and arthropods, but generally inactive against systemic or digestive protozoans (Campbell et al. 1983, Campbell 1985, Rey 1991, Cimermann & Cimermann 1999, Barbosa & Campos 2001).

For the control of Triatominae, it is well recognised that improved approaches are required for the elimination of peridomestic bug populations (Dias et al. 2002, WHO 2002) and various techniques involving application of insecticides to the peridomestic animals are being tried – including the use of pour-on, spray-on, and dust formulations – as a means of xenointoxication of the bug populations. In the veterinarian practice, the use of a systemic product such as ivermectin offers a series of potential advantages, because of strong action against gastro-intestinal nematode parasites, and against ectoparasitic arthropods such as ticks, lice and fleas (Campbell et al. 1983, Campbell 1985, Paradis 1998, Barbosa & Campos 2001). However, although our results endorse the effect of ivermectin against *R. sanguineus*, we found only a limited effect against Triatominae feeding on the treated dogs. Although the treatment induced high mortality of *Triatoma* and *Rhodnius*, this was largely restricted to bugs feeding within 24 h of the ivermectin treatment. It would appear that metabolism of ivermectin during the first 48 h after treatment (Azambuja et al. 1985, Paradis 1998), results in concentrations too low to offer a sustained effect against Triatominae.

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