Auto-inoculation of blood for tick control in infested sheep

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SUMMARY
Ticks are blood-sucking ectoparasite of livestock that severely impairs their production. The aim of this study is to report the results of auto-inoculation of blood to tick-infested sheep as a possible tick control method.
Forty naturally infested sheep were selected for the study. The sheep were divided random in two groups: group A and group B (control group). All animals during the shearing period were subjected to the count of the ticks, annotated as T₀ value. All animals of the group A were subjected to the blood sampling. The collected blood was immediately inoculated at the dosage rate of 1 ml/10 kg body weight subcutaneously to the tip of the chest. In subjects of group B was not carried out any treatment.
The ticks count were performed on days 7 (first control: T₁) and 21 (second control: T₂). At the first control the sheep of group A were divided into two sub-groups (group A₁ and A₂); in animals of group A the auto-inoculation of blood was repeated. Statistical analysis (ANOVA) showed the effect of time on the studied parameters, in relation to the treatment (group A₁, A₂, B). A mean reduction of ticks was noticed on day 7 and 21, respectively 47% and 49%, in the sheep belonging to the group A.
In animals that had received a second treatment (group A₁) a higher percentage of reduction of ticks was observed (50%) 2 weeks after the second inoculation.
In our study, the inoculation of blood containing parasitic antigens and specific antibodies in the subcutaneous tissue have likely stimulated the immune system more strongly, resulting in the rejection of a large number of ticks feeding on sheep. Although the sample size is small in the number of sheep enrolled, our data make a contribution to the study of alternative tick control strategies in food animals.

KEY WORDS
Auto-inoculation of blood; sheep; Rhipicephalus bursa; TBPs; ticks control.

INTRODUCTION
Ticks are blood-sucking ectoparasite of livestock that severely impairs their production, with a significant economic impact on animal breeding industry worldwide, causing a variety of deleterious effects in animals, mainly as result of bodyweight reduction, blood loss and the transmission of disease-causing agents, such as deadly protozoan and rickettsial diseases (TBD: Tick Borne Disease)¹;².
The intensive use of chemical acaricides in order to control tick infestation is not sustainable due to increasing tick resistance, blood loss and the transmission of disease-causing agents, such as deadly protozoan and rickettsial diseases (TBD: Tick Borne Disease)¹;².
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So, the development of more effective and sustainable control methods is required as alternative strategy.
Various experimental investigations report the use of a tick vaccine, as an alternative tick control method in livestock¹;⁴.
It has been repeatedly demonstrated that the stimulation of bovine immune system by tick proteins vaccination induces a protective immune response against the same ticks.
Furthermore, various studies showed that the animal diseases can be treated using the so-called autovaccines, or autologous vaccine, today commonly used in veterinary medicine as a therapeutic and preventative treatment of diseases occurring in populations of domestic animals (stable or herd specific vaccine)⁵.
Representative examples of the use of autovaccines in veterinary medicine include the treatment of post partum metritis caused by Actinomyces pyogenes in dairy cows, staphylococcal conjunctivitis in rabbits and ewe mastitis caused by Pasteurella hemolytica. Autovaccines have been also used to treat a diversity of viral infections including infectious kertitis in cattle, canine oral papillomavirus infection in dogs or papilloma virus infection (equine sarcoids) in horses⁶.
On basis of the above mentioned concepts, the aim of this study is to report the results of a preliminary attempt of auto-inoculation of blood to tick-infested sheep in order to demonstrate the possibility of its application in tick control.

MATERIAL AND METHODS
Forty infested Pinzirita sheep, 36 male and 4 female, 2-10 years old, with natural infestation of ticks were selected for the study. The sheep were divided random in two groups: group A with 30 subjected and group B with 10 sheep (control group). All animals during the shearing period were subjected to the count of the ticks located at head, axillar and inguinal areas and perineal region (Figure 1).
The numbers of ticks on each sheep were annotated as T0 value. From 5 infested sheep, not belonging to the experimental groups, 28 ticks were collected and maintained, at room temperature, into tubes containing cotton wool moistened with saline solution until the analysis. The samples were sent to C.R.A.Ba.R.T. (National Reference Center for Anaplasma, Babesia, Rickettsia and Theileria) in order to typing the ticks and to determine the presence of tick borne pathogens (TBPs).

Ticks were microscopically identified using morphological keys of Khoury and Lezzerini in 1980, based on the different morphological characteristics of adanal plates, for males, and genital aperture for females, as described by Estrada-Penä et al. in 2004.

Polymerase Chain Reaction (PCR) analysis was used for detection of Anaplasma spp. and Rickettsia spp. and Reverse Line Blot (RLB) for detection of Babesia/Theileria spp., as previously described.

All animals of the group A were subjected to the blood sampling. The required quantity of fresh blood was directly drawn from the jugular vein using sterilized syringes and needles. The collected blood was immediately inoculated at the dosage rate of 1 ml/10 kg body weight subcutaneously to the tip of the chest (Figure 2). In subjects of group B was not carried out any treatment. The animals were observed for 21 days; the ticks count were performed on days 7 (first control: T1) and 21 (second control: T2).

At the first control the sheep of group A were divided into two sub-groups consisting of 15 subjects (group A1 and A2); in animals of group A2 the auto-inoculation of blood was repeated. All inoculated and control sheep were allowed to graze with the rest of the herd animals, exposing them to equal and uniform chances for tick infestation.

Statistical analysis of the data was performed by applying the two-way Analysis of Variance (ANOVA) for repeated measures in order to evaluate the effect of time on the studied parameters, in relation to the treatment (group A, A1, A2, B). All results were expressed as mean± standard deviation (SD). P value <0.05 was considered statistically significant.

The percentage of efficacy of auto-inoculation of blood in reducing tick infestation was calculated applying the formula: 

\[1 - (T_1 / T_0) \times (C_0 / C_1)\] \times 100, where T0 is pre- and T1 is post-inoculation (first or second control) means of tick count in sheep belonging to group A (A1 and A2), while C0 and C1 are the corresponding means of the control group (B).

RESULTS

Mean and standard deviation of the tick count performed on sheep of A and B groups at the different step of the trial (T0, T1 and T2) and the statistical analysis are reported in Table 1 and in Graphic 1.

The statistical analysis showed a significant effect of time in both experimental groups, in relation to the performed treatment.

The comparison between the means of the ticks number in group A and in group B at baseline (T0) showed no significant differences (p>0.05), demonstrating the homogeneous degree of tick infestation in all animals.

After 7 days from auto-inoculation of blood (1st control) a significant reduction in the number of ticks in the treated animals (group A) was observed (T1 vs T0: p<0.001).

After 21 days, the number of ticks counted in animals treated was further decreased (T2 vs T1: p<0.001), both in the group of animals that had received only one treatment (group A2) and in the group that had received two treatment (group A1). At first control (T1) the comparison between the groups (A vs B) showed a significant decrease of the ticks number (p<0.001).

At second control (T2) statistically significant differences were observed comparing the group A1 and the group A2 versus the group B (p<0.001 and p<0.01 respectively). No

Table 1 - Mean± standard deviation (SD) of ticks number before the trial (T0), at 1 week (T1) and 3 weeks (T2) from the auto-inoculation of blood in treated sheep (Group A1: double treatment; Group A2: single treatment) and in not-treated sheep (Group B).

<table>
<thead>
<tr>
<th>Group</th>
<th>T0</th>
<th>T1</th>
<th>T2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>18.2 ± 9.99</td>
<td>8.15 ± 5.33</td>
<td>6.93 ± 4.29</td>
</tr>
<tr>
<td>Group A1</td>
<td>16.4 ± 8.73</td>
<td>7.92 ± 4.5</td>
<td>6.93 ± 4.29</td>
</tr>
<tr>
<td>Group A2</td>
<td>20 ± 11.43</td>
<td>8.38 ± 6.41</td>
<td>8.62 ± 6.09</td>
</tr>
<tr>
<td>Group B</td>
<td>21.9 ± 13.3</td>
<td>18.7 ± 14.45</td>
<td>18.4 ± 9.31</td>
</tr>
</tbody>
</table>
significant differences (p>0.05) were observed comparing animals subjected to single or double treatment (group A vs group A1).

A mean reduction of ticks was noticed on day 7 and 21, respectively 47% and 49%, in the sheep belonging to the group A. In animals that had received a second treatment (group A1) a higher percentage of reduction of ticks was observed (50%) 2 weeks after the second inoculation.

The morphological examination of ticks, collected at random on some subjects of the flock, allowed the identification of arthropods as feeding or not feeding adults belonging to the family Ixodidae, genus *Rhipicephalus* and species *R. bursa*, both female and male.

On 28 ticks examined by PCR and RLB the following pathogens were isolated: *Babesia/Theileria* spp. on 22 ticks and *Anaplasma* spp. on 6 ticks, as summarized in Table 2.

Ticks observed in animals of group A appeared wrinkled, small in size, pale or bluish and the fully engorged ones were rarely seen.

**DISCUSSION AND CONCLUSION**

The microscopically examination performed on ticks collected from Sicilian sheep showed the presence of single tick specie *Rhipicephalus bursa*, in agreement with what observed in previous studies carried out by Torina et al. in 2006 and in 2010 in Sicily.

Furthermore, all ticks were found infected with TBPs, *Babesia/Theileria* spp. and *Anaplasma* spp., confirming that these pathogens are endemic in Sicily as well as in other regions of Italy and Europe.

The morphological changes of ticks and the reduction of their number noted in the present study may have been influenced by immunological reactions, such as assumed in another study conducted on calves in India.

It is known that sufficient salivary secretion of ticks enters the circulation of infested hosts at every blood meal. The subsequent antigen-antibody reactions determine a partial immunity and the antibodies against the salivary antigens are non-protective in natural conditions, as can be concluded by the persistence of infestation in animals.

In our study, the inoculation of blood containing parasitic antigens and specific antibodies in the subcutaneous tissue have likely stimulated the immune system more strongly, resulting in the rejection of a large number of ticks feeding on sheep.

Recent research conducted on the use of auto-vaccines in animals affected by metritis support this hypothesis, demonstrating that the subcutaneous administration of antigens leads to the activation of immunologic effector mechanisms which contribute to recovery of the diseased animals. In particular, the Authors showed a decrease in activated CD4+ lymphocytes and an increase of specific receptors on T cells, which contribute to recovery of the diseased animals.

The different alternative control strategies against tick infestations demonstrate that the immunization of infested animals using anti-tick vaccines (consisting of recombinant tick proteins as antigens) elicits a variable immune-protection in vaccinated animals, measured by the parasite reproductive potential, including reduction in number and weight of engorging ticks and in egg weight and hatchability. This is in agreement with the results of this study.
We believe that the present study may represent a practical contribution to the study of alternative control method against tick infestation, which has an economic, health and environmental impact.

The immunization by auto-inoculation of blood is a simple and economic method allowing to significantly reducing the parasitic charge, as demonstrated by our results. This technique has several advantages, such as the environmental security, the absence of intolerance reactions which may occur with vaccines and the absence of residues in food. The lack of withdrawal periods allows to not interfering with the productive performance, the main objective of breeding livestock.

Although the sample size is small, particularly in the number of sheep enrolled, our data make a contribution to the study of alternative tick control strategies in food animals, specifically in organic farming, a constantly growing practice.

References