Gastrointestinal strongyles burden monitoring in a flock of Zerasca sheep treated with homeopathy

L. GIULIOTTI1*, F. PISSEI2, P. ROBERTI DI SARSINA3, B. M. AZZARELLO4, G. TERRACCIANO5, M. N. BENVENUTI1

1Department of Veterinary Science, University of Pisa. Viale delle Piagge 2, 56124, Pisa, Italy.
2Veterinarian, CIMI, Roma, Italy.
3Non Conventional Medicine, High Council of Health, Ministry of Health, Italy
4Veterinarian, Auxerre, France.
5Animal Prophylaxis Research Institute for Lazio and Toscana. S.S. dell’Abetone e del Brennero 4, 56123 Pisa, Italy.

* Corresponding author: Email: novella.benvenuti@unipi.it

ABSTRACT
Introduction

The widespread use of conventional drugs in farm animals has resulted in anthelmintic resistance as well as the contamination of deleterious molecules in animal products and in the environment. Researchers are thus focusing on production systems that rely less on chemicals. The aim of this study was to monitor the gastrointestinal strongyle burden, blood count, body condition scores (BCS), and FAffà MAlan CHArt (FAMACHA) in a local Italian breed reared in natural conditions.

Methods

The study was carried out in a farm where homeopathy was applied. Over a one-year period, faeces were sampled six time from ten Zerasca ewes to evaluate the
faecal eggs count with a modified McMaster technique. At the same time, blood samples were collected to evaluate white blood cells, red blood cells, haemoglobin, packed cell volume, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, and red cell distribution width. BCS and FAMACHA were also recorded.

Results
Results showed low parasite levels in most of the samples with the highest value in the spring. Blood parameters were within the normal range, with significant fluctuations during the sampling period. BCS values corresponded to an adequate nutritional condition of the animals and FAMACHA scores did not suggest a worrying state of anaemia.

Conclusions
In this farm, a thorough monitoring of the gastrointestinal parasite burden together with a BCS and FAMACHA evaluation allowed the amount of chemical treatments to be limited, normally administered twice a year without laboratory tests.

**Keywords:** blood count, BCS, FAMACHA, gastrointestinal strongyles, homeopathy, monitoring, sheep, Zerasca breed.

**Introduction**
In small ruminants, breeding management is carried out on extensive systems mainly based on pasture that represents the environment where gastro-intestinal parasites complete their biological cycle. Controlling parasitic diseases is thus important for animal health and welfare. In fact, gastrointestinal parasites are one of the most important challenges for health management in sheep breeding and
can lead to significant losses in productivity, and can even be lethal when infection becomes severe [1]. Gastrointestinal parasitism greatly impairs animal productivity through reduction in voluntary food intake and/or decrease in the efficiency of nourishments, particularly, in the inefficient use and absorption of nutrients. Disturbance in protein metabolism and reduced assimilation and/or retention of minerals are particularly significant. As a consequence, growth, milk, wool production and reproductive efforts could be reduced in parasitized animals [2].

Chemical drugs are broadly applied without laboratory tests. Their abuse has had a great impact on the rural environment and they have become a worrying problem regarding food safety [3;4]. Anthelmintics offer a short-term, cost-effective method of controlling nematodes. These drugs kill existing parasites and reduce eggs production. Unfortunately, reports of anthelmintic resistance date back to the early 1960s, when the modern chemical assault on nematode parasites began [3]. Another imperative for change is the increasing consumer demand for uncontaminated agricultural products [5]. In fact, consumer concern regarding food quality is increasing together with the preference for meat with minimal chemical contamination [6].

Thus, the best approach to tackling endoparasite problems in extensive farming involves the effective management of pastures, the use of breeds well adapted to the environment [7], the use of complementary and alternative medicine (CAM), [8], and the monitoring of the parasite burden. The frequent use of anthelmintic drugs however, is no longer considered sustainable. The use of CAMs is widespread in humans and is increasingly being applied for animal care. The CAMs are based on salutogenic principles, in which the generation of health is consequent to the stimulation of innate self-healing abilities [9].

An ecological approach to parasitosis takes into account the complex relationship among plants, animals and the environment, in fact the parasite is only able to cause damage in the host when an imbalance in the system occurs [8]. The use of CAMs could be driven by an approach towards the system complexity [10].
Medical knowledge and practice should address not only how to treat pathology, but also how to generate health [9].

Homeopathic medicines share a holistic view of the patient-environment relationship taking into account the complexity of the natural phenomena [11]. Recent studies have highlighted the possibility of preventing the chemical treatment of the whole flock by monitoring the faecal egg count (FEC) and using indirect indicators of parasite damage: Body Condition Score (BCS) and FAffA MAlan CHArt (FAMACHA) [12].

A BCS is helpful as an indicator of the nutritional and health status of animals [13]. FAMACHA is a system that measures anaemia on the basis of the color of the lower eyelid mucous membrane in small ruminants, as a morbidity marker for *Haemonchus contortus*. FAMACHA could represent a good tool in targeting selective treatments in order to reduce anthelmintic usage [14;15]. Blood parameters are also important and reliable media for assessing the physiological and health status of animals [16].

The aim of this study was to monitor the gastrointestinal parasite burden together with the anaemic and nutritional status in a flock of a native Italian breed, where homeopathic medicine was applied in the health management, in order to evaluate the real need for anthelmintic treatment that is normally administered without previous laboratory tests.

**Materials and methods**

The study involved the native Zerasca sheep breed, named after the homonymous area (Zeri), located in north western Tuscany at 800 m a.s.l [17]. The flock consisted of 50 sheep reared in extensive natural conditions and fed on grass and shrub pasture with supplementation provided all year. Pasture area was 11 ha managed with rotation based on grass availability. During the night and under unfavourable weather conditions, the animals were kept in a barn with appropriate animal density, good ventilation and dry litter in sufficient quantities. Previous study [2] carried out in Zeri district showed the presence of various *genera* of gastrointestinal strongyles such as Ostertagia, Trichostrongylus,
Oesophagostomum, Haemonchus and Chabertia. Chemical anthelmintic treatment had not been administered in the six months leading up to the beginning of the trial. The study lasted 12 months, from April 2012 to March 2013, and involved an unchanged sample of ten randomly-selected pluriparous ewes, considered a statistically representative sample. During the sampling period all the ewes gave birth; the incidence of twin birth was 40%. The animal care procedure followed the European Directives for the Protection of Experimental Animals (Directive 2010/63/EU).

Unicist homeopathy was applied. This method is based on the application of a single medication which includes the totality of the symptoms and characteristic of the patient.

The homeopathic examination of both farm and animals was performed at the beginning of the study in order to identify the right remedy to administer. Information on the behaviour of the flock, management practices, animal-human interactions and pathological farm history was collected in order to fill the repertory schedule for the appropriate remedies, applying the “similarity principle” [18].

The homeopathic repertory consists of a database that includes the results of treatments used in homeopathic tests. The entire flock was considered as a single individual and the various pathologies encountered were interpreted as the expression of the pathological tendency of the farm. The veterinarian then chose the remedy from the repertory that showed the greatest similarities with the symptoms. The farmer kept the animals inside during bad weather conditions; this situation is most frequent in winter and the animals spend considerable time in the barn, consequently becoming nervous. Some ewes were thin but in general were in good condition; the state of the fleece appeared excellent. At clinical inspection some animals revealed bronchitis or rhinitis, respiratory problems with transparent but abundant mucus, chronic cough and sporadic lameness. These symptoms were used for the repertorisation.
The *Cina* remedy was chosen from the list of the Mac Repertory programme [19]. This remedy appeared to be the most suitable for the characteristics collected [20] due its close analogy with the flock.

*Cina* MK (1000 Korsakovian) was diluted in natural water and administered individually by an oral syringe (5ml/animal) every two weeks for the first two months, then twice during the remaining 10 months.

Faecal and blood samples were collected every two months. Faeces were collected directly from the rectal ampoule and individually examined to estimate the faecal egg count of gastrointestinal nematodes, expressed as eggs per gram (EPG), using a modified McMaster technique [21]. Blood samples were drawn from the jugular vein and a complete blood count was measured using an automated hematology analyser (HeCo SEAC): white blood cells (WBC), red blood cells (RBC), hemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), and red cell distribution width (RDW).

On the same dates, BCS and FAMACHA were measured following the five-point scale method suggested by Russel [22] and Malan [14], respectively. From a visual and tactile examination of the adipose tissue around and on the vertebrae of the lumbar region, BCS attributes the score 1 as emaciated and 5 as obese. FAMACHA attributes the score 1 as optimum and 5 as extremely anaemic, by assessing the colour of the conjunctival membrane.

Statistical analysis of the gastrointestinal strongyles burden was performed by ANOVA with JMP statistical software [23]. The factor included in the model was the date of sampling. Tuckey test for LSD was performed after ANOVA to find the differences between means. Data regarding FECs were logarithmically transformed \[y = \log(\text{EPG} + 25)\] to normalize error [24]. Means and standard deviations were calculated for BCS and FAMACHA. Correlation between FEC and PCV was estimated via Pearson’s correlation. Non-parametric Spearman's *rho* correlations were calculated between FEC, FAMACHA and BCS.
Results and discussion

The overall mean FEC, BCS and FAMACHA were 110±161.35, 2.9±0.38, 2.8±0.46 respectively. The strongyles burden (Table 1) showed a significant variation (P<0.01), in relation to the date of sampling.

FEC output displayed an increase in spring in accordance with “spring rise” [25;26;27]. EPG mean values showed a low level of gastrointestinal strongyle burden, preventing negative repercussions on welfare, productivity, and health of animals, which are impaired at over 600 EPG. Only a few ewes at the last sampling got close to the level of infestation corresponding to a high risk for animal health (>600 EPG) [28].

The obtained mean FEC values were lower than those reported in previous studies conducted in two different farms of Zerasca sheep raised under similar condition and not chemically treated (533 and 360 EPG ) [29; 30].

The values of BCS close to 3 appeared to be ideal to ensure nutritional and metabolic welfare, while a score below 2 would indicate a susceptibility to metabolic imbalances [13].

FAMACHA values scored in the tested ewes below 3 during the monitoring seemed to avoid problems of anaemia, since this value is considered as borderline [31]. FEC correlations between BCS and FAMACHA were not significant.

The low level of infestation during the study was likely responsible for the lack of significative correlation with BCS and FAMACHA.

Table 1. EPG, BCS and FAMACHA recorded in the sampled period.

Data on blood count revealed values within the normal range, suggesting adequate conditions of the flock and an absence of noticeable health problems (Table 2). WBC, Hgb, MCH, Plt showed significant (P<0.01) fluctuation during the sampling period, however the overall parameters were within the normal range.

FEC and PCV showed a significant negative correlation (r= -0.3409). These results, due to the fact that PCV values were in the physiological range, could indicate that no damage related to parasitism was found.
Table 2. Blood parameters recorded in the sampling period.

CONCLUSIONS
This study highlighted that the thorough monitoring of parasites can be used as a valid tool in controlling drug use, thus safeguarding animal health, welfare and productions. The control of the nutritional and anaemic status of the sheep by the BCS, complete blood count and FAMACHA could be an integration of the information on the health status of the flock; in the farm practice it would be advantageous limiting the frequency of the samplings to the period of higher risk of infestation. The sensitivity towards the consequence of the abuse of chemical molecules should motivate the veterinarian in arranging new alternative strategies in agreement with the farmer. Infact, a sustainable control of gastrointestinal parasites, such as the selective treatments, could be of great value to safeguard animal health and beneficial to the environment. Based on this study, twice a year routine deworming is not needed to maintain the health of the animals.

Authors: all research done by the authors.
Financial support: research supported by an ARSIA (Tuscany Region) grant. The homeopathic remedy was provided by UNDA (Cemon, Naples, Italy). The authors declare no conflicts of interest.

References


Table 1. EPG, BCS and FAMACHA recorded in the sampling period.

<table>
<thead>
<tr>
<th>Date of sampling</th>
<th>EPG</th>
<th>BCS</th>
<th>FAMACHA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mean</td>
<td>s.d.</td>
<td>mean</td>
</tr>
<tr>
<td>April</td>
<td>160A</td>
<td>151.4</td>
<td>2.9</td>
</tr>
<tr>
<td>July</td>
<td>32B</td>
<td>55.9</td>
<td>2.9</td>
</tr>
<tr>
<td>September</td>
<td>36B</td>
<td>63.8</td>
<td>2.9</td>
</tr>
<tr>
<td>November</td>
<td>58B</td>
<td>53.3</td>
<td>2.9</td>
</tr>
<tr>
<td>January</td>
<td>115B</td>
<td>91.8</td>
<td>2.6</td>
</tr>
<tr>
<td>March</td>
<td>268A</td>
<td>276.5</td>
<td>3.0</td>
</tr>
</tbody>
</table>

A,B:P<0.01
<table>
<thead>
<tr>
<th>Parameter</th>
<th>April</th>
<th></th>
<th>July</th>
<th></th>
<th>September</th>
<th></th>
<th>November</th>
<th></th>
<th>January</th>
<th></th>
<th>March</th>
<th></th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mean</td>
<td>s.d.</td>
<td>mean</td>
<td>s.d.</td>
<td>mean</td>
<td>s.d.</td>
<td>mean</td>
<td>s.d.</td>
<td>mean</td>
<td>s.d.</td>
<td>mean</td>
<td>s.d.</td>
<td></td>
</tr>
<tr>
<td>WBC (K/μL)</td>
<td>9.1B</td>
<td>1.90</td>
<td>13.3A</td>
<td>3.73</td>
<td>9.4B</td>
<td>3.32</td>
<td>7.1B</td>
<td>1.72</td>
<td>7.9B</td>
<td>2.25</td>
<td>7.9B</td>
<td>2.00</td>
<td>5.2-13.9</td>
</tr>
<tr>
<td>RBC (M/μL)</td>
<td>8.8</td>
<td>1.26</td>
<td>8.9</td>
<td>1.12</td>
<td>8.5</td>
<td>0.90</td>
<td>8.7</td>
<td>1.04</td>
<td>9.1</td>
<td>0.45</td>
<td>7.9</td>
<td>0.80</td>
<td>6.9-10.9</td>
</tr>
<tr>
<td>Hb (g/dL)</td>
<td>9.4BC</td>
<td>1.17</td>
<td>9.4BC</td>
<td>0.89</td>
<td>9.0C</td>
<td>0.85</td>
<td>10.3AB</td>
<td>1.21</td>
<td>10.6A</td>
<td>0.76</td>
<td>9.3</td>
<td>0.93</td>
<td>7.4-11.8</td>
</tr>
<tr>
<td>PCV (%)</td>
<td>28.3</td>
<td>3.98</td>
<td>28.4</td>
<td>2.83</td>
<td>27.2</td>
<td>2.88</td>
<td>29.3</td>
<td>3.85</td>
<td>29.4</td>
<td>2.32</td>
<td>25.5</td>
<td>3.01</td>
<td>22.0-34.2</td>
</tr>
<tr>
<td>MCV (fL)</td>
<td>32.1</td>
<td>1.66</td>
<td>32.1</td>
<td>1.91</td>
<td>32.2</td>
<td>2.20</td>
<td>33.6</td>
<td>1.81</td>
<td>32.3</td>
<td>2.29</td>
<td>32.3</td>
<td>1.64</td>
<td>28.4-37.0</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>10.7CB</td>
<td>0.52</td>
<td>10.6D</td>
<td>0.57</td>
<td>10.6D</td>
<td>0.71</td>
<td>11.8A</td>
<td>0.55</td>
<td>11.6AB</td>
<td>0.73</td>
<td>11.2BC</td>
<td>0.61</td>
<td>9.4-12.4</td>
</tr>
<tr>
<td>MCHC (%)</td>
<td>33.3C</td>
<td>0.93</td>
<td>33.1C</td>
<td>0.77</td>
<td>32.9C</td>
<td>0.61</td>
<td>35.2B</td>
<td>0.61</td>
<td>35.9A</td>
<td>0.82</td>
<td>34.8B</td>
<td>0.87</td>
<td>30.4-36.3</td>
</tr>
<tr>
<td>RDW (%)</td>
<td>16.0</td>
<td>0.31</td>
<td>16.2</td>
<td>0.48</td>
<td>16.1</td>
<td>0.50</td>
<td>16.1</td>
<td>0.40</td>
<td>15.7</td>
<td>0.28</td>
<td>15.9</td>
<td>0.67</td>
<td>15.1-17.2</td>
</tr>
<tr>
<td>Plt (K/μL)</td>
<td>167.1AB</td>
<td>83.3</td>
<td>198.9A</td>
<td>64.71</td>
<td>123.2B</td>
<td>57.78</td>
<td>128.4B</td>
<td>58.89</td>
<td>206.3A</td>
<td>39.33</td>
<td>170.7AB</td>
<td>56.53</td>
<td>107.6-324.8</td>
</tr>
</tbody>
</table>

A,B, C, D: P<0.01