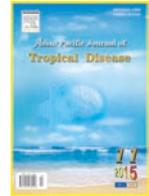




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Impact of the carrier state by *Theileria annulata* on milk yield in Tunisian crossbred (*Bos taurus*) cattle

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ABSTRACT

Objective: To estimate milk yield losses in *Theileria annulata* (*T. annulata*) carrier cows by a trial which was made in two cattle farms in the north of Tunisia.

Methods: Eight non-infected cows were randomly and equally divided into two groups: the treated and control groups. Eight *T. annulata* carrier cows were also randomly and equally divided into two groups: the treated and control groups.

Results: There was a negative correlation between milk yield and parasitemia in both treated ($r = -0.495$, $P = 0.037$) and control carrier cows ($r = -0.662$, $P = 0.003$). Average adjusted lactation curve showed that milk yield in treated carrier cows was higher than that in control carrier cows. Daily milk yield losses due to *T. annulata* infection have been estimated at 0.77 kg per of each *T. annulata* carrier cow.

Conclusions: Since *T. annulata* infection induces a small but persistent decrease of milk yield in *T. annulata* carrier cows, further studies are needed in a larger animal group to confirm and improve this estimation.

1. Introduction

Tropical theileriosis [*Theileria annulata* (*T. annulata*) infection] is a protozoan disease transmitted by tick species belonging to the genus *Hyalomma*. In enzootic countries, *T. annulata* caused important losses, such as mortality, weight losses, abortions, milk yield losses, and control costs[1,2]. The impact of the carrier state of *T. annulata* infection on milk production was shown in a field study in 1991 by Singh[3]. It is estimated that the milk losses caused by the asymptomatic infection (carrier state) in zebu-taurine crossbred cows treated with buparvaquone at a daily average of 1.4 L, *i.e.* 126 L over a period of 90 days[3]. Minjauw and McLeod estimated

these losses at 386 L/lactation period in carrier zebu-taurine crossbred cows[4]. Considering the prevalence of the carrier state in lactating cows from enzootic regions for tropical theileriosis[5], the estimation of the financial losses due to this infection state is an important step toward an accurate analysis of the financial impact of tropical theileriosis and to rank this infection among other animal health problems[6]. To our knowledge, the present study is the first in Africa to evaluate milk losses of *T. annulata* carrier cows (*Bos taurus*) by using a protocol based on injection of theilericidal drugs.

2. Materials and methods

The present trial was carried out from February to April 2009. *Bos taurus* cows in two farms were in sub-humid regions, cows were milked twice a day. The first was enzootic instability for tropical theileriosis in El Khetmine village (Gouvernorate of Bizerte, North of Tunisia). It had a small herd of 17 taurine crossbred cows with a mean daily milk yield of 10.6 kg. Eleven of these cows had already presented clinical episodes of tropical theileriosis during the summer season of 2008. The cows were lodged in a building which

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was favourable for the hibernation of tick nymphs and for females to lay eggs. The second was Holstein-Frisian crossbred cattle farm located in Sidi Thabet (Gouvernorate of Ariana, North Tunisia) which was free of both ticks and tropical theileriosis. The mean daily milk yield in this farm was 10.7 kg.

During the first visit (Day 0), all the cows were clinically examined to detect health troubles, their udders were examined for clinical mastitis and the California mastitis test was performed to check sub-clinical mastitis. Cows were drenched with fenbendazole (Vermicur® 2.5%, Médivet) at the conventional dose of 7.5 mg/kg.

In order to determine the status of the cows for tropical theileriosis, 5 mL of blood were collected in vacutainer tubes for serum extraction, and in vacutainer ethylene diamine tetraacetic acid tubes for blood smears and DNA extraction. In order to monitor the intensity of *T. annulata* infection, Giemsa-stained blood smears were examined under the microscope with the 100× immersion objective. Hemopathogens were screened for each animal in 100 microscope fields per slide. In order to confirm *T. annulata* infection, an indirect fluorescent antibody test was performed[7]. DNA was extracted from blood samples and a PCR was run using the Tams primers[8]. Only cows with positive blood smears, indirect fluorescent antibody test, and PCR were included in the trial. A total number of 8 cows from the infected farm were exclusively positive to *T. annulata* and 8 cows from the non-infected farm were included in the trial. Cows were randomly divided into four groups, each composed of 4 animals, as follows:

Group 1: infected by *T. annulata*, treated with buparvaquone (T1B1).

Group 2: infected by *T. annulata*, not treated with buparvaquone (T1B0).

Group 3: not infected by *T. annulata*, treated with buparvaquone (T0B1).

Group 4: not infected by *T. annulata*, not treated with buparvaquone (T0B0).

At Day 0, four carrier cows from the infected farm and 4 from the non-infected farm received a single intramuscular injection of buparvaquone (Teldex®, Médivet) at the conventional dose of 2.5 mg/kg. The 8 control cows (4 from each farm) received no treatment and were monitored in the same way as the treated group (parasitemia and milk yield estimation).

The parasitemia has been monitored at Day -15 and 0, then daily during 15 days and each two days during an additional period of one month. In order to confirm the absence of infection during the whole survey in cows from the non-infected farm, the parasitemia was performed up to Day 45 post-treatment. Milk yield was assessed individually at the evening milking by using scales with a precision of 100 g. Since the interval between the two milking is roughly 12 h, we have considered that the milking of the evening represented half of the daily milk yield. The production was then converted to litres (1 L of milk weighs 1.032 kg). Milk yield was recorded daily in the four groups 15 days before and after buparvaquone injection. After this time period, milk yield was recorded every two days during 30 days. The evolution of milk yield was illustrated by using

observed and predicted yields before and after the buparvaquone injection. Predicted yields to a 305-day period were derived from fitting the Wood function to milk data points recorded during the trial period[9,10]. Adjusted lactation curves should show the evolution (increase or decrease) of milk production following the treatment. Milk yield losses were calculated by comparing yields recorded between treated and control animals during two periods.

In order to decrease the heterogeneity, the milk yields were log transformed. The comparison of means of milk yield from Day 1 to Day 12 after buparvaquone injection was performed by using the student's *t* test with a cut-off value of 0.05 following One-way ANOVA where groups of cows (T0B0, T0B1, T1B0, and T1B1) were levels of the explanatory variable. Similar analysis was conducted for parasitemia. The correlation between parasitemia and milk yield was also computed (SPSS 13 for Windows).

3. Results

Before treatments, parasitemia was relatively high (maximum value: 20 parasites/100 fields in both control and treated animals). After buparvaquone injection, the parasitemia decreased in treated animals and remained fluctuant in control cows (Figure 1).

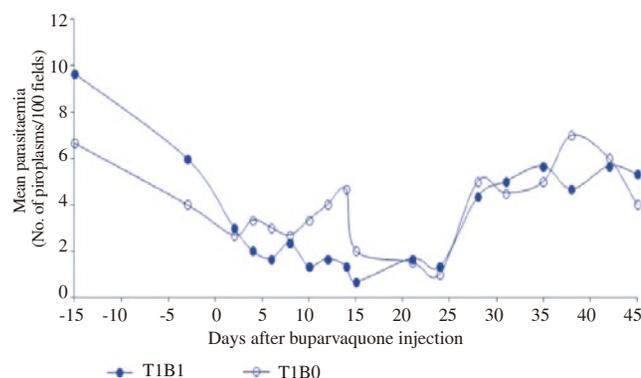


Figure 1. Mean parasitaemia in cows infected with *T. annulata* and treated with buparvaquone (T1B1) and infected control cows (T1B0).

Before buparvaquone injection, milk yield was slightly fluctuating. There was no difference between milk yield in control and treated cows in both infected and non-infected groups ($P > 0.05$) (Figure 2). From Day 15 post-treatment, milk yield was constantly higher in the treated group as compared to the control group (Figure 2). Milk yield in control cows presented slight fluctuations. Average adjusted lactation curves showed that data points collected during the trial period, corresponding to the declining phase of a typical lactation curve for most of the sampled cows[9,10], resulted in increasing yields in treated cows as compared to control cows (Figure 3). Groups of cows accounted for roughly 42% of variation of milk yield (coefficient of determination = 0.42). This increase in milk yield detected by the Wood function mirrored an increase in the effective yield following the treatment of cows even though cows were supposed to have their production declining (they were in the phase following the peak of lactation). Because of the short period of milk monitoring in the present study and since the recorded yields were

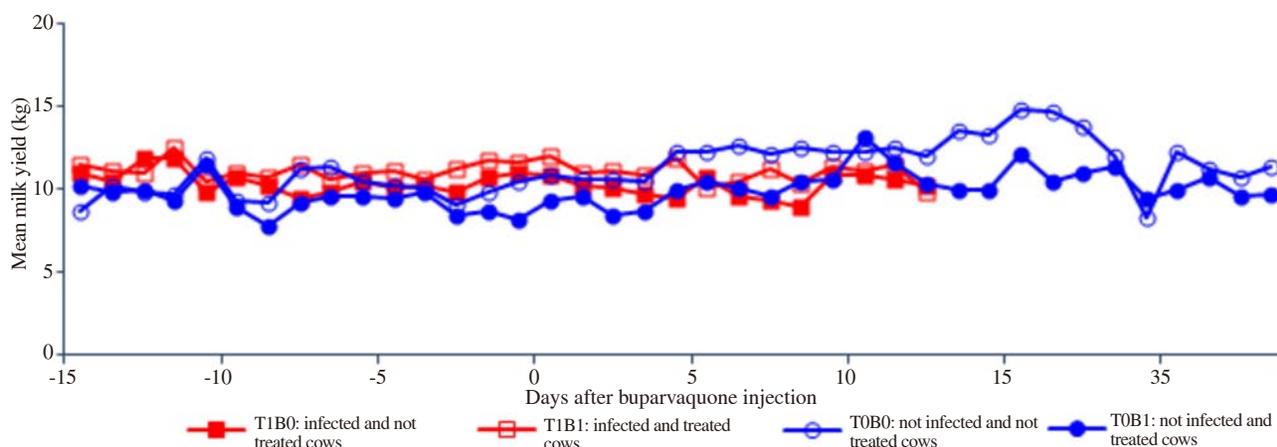


Figure 2. Mean milk yield of control and buparvaquone-treated cows in *T. annulata* infected and *T. annulata* non-infected farms.

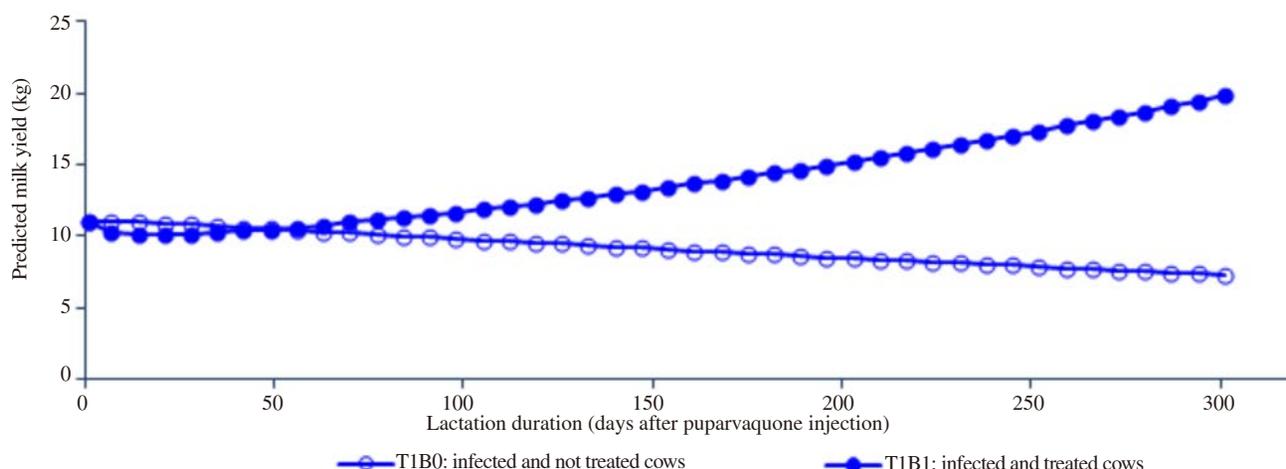


Figure 3. Adjusted lactation curves for buparvaquone treated and control *T. annulata* carrier cows.

carried out in the decreasing period of the lactation curve, the curves did not show the three phases of typical curves (increasing period, peak and decreasing lactation curve). Relative variation of milk yield in treated cows (11.63%) was higher than that in control cows (4.84%) ($P < 0.05$). The relative difference in milk yield was 6.79% with a mean daily loss per cow estimated at 0.77 kg. There was a significantly negative correlation between milk yield and parasitemia in both treated ($r = -0.495$, $P = 0.037$) and control carrier cows ($r = -0.662$, $P = 0.003$).

4. Discussion

In Tunisia, M'barek estimated milk yield losses in clinically infected cows during the month after treatment at 300 L per cow[11]. The present trial was carried out to estimate the milk yield losses due to the carrier state by *T. annulata* in the Tunisian context. To our knowledge, the only study that estimated such losses was carried out by Singh in crossbred taurine-zebu cattle[3], which was more resistant to *T. annulata* infection than pure taurines[12]. Another difference was that Tunisian cattle were exposed to the vector tick (*Hyalomma scupense*) from late June to early August[13], while in India the challenge occurs throughout the year. This difference had a high impact on the host-parasite relationship and the epidemiological features of the disease.

The number of cows was low. That was due to the difficulties to find

farmers accepting the protocol since buparvaquone was associated with a withdrawal period for the milk and to recruit cows with the same characteristics (animals exclusively infected by *T. annulata*, same milk production level, same lactation stage, etc.). The cows were monitored for 45 days before buparvaquone injection and their lactation curves were adjusted by the Wood function to detect milk yield evolution before and after the buparvaquone injection for several reasons: daily high fluctuation of both milk yield and parasitemia (interference of several factors leading to a dynamic evolution between the host's immune system and the parasite), a base line value and the low number of included cows.

A significant negative correlation was documented between parasitaemia and milk yield. The losses due to *T. annulata* infection showed a difference of milk yield of 6.79% between treated and control cows. In Tunisia, the mean annual milk yield was estimated by Ben Salem and Bouraoui [14] at 4662 L/305 days (range: 1900 to 8170), therefore a carrier cow should present in Tunisia a mean annual loss of 316.55 L (range: 129.01–569.45).

The buparvaquone treatment has been used as a tool to annihilate the effect of *T. annulata* on milk yield. The non-infected group was included to identify non-specific effect of buparvaquone on milk yield. After buparvaquone injection, the cows presented a parasitemia decrease which reached a mean of 0.667 piroplasm/100 microscope

fields at Day 15. By Day 45, the parasitaemia regained its initial values. This evolution emphasizes the role of preventive measures as the best tools of *T. annulata* infection control, instead of treatment of clinical cases.

In India, Singh reported a more significant decrease of parasitemia passing from 0.0155% at Day 0 to 0.007 and 0.002% at Day 5 and 7, respectively[3]. This is due to a more innate resistance to *T. annulata* infection of these animals when compared of the present cow population[12]. In India, the animals are challenged throughout the year representing a fundamental difference when compared to the epidemiology of the disease in Tunisia, which occurs exclusively in the summer season (from June to August)[13]. Accordingly, the cows monitored in the present study were true carriers since they were not subject to any re-infection. This discrepancy with the milk yield level in the two countries should be taken into account when comparing the increase record in milk yields with the values reported in India[3]. The effect of buparvaquone injection is not comparable to its effect on clinically infected cows. In these animals, a dramatic and rapid decrease of parasitaemia is usually reported, and the disease can lead to a drying up of the cow[11]. In a study in Egypt, milk yield in *T. annulata* Friesian cows varied between 2.5 and 5.0 kg/day during the production peak in treated animals and between 3 and 3 kg/day in non-treated animals[15]. Compared to our results, these losses are very high, which is due to the fact that these cows develop clinical episodes of tropical theileriosis while our animals were asymptomatic. During the 10-day post-injection, a progressive significant increase of milk yield was observed with a statistically significant negative correlation between milk yield and parasitemia.

The results obtained herein were lower than those reported in India[3], where the mean daily losses were estimated to 1.4 L. This difference can be explained by the fact that these animals were challenged several times during the trial leading to higher parasitaemia levels. A significant effect of the carrier state on milk yield was identified in our study. However, it was carried out on a very low number of cows, and further trials are needed to confirm this decrease.

Conflict of interest statement

We declare that we have no conflict of interest.

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References

- Gharbi M, Sassi L, Dorchies P, Darghouth MA. Infection of calves with *Theileria annulata* in Tunisia: economic analysis and evaluation of the potential benefit of vaccination. *Vet Parasitol* 2006; **137**(3-4): 231-41.
- Gharbi M, Touay A, Khayeche M, Laarif J, Jedidi M, Sassi L, et al. Ranking control options for tropical theileriosis in at-risk dairy cattle in Tunisia, using benefit-cost analysis. *Rev Sci Tech* 2011; **30**(3): 763-78.
- Singh DK. Theileriosis in India. In: Singh DK, Varshney BC, editors. Proceedings of the second EEC workshop on orientation and coordination of research on tropical theileriosis; 1991 Mar 18-22; Anand, India. Anand: National Dairy Development Board; 1991, p. 23-8.
- Minjauw B, McLeod A. Tick-borne diseases and poverty: the impact of ticks and tick-borne disease on the livelihoods of small-scale and marginal livestock owners in India and eastern and southern Africa. Edinburgh: DFID animal health programme, University of Edinburgh; 2003. [Online] Available from: http://r4d.dfid.gov.uk/PDF/Outputs/RLAHTickBorn_Book.pdf [Accessed on 10th March, 2015]
- Gharbi M, Rjeibi MR, Darghouth MA. [Epidemiology of tropical bovine theileriosis (*Theileria annulata* infection) in Tunisia: a review]. *Rev Elev Med Vet Pays Trop* 2014; **67**: 241-7. French.
- Gharbi M, Darghouth MA. Control of tropical theileriosis (*Theileria annulata* infection in cattle) in North Africa. *Asian Pac J Trop Dis* 2015; **5**(7): 505-10.
- Rajendran C, Ray DD. Diagnosis of tropical bovine theileriosis by ELISA with recombinant merozoite surface protein of *Theileria annulata* (Tams1). *J Parasit Dis* 2014; **38**(1): 41-5.
- Mans BJ, Pienaar R, Latif AA. A review of *Theileria* diagnostics and epidemiology. *Int J Parasitol Parasites Wildl* 2015; **4**(1): 104-18.
- Rekik B, Ben Gara A, Ben Hammouda M, Hammami H. Fitting lactation curves of dairy cattle in different types of herds in Tunisia. *Livestock Prod Sci* 2003; **83**(2-3): 309-15.
- Wood PDP. Algebraic model of the lactation curve in cattle. *Nature* 1967; **216**(5111): 164-5.
- M'Barek M. [Impact of tropical theileriosis on milk yield: preliminary estimation in the Lower Valley of Medjerda] [dissertation]. Tunisia: National School of Veterinary Medicine of Sidi Thabet; 1994. French.
- Glass EJ, Preston PM, Springbett A, Craigmile S, Kirvar E, Wilkie G, et al. *Bos taurus* and *Bos indicus* (Sahiwal) calves respond differently to infection with *Theileria annulata* and produce markedly different levels of acute phase proteins. *Int J Parasitol* 2005; **35**(3): 337-47.
- Gharbi M, Darghouth MA. A review of *Hyalomma scupense* (Acari, Ixodidae) in the Maghreb region: from biology to control. *Parasite* 2014; **21**: 2.
- Ben Salem M, Bouraoui R. Milk production and composition of dairy cows raised under landless small-scale dairy system in Tunisia. *Livest Res Rural Dev* 2009; **21**(11): 196.
- Michael SA, el Refaï AH, Mchardy N, Rae DG. Effect of treatment of chronic theileriosis with buparvaquone on milk yields. *Trop Anim Health Prod* 1989; **21**(4): 218-22.