A comparative study on susceptibility, course of parasitemia, and pattern of infection with *Trypanosoma evansi* between different laboratory animals

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**ABSTRACT**

This study investigated the susceptibility of eight different animal species (rat, mice, guinea pigs, hamsters, rabbits, sheep, goats, and chicken) experimentally infected with *Trypanosoma evansi* isolated from camels. In all laboratory animals, the number of trypanosomes was standardized according to the weight of the animal, and daily examination of the blood was conducted to assess the presence of trypanosomes to determine the prepatent period and the peak of parasitemia. Results suggested that mice and rats were the most susceptible laboratory animals to infection, whereas hamsters and guinea pigs displayed a certain degree of tolerance to infection. Rabbits exhibited a chronic course of infection, but the level of parasitemia was very low. In sheep and goats, trypanosomes were detectable only by subpassage to rats and mice, whereas all the chickens remained uninfected even with increasing doses of infection.

**1. Introduction**

Trypanosome diseases are considered as one of the most fatal disasters on the civilization of tropical Africa affecting both humans and animals. Like several protozoan diseases, *Trypanosoma evansi* is transmitted from one animal to another through a third vector, which is generally a completely different animal such as an arthropod ectoparasite belonging to the genera *Tabanus*, *Stomoxys*, *Atylotus*, and *Lyperosia*. The course of infection with *T. evansi* depends on numerous factors such as the susceptibility of the host, the strain of parasite, and the severity of disease, which vary in diverse mammals and in different localities. The infection is characterized by a succession of crises and relapses, and after an incubation period of 4–9 days, the trypanosomes invade the blood and parasitemia increases with the temperature of the host and declines when it decreases [1]. Furthermore, the species, age, and physiological status of the host determine the susceptibility to the infection and the clinical manifestations of the disease. Moreover, the degree and type of infection (acute or chronic), similar to the level of parasitemia, affect the transmission through insect vectors [2].

Several studies on *T. evansi* have been conducted using various experimental animals. Several animal models have also been used in various studies to investigate the effect of *T. evansi*, such as chicks and fowls for examining infectivity [3], different mouse strains for determining susceptibility and resistance to infection [4], and rats for exploring the immunological aspects [5].

Even larger animals such as goats, pigs, horses, cattle, and buffaloes have been used by several researchers for investigating *T. evansi* infections [1].

Surra is diagnosed at a later stage due to the lack of simple and accurate diagnostic methods and standardized description of clinical signs [6]. Several techniques have been developed to detect *T. evansi* infection, ranging from the most inexpensive and primitive methods such as blood smear examination to the most advanced methods such as enzyme-linked immunosorbent assay (ELISA). However, each method has its own defects; for example, ELISA may be used for large-scale animal tracking but can show cross-reactions between different species of trypanosomes. In fact, ELISA cannot be used to differentiate between sick and healthy animals. Regarding blood smear examination, although it is a simple, inexpensive, and rapid method, it has limited sensitivity and is not quite helpful in detecting parasites in the early stages of infection due to low rates of parasitemia [7].

The only valid diagnostic test available for detecting *T. evansi* is its isolation from the blood of the infected host. However, this strategy is insensitive primarily because of the periodic cryptic nature of parasitemia, which results in long periods during which the host shows no detectable parasites in its blood [8]. Rodent inoculation is commonly considered to be the most effective method for detecting trypanosomosis in animals. In addition, a previous survey [2] reported about some routinely used parasitological tests based on a descending order of their sensitivities as follows:
rodent inoculation, miniature anion-exchange centrifugation technique (mAECT), microhematocrit centrifugation technique (MHCT), and microscopic examination of fresh or stained blood smears.

Our study supports the rodent inoculation method with a dose in correlated with animal’s weight as a reliable strategy, especially for the detection of surra in sheep and goats and any other domestic animal that may exhibit conflicting pathogenicity.

2. Materials and methods

2.1. Collection of blood samples

This study was conducted in Dairout city (27°34’ N and 30°49’ E, located on the west bank of the Nile River, in Assiut Governorate) in upper Egypt. Blood samples (20 ml) were collected under complete aseptic conditions from the jugular vein of camels reared in the rural areas of Dairout city.

2.2. Examination of fresh blood for T. evansi:

Fresh blood was examined as a first step before the inoculation of trypanosomes into laboratory animals. A drop of blood was immediately examined under the low power of microscope. In some cases, the blood was immediately centrifuged for approximately 15 min at 3000 × g using a laboratory centrifuge (SM 800B, Surgifriend Medicals, England), and the pellet was examined for the presence of trypanosomes, which are generally identified by their characteristic movements as described previously [9].

2.3. Susceptibility of laboratory animals

In all laboratory animals, the number of trypanosomes was standardized according to the weight of the animal (15 trypanosomes/g body weight). The data are summarized in Table 1.

All animals were examined to be free from parasitic infection to avoid the production of antibodies before the experiments. Sheep and goats were treated orally with Ergamisol (a medication used to treat parasitic worm infections), and after 10 days, fecal examinations were performed to ensure that they are free of any gastrointestinal parasites. The animals were raised in the animal house of the Veterinary College of Assiut University and maintained in individual cages. They were provided ad libitum access to food and water. All animal experiments were conducted according to the Institutional Animal Care and Use Committee, National Research Centre Animal Care Unit, compatible with the guidelines of the International Animal Ethics Committee and according to the local laws and regulations.

2.4. Daily follow-up

Daily examination of the blood of laboratory-infected animals was conducted using wet smears as well as thin and thick films for the presence of trypanosomes under a microscope at 100 × magnification according to the Pizzi-Brenner method [10] to determine the prepatent period and the peak of parasitemia. Furthermore, the phase of multiplication and the duration of parasitemia were taken into consideration. The prepatent period is believed to be the time elapsed from the time of inoculation to the first appearance of trypanosomes in blood.

2.5. Method of counting trypanosomes

The blood of laboratory-infected animals was examined daily using wet smears and thin and thick films to assess the presence of trypanosomes and determine parasitemia according to a previously described method [11].

3. Results

3.1. Susceptibility, pattern of infection, and course of parasitemia in different laboratory animals

3.1.1. Rats

All the 10 rats that were inoculated intraperitoneally contracted the infection. The average prepatent period ranged from 2 to 3 days. Initially, there were only a few trypanosomes, and then the parasitemia increased rapidly till the death of the animals on the 8th to 10th day of infection. No periodicity of parasitemia was observed. As shown in Figure 1, trypanosomes started to appear in the blood on the 3rd day at a

| Table 1. Experimental animals (numbers and route of injection). |
|---------------------------------------------------------------|
| **Experimental animal** | **No.** | **Dose/ gm body weight** | **Blood collection site** | **Route of injection** |
|------------------------|---------|------------------------|--------------------------|-----------------------|
| Rats                   | 10      | 15                     | Caudal vein              | Intrapitoneal injection |
| Mouse                  | 10      | 15                     | Caudal vein              | Intrapitoneal injection |
| Hamsters               | 5       | 15                     | Jugular vein             | Intrapitoneal injection |
| Rabbids                | 4       | 15                     | Marginal ear vein        | Intrapitoneal injection |
| Guinea pig             | 5       | 15                     | Jugular vein             | Intrapitoneal injection |
| Sheep                  | 3       | 15                     | Jugular vein             | Intramuscular injection |
| Goat                   | 3       | 15                     | Jugular vein             | Intramuscular injection |
| Chickens               | 2       | 15                     | Right-sided jugular vein | Intramuscular injection |
|                        | 2       | 100                    |                          | Intravenous injection  |
|                        | 2       | 50000                  |                          | Subcutaneous injection |
count of $42 \times 10^4 / \text{mm}^3$ that gradually increased to $52 \times 10^4 / \text{mm}^3$, $86 \times 10^4 / \text{mm}^3$, and $135 \times 10^4 / \text{mm}^3$ on 4th, 5th, and 6th days, respectively. The number of trypanosomes continued to increase very rapidly on subsequent days till it reached $625 \times 10^4 / \text{mm}^3$ on the 10th day when all the infected rats in the experiment died.

3.1.2. Mice

Laboratory mice exhibited almost a similar pattern of infection as shown by the rats, but the incubation period was shorter (2 days) than that in rats. The average survival time was 5–7 days. The course of parasitemia was almost similar to that in rats. Parasitemia raised rapidly till the death of the animals. It can be clearly observed in Figure 2 that the pattern of infection and the course of parasitemia exhibited a more or less similar course in all the infected mice throughout the observation period. The blood remained negative till the 2nd day when trypanosomes began appearing in the blood starting from $32 \times 10^4 / \text{mm}^3$ and continuing to flare up very rapidly to $400 \times 10^4 / \text{mm}^3$ on the 7th day when all the infected mice died.

3.1.3. Hamsters

Hamster appeared to be comparatively resistant to the infection compared with rats and mice. The average prepatent period was 5–7 days after which the parasitemia reached the peak on the 8th to 9th day of infection. As shown in Figure 3, the experimental infection of hamsters with *T. evansi* exhibited a chronic course with three peaks of parasitemia on the 5th day of infection when trypanosomes began appearing in the blood with a mean count of $5 \times 10^5 / \text{mm}^3$. The number of trypanosomes then gradually increased till it reached the first peak on the 10th day when the mean count increased to $82 \times 10^4 / \text{mm}^3$. On the 11th day, there was a decrease in the mean count of trypanosomes, reaching merely $15 \times 10^3 / \text{mm}^3$. Thereafter, the blood remained negative for 4 days. On the 16th day, the trypanosomes reappeared in the blood and continued to increase gradually in number resulting in a second peak on the 20th day (mean count = $78 \times 10^4 / \text{mm}^3$), which was more or less equal to the first peak. There was a sudden drop in the mean count of trypanosomes to $27 \times 10^3 / \text{mm}^3$ on the 22nd day, becoming negative on the 23rd day. The third peak of parasitemia began appearing on the 25th day and continued to increase rapidly to $92 \times 10^4 / \text{mm}^3$ on the 30th day when all the animals died.

3.1.4. Rabbits

Rabbits displayed a chronic course of infection similar to that in hamsters, showing a very low level of parasitemia. The blood of infected rabbits contained very low number of trypanosomes. However, when the blood collected from experimentally infected rabbits
was inoculated into rats and mice, it was highly infective as shown in Figure 4.

3.1.5. Guinea pigs

The pattern of infection in guinea pigs infected with *T. evansi* was similar to that in rabbits and hamsters, but the incubation period was shorter and the survival time was 35–40 days. Guinea pigs also displayed the same course of infection as in rabbits. However, the parasitemia was slightly higher than that in rabbits, with a trypanosome count of 20,200/mm³ in the terminal stage of the disease. Moreover, the blood was highly infective to rats and mice in all stages of the disease even during the negative period of parasitemia as depicted in Figure 5.

3.1.6. Sheep and goats

Sheep and goats exhibited a cryptic course of the disease, with no parasites being detectable in the blood for up to 4 months. However, the blood of sheep and goats was infective to rats and mice for up to 2 months. During this period, inoculation of the blood of sheep or goats to rats or mice was successful in isolating the parasite.

3.1.7. Chickens

All chickens inoculated by different routes did not contract the infection. Increasing the dose of infection from 15 organisms/g body weight to $5 \times 10^4$/g was also not successful in initiating the infection in chickens. The chickens were kept under observations for 3 months. During this period, the inoculation of their blood to rats and mice proved to be negative. Therefore, it be concluded that chickens are resistant to *T. evansi* infection.

4. Discussion

Mice and rats appeared to be the most susceptible laboratory animals to infection with *T. evansi*. Parasitemia was found to increase progressively, killing the animals within a few days. Accordingly, the antibody production in rats and mice was insignificant, with no periodicity of parasitemia being observed, but the trypanosomes multiplied without hindrance until the animal died [5]. The mean prepatent period observed in our study was similar to that reported in a previous study [12] on mice, in which the prepatent period was in the range of 1–3 days post infection, with parasitemia progressing to the peak within 3 days, and the survival period was 10 days.

In rats, trypanosomes started to appear in the blood on the 3rd day and continued to increase very rapidly on subsequent days. This result was in agreement with a previous study [3], in which the authors reported a prepatent period of 2–4 days. Another study [13] reported that when *T. evansi* was inoculated into albino rats, the parasite appeared in the blood on the 5th day, the density of trypanosomes in the blood reached its peak on the 6th day, and all animals succumbed to infection between the 6th and 8th day of inoculation. The susceptibility of small rodents such as mice and rats correlated with the immunosuppression that was associated with an elevation of hormone levels such as corticosterone (CT) and corticosterone dehydroepiandrosterone-sulfate (CT/DHEA-s) as described previously [14] in mice and rats infected with *T. cruzi*.

In the present study, the blood of infected rabbits contained very low number of trypanosomes, which was consistent with a previous research [15], which reported that rabbits were most resistant to trypanosome infection. Hamsters and guinea pigs also displayed a certain degree of resistance to infection. Due to limited information regarding the susceptibility of hamsters to infection with *T. evansi*, we compared our results with those obtained from hamsters infected with other species of trypanosomes and found that in *T. cruzi*-infected Syrian hamsters, the prepatent period ranged from 3 to 5 days and parasitemia appeared on 4, 17, 13, and 21 days post infection [16]. There is also a lack of detailed studies on guinea pigs regarding their susceptibility or pathogenicity. They are primarily used for maintaining the *T. evansi* strain. A previous study [13] investigated the effect of *T. evansi* on guinea pigs and reported that after the intraperitoneal inoculation of *T. evansi*, the parasites exhibited some regular peaks, but later the parasitemia became irregular, and the animals died after the first peak of infection and some survived till 4 months. These results were partially consistent with our results, which revealed some parasitemia peaks but the survival time was 35–40 days. The resistance in rabbits, hamsters, and guinea pig could probably be due to increased IgG antibody synthesis and estradiol levels [14].
Periodicity, fluctuation, and appearance of peaks in the course of infection with *T. evansi* in these animals may be attributed to the exhaustion of the host’s defense mechanism as mentioned earlier [17]. It has been reported that during the ascending phase of parasitemia, the majority of multiplying parasites (e.g. long slender forms) belong to the same antigenic type known as the homotype [18]. A peak of parasitemia reaches when the long slender forms differentiate into nondividing, short stumpy forms, which have a relatively short in vivo half-life of 24–36 h and release variant surface glycoproteins (VSGs) (a dense immuno- 
genic surface coat of 12–15 nm thickness with a single polypeptide species covering the parasite in the mammalian host) in the circulation upon degeneration. These degenerating parasites allow the host to develop an antibody response to the homotype. Subsequently, the parasitemia enters a descending phase as the trypanosomes of the major variable antigen type are eliminated.

Because the experimental infection of sheep and goats with trypanosomes exhibited a cryptic course as the trypanosomes were detectable only by subpassage to rats and mice, these animals can act as a reservoir host for surra trypanosomes. The long lasting period during which trypanosomes can be detected in the blood for approximately 4 months provides further evidence that these animals are healthy carriers of these trypanosomes. Our results are partially in agreement with a previous study [19] where the authors reported that goats were also most often less susceptible when experimentally infected with a camel trypanosome isolate from the Canary Islands. In such cases, there is a complex interaction between the host immune response and the parasite’s survival strategies. As such, natural selection has enabled African trypanosomes to develop highly advanced mechanisms to evade immune killing to survive in the chronically infected host and thereby allow transmission to the next host [18]. Antigenic variation remains one of the most spectacular adaptive mechanisms exhibited by African trypanosomes and is the central most important immune escape mechanism of these parasites [20, 21]. These conditions have all the attributes of concomitant or tolerance immunity, better known as premonition. In this type of immunity, the parasites are not eliminated but prevented from multiplying excessively, ultimately resulting in a latent infection.

Based on the abovementioned data and the results obtained in the present study, it can be concluded that sheep and goats appeared to be an important reservoir host of surra trypanosomes. In addition, the infection persists in these animals, and consequently, they may harbour the trypanosomes long enough to serve as a source of infection for insect vectors (*Tabanus* and *Stomoxys*).

Our attempts to infect chickens with *T. evansi* in the present study had failed. This result was different from than obtained in a previous study [22] where the parasite was detected in peripheral blood films only after the 9th day of inoculation; However, another previous research [23] had also reported a similar result, in which the author attempted to transmit a freshly isolated strain of *T. evansi* to different laboratory and domestic animals, including two domestic fowls. A later study [24] reported that although infection with *T. cruzi* can be initiated in the egg, the parasite persistence was precluded.

Our failure to infect chickens with *T. evansi* might be explained on the basis of several factors. One of them might be the higher temperature of the body of poultry than that of mammals. Moreover, the physiological and biochemical characteristics of the blood of poultry diver markedly from those of mammals. As previously mentioned regarding the attempts to cultivate *T. evansi*, these characteristics are the most important factors that control the survival and growth of trypanosomes in mammals. Finally, the presence of innate immunity in birds against the trypanosomes of mammals might be an important lethal factor to trypanosomes. This explanation is supported by the previous findings [23] that the natural resistance of chickens to *T. cruzi* infection and the capacity of their sera to lyse blood forms (try- pomastigotes) of the parasite in vitro were found to be complement-dependent phenomena.

5. Conclusion

Mice and rats appeared to be the most susceptible laboratory animals to *T. evansi*. Rabbits, hamsters, and guinea pigs displayed a certain degree of resistance to infection, although their blood could infect other animals even during the negative period of parasitemia, so we recommended it for preserving the parasite for a long period of up to a month or more. Sheep and goats act as a perfect reservoir host for surra trypanosomes. Our attempts to infect chickens with *T. evansi* had failed. Our study confirms the reliability of the laboratory animal inoculation strategy with a dose in correlated with animal’s weight for the identification of *T. evansi* in infected animals even when the parasitological results are negative.

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Disclosure statement

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