Serum and saliva antibody isotypes against *Haemonchus contortus* in Żelaźnieńska and Wrzosówka sheep naturally infected with gastrointestinal nematodes over 2004 and 2005 grazing seasons

**Abstract**

Gastrointestinal nematodes from family *Trichostrongylidae* are probably the most important parasites of small ruminants worldwide, causing significant morbidity and loss of production. Also in Poland these parasites cause important health problems of sheep. The immune response of two breeds of sheep – Wrzosówka (Polish Heath Sheep) and Żelaźnieńska (Polish Lowland Sheep) to somatic antigens of gastrointestinal nematode *Haemonchus contortus* were compared. Female sheep of both breeds were kept in the same conditions. Faecal probes were collected four times during the grazing season, and then examined using flotation and sedimentation methods. Serum and saliva samples were collected at the beginning and the end of grazing season. The levels of both serum and saliva IgA, IgG and IgM antibodies reacting with antigens of *H. contortus* were estimated using ELISA method. The Wrzosówka Sheep have shown lower prevalence of infection and lower number of nematode eggs in faeces than the Żelaźnieńska Sheep. Negative correlations between the level of all isotypes of serum and saliva antibodies and prevalence of invasion (also with a number of eggs in faeces) have been found in both breeds of sheep.

**Key Words**: sheep, gastrointestinal nematodes, antibody isotypes

**Introduction**

Nematodes from superfamily *Trichostrongylidea* (including families *Trichostrongylidae, Haemonchidae* and *Cooperidae*) invade predominantly ruminants like cattle and sheep, and some species can parasitise in horses or rodents. The most common (and most important) in Poland are: *Trichostrongylus sp., Nematodirus sp., Haemonchus contortus* and *Teladorsagia circumcincta*.

Gastrointestinal nematodes are probably the most important parasites of small ruminants worldwide, causing significant morbidity and loss of production. Also in Poland these parasites can be the reason of serious veterinary problems. They can be treated by anthelmintic chemotherapy, however treatment is costly and drug resistance has evolved in all major gastrointestinal nematode species (ROOS, 1997). Because of this and the growing concentration of pesticides in the environment and in food, it is becoming very important to develop other ways of controlling nematode infections in sheep. One option is selective breeding for a reduction in faecal egg counts following natural infection (BISSET et al., 1996). Another idea is to identify the genetic basis for naturally occurring resistance of some breed of sheep against nematode infections. It is known that some breeds of sheep like Red Massai Sheep in East Africa or Polish Longwool (BAKER, 1995; BOUIX et al., 1998) are more resistant than others. NOWOSAD et al. (2003) and GORSKI et al. (2004) found significant differences in prevalence of some parasitic infections among various sheep breeds in Poland. Some of these breeds are typical Polish, rather not raised in other countries. Wrzosówka (Polish Heath Sheep) is a primitive, small fur breed of north short-tailed sheep groups. Sheep of this breed are very well adapted to the difficult environmental conditions,
resistant to diseases and unseasonal. There were many studies undertaken on this old breed, concerning its immune response to infections of gastrointestinal nematodes (MOSKWA, 1999, 2000; MOSKWA et al., 2002). The second breed – Żelaźnieńska sheep (Polish Lowland Sheep) was developed from the Polish Merino crossed with the Leicester Longwool and Łowicz sheep.

The most important aim of our studies was to compare humoral immune response of these two breeds of sheep (kept in the same conditions) to *Haemonchus contortus* somatic antigens. We also observed the changes in level of serum and saliva antibodies in both breeds during the grazing season.

**Materials and Methods**

The study was carried out on the Polish Wrzosówka sheep and the Żelaźnieńska sheep maintained on a sheep farm in Żelazna (central Poland) and run by the Department of Sheep and Goat Breeding, Faculty of Animal Sciences, Warsaw Agricultural University. All animals were over 2-year-old ewes. In 2004 the flock of about 70 Żelaźnieńska sheep was investigated, and 22 to 27 specimens of this breed in 2005. The Wrzosówka sheep (from 30 to 40 ewes) were examined only in 2005. The sheep from this farm were treated with levamisol before and after (Żelaźnieńska sheep), or only after (Wrzosówka sheep) the grazing season.

Faecal samples were collected from the rectum of all ewes four times in the year – at the beginning (before treatment with anthelmintic) of grazing season (April or May), in June, August and at the end of season (September or October). The prevalence of gastrointestinal nematodes invasion was estimated using saturated NaCl flotation method. Faecal egg counts per gram of faeces (EPG) were made from a one-g sample of faeces, using the modified flotation method described previously (DOLIGALSKA et al., 1997).

Blood samples were collected by jugular venepuncture using 9ml evacuated tubes (SARSTEDT Monovette, EDTA KE/9ml). Two times in the year (before and after grazing season) Saliva samples were collected from mouth of sheep by sponges also two times yearly. Serum and saliva samples were stored at -20°C before used. Somatic antigens of adult (both sexes) *Haemonchus contortus* stages in concentration 5 µg/ml were used in ELISA tests. IgA, IgM and IgG isotypes of serum and saliva antibodies were detected using HRP-labelled anti-sheep antibodies (ROCKLAND for IgM and IgG, and EIVAI BOIS Laboratories for IgA), and the absorbance was determined using MRX plate reader (Dynamech Laboratories) with the 450 nm filters.

**Results**

The prevalence of infection with gastrointestinal nematodes was increasing during the grazing season in both breeds. In the Żelaźnieńska sheep it was growing from 29.4% to 97.1% in 2004 and from 92% to 100% in 2005. The prevalence was lower in the Wrzosówka sheep and it was increasing from 57.5% to 93.8%. Also the egg number per gram of faeces connected with the prevalence was significantly lower in the Wrzosówka sheep than in Żelaźnieńska. This parameter for the Żelaźnieńska sheep increased from 0.86 to 18.14 in 2004 and from 7.55 to 84.86 in 2005. In Wrzosówka the number of eggs per gram of faeces increased from 1.26 to 23.75 (Fig. 1).
Fig. 1: Changes in number of gastrointestinal nematode eggs in 1 gram of faeces of Zelaznienska and Wrzosówka Sheep. EPG = Eggs Per Gram.

Both examined breeds developed the humoral immune response to somatic nematode antigens. Negative correlation between the level of all antibody isotypes (in serum and saliva) and the faecal egg count (Table 1) was observed.

Table 1
Correlation between the level of serum and saliva antibodies and the faecal egg count.

<table>
<thead>
<tr>
<th>Year</th>
<th>Antibodies (Optical Density)</th>
<th>Eggs per gram of faeces</th>
</tr>
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<tbody>
<tr>
<td>2004</td>
<td>Zelaźnieńska sheep</td>
<td>Wrzosówka sheep</td>
</tr>
<tr>
<td>IgA</td>
<td>Serum 0.105 - 0.05</td>
<td>IgA Serum 0.06 - 0.01</td>
</tr>
<tr>
<td></td>
<td>Saliva 0.2 - 0.1</td>
<td>Saliva 0.16 - 0.0</td>
</tr>
<tr>
<td>IgG</td>
<td>Serum 1.17 - 1.09</td>
<td>IgG Serum 0.42 - 0.41</td>
</tr>
<tr>
<td></td>
<td>Saliva 0.3 - 0.2</td>
<td>Saliva 0.21 - 0.17</td>
</tr>
<tr>
<td>IgM</td>
<td>Serum 1.05 - 0.7</td>
<td>IgM Serum 1.1 - 0.4</td>
</tr>
<tr>
<td></td>
<td>Saliva 0.04 - 0.07</td>
<td>Saliva 0.04 - 0.16</td>
</tr>
</tbody>
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<table>
<thead>
<tr>
<th>Year</th>
<th>Antibodies (Optical Density)</th>
<th>Eggs per gram of faeces</th>
</tr>
</thead>
<tbody>
<tr>
<td>2005</td>
<td>Zelaźnieńska sheep</td>
<td>Wrzosówka sheep</td>
</tr>
<tr>
<td>IgA</td>
<td>Serum 0.05 - 0.003</td>
<td>IgA Serum 0.06 - 0.01</td>
</tr>
<tr>
<td></td>
<td>Saliva 1.4 - 0.5</td>
<td>Saliva 1.6 - 0.0</td>
</tr>
<tr>
<td>IgG</td>
<td>Serum 0.7 - 0.4</td>
<td>IgG Serum 0.21 - 0.17</td>
</tr>
<tr>
<td></td>
<td>Saliva 0.04 - 0.07</td>
<td>Saliva 0.04 - 0.16</td>
</tr>
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</table>

Also, both breeds showed the decreasing level of the serum and saliva of all antibody isotypes during the season. The only exception was IgM in saliva which increased from Optical Density 0.04 to 0.07 or 0.16 in both breeds. In all the remaining cases the Wrzosówka sheep have shown the lower level of antibodies than Zelaźnieńska ones. ELISA results are shown in Figs. 2, 3, 4 and 5.
Fig. 2: Serum antibodies of Zelaznienska Sheep reacting with *Haemonchus contortus* somatic antigens. OD = Optical Density. Sampling I – before, Sample II – after grazing season

Fig. 3: Saliva antibodies of Zelaznienska Sheep reacting with *Haemonchus contortus* somatic antigens. OD = Optical Density. Sampling I – before, Sample II – after grazing season

Fig. 4: Serum antibodies of Wrzosowka Sheep reacting with *Haemonchus contortus* somatic antigens. OD = Optical Density. Sampling I – before, Sample II – after grazing season
Discussion
The faecal egg count increased during the grazing season and the highest was at the beginning of autumn in both breeds. Similar phenomenon was observed in 1997 and 1998 in the Wrzosówka flock maintained at the same farm in Żelazna in Central Poland. Otherwise in 1996 the number of eggs per gram of faeces was higher in the middle of the season (MOSKWA, 1999). The author of these studies has found a considerable variation in faecal egg counts in different years (however, in general the numbers of eggs per gram of faeces were higher than found in our studies), so we cannot treat our results as invariable phenomenon.

We found negative correlation between the level of antibodies and faecal egg counts. It is well known phenomenon typical for gastrointestinal nematode infections (BAKER et al., 1996; DOUCH et al., 1996; MOSKWA, 1999). Sheep grazing on the pasture are exposed to natural gastrointestinal nematode infections and acquire resistance slowly, during the grazing season. The state of immunity can be assessed by measuring the number of nematode eggs passed by infected sheep. A reduction of the number of nematode eggs in faeces is associated with the development of acquired immunity against nematode infection. Because serum antibody level may be negatively correlated with faecal egg counts, the measurement of specific antibody production in infected animals may provide another useful selection criterion and be used to complement faecal egg counts (MOSKWA et al., 2002).

In both the Żelaźnińska and Wrzosówka sheep the IgM level in saliva increased during the season, whereas the level of other isotypes decreased. Probably the reason of increasing of saliva IgM level was summer infection with *Haemonchus contortus* infective larvae not many days before the second sampling.

The level of serum and saliva antibodies was somewhat higher in the Wrzosówka than Żelaźnienska sheep, but similar. Because of lower prevalence and lower number of nematode eggs in faeces of Wrzosówka, proportionally the humoral response of this breed is stronger and probably more effective. Some studies have already established the importance of host genotype on resistance of host to parasite infection (STEAR et al., 1995). Some primitive, old breeds of sheep are known to be more resistant to gastrointestinal nematode infections. It was confirmed in such breeds as the African
Red Maasai and Djallonke sheep (BAKER, 1995), or Scottish Soay sheep (COLTMAN et al., 2001). In the last case the authors have shown that resistance to gastrointestinal nematode infections of naturally parasitized animals is associated with the microsatellite polymorphism in gamma interferon gene. Summarising, it can be noticed that it is possible to certify that Wrzosówka, primitive breed of sheep is more resistant to gastrointestinal nematode infections than the highly selected Żelaźnieńska sheep, however further studies on that problem should be undertaken in more seasons.

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