Genetic resistance of three genotypes of goats to experimental infection with *Haemonchus contortus*

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Abstract

A total of 46 weaned kids of three genotypes aged about 4–5 months were used to evaluate the effects of trickle infection with a sheep strain of *Haemonchus contortus*. A completely randomized 3 × 2 factorial design was used. Factors were genotype (Thai native (TN), 75% TN × 25% Anglo-Nubian (AN) and 50% TN × 50% AN) and parasite (control and infected). The animals were infected with 750 infective larvae (L3) of *H. contortus* three times a week for 3 weeks, with a total of 6750 larvae. The experiment lasted 9 weeks. Each week animals were weighed, faecal egg counts done and blood examined for haematological and biochemical variables. Twenty-seven kids were slaughtered at the end of experiment for worm recovery. Weight gain of infected animals was lower than those of uninfected controls (P < 0.05). The genotype 50% TN × 50% AN had higher growth rate than TN and 75% TN × 25% AN genotypes (P < 0.05). Eggs per gram of faeces (EPG) were significantly higher in 50% TN × 50% AN kids than in TN (P < 0.0005) and 75% TN × 25% AN (P < 0.0001) kids. There was a large variation in the EPG of individual animals within a genotype. The percent establishment of L3 was 8.2% in TN, 16.97% in 50% TN × 50% AN and 17.91% in 75% TN × 25% AN kids. TN kids had worm counts lower than 50% TN × 50% AN (P < 0.05) and 75% TN × 25% AN (P = 0.07) kids. Infection had a significant effect on packed cell volume (PCV), haemoglobin, total protein and albumin. The decrease in the level of these blood parameters was less in TN kids than in 50% TN × 50% AN and 75% TN × 25% AN kids. There was no significant difference between genotypes in the values of total and differential leucocyte counts and mean corpuscular volume (MCV). It can be concluded that TN goats are more resistant to *H. contortus* than 50% TN × 50% AN.
AN goats. However, under the present experimental conditions, the liveweight gain of 50% TN × 50% AN was higher than the TN goats.

Keywords: Haemonchus contortus; Goat; Genetic resistance; Thailand

1. Introduction

Gastro-intestinal nematodes, and particularly the stomach worm *Haemonchus contortus*, are a major constraint in small ruminants production in the humid tropics in South East Asia and elsewhere (Kochapakdee et al., 1991; Pandey and Sivaraj, 1994; Dorny et al., 1995; Romjali et al., 1996b). Due to favourable climatic conditions, the transmission of parasites occurs throughout most of the year. Current control practices rely heavily upon chemotherapy, which leads to the development of anthelmintic resistance in parasite populations (Pandey and Sivaraj, 1994; Sivaraj and Pandey, 1994; Sivaraj et al., 1994; Kochapakdee et al., 1995).

Comparative studies have shown that goats are more susceptible to gastro-intestinal nematodes than sheep (Le Jambre and Royal, 1976; Le Jambre, 1984; Pomroy et al., 1986). Recommended drug dosage to goats are the same as for sheep but due to differences in the pharmacokinetics of drugs between sheep and goats, the anthelmintics are less efficacious in goats and may lead to rapid selection of anthelmintic resistant worms (Sangaster et al., 1991). Thus, due to a higher susceptibility to infection and the possibility of rapid development of anthelmintic resistance, goats may even pose a threat to sheep where both species are grazed together, as is the case in many countries.

Considerable effort has recently been devoted to searching for alternative or complementary methods to chemotherapeutic control of nematodes. Breeding animals with higher genetic resistance is one such alternative (Gray, 1991; Pandey et al., 1994).

Studies on genetic variation in resistance to nematodes and its exploitation for selective breeding for resistant animals has been reported mainly for sheep (Woolaston, 1990; Woolaston et al., 1991; Baker et al., 1991; Gray et al., 1995; Romjali et al., 1996b). Less work has been done on goats. Studies in Kenya with *H. contortus* (Preston and Allonby, 1978; Shavulimo et al., 1988; Waruiru et al., 1994) and in France with gastro-intestinal strongyles (Richard et al., 1990) have shown the existence of genetic variation in susceptibility of nematode infections in goats. However, in a study in Fiji Woolaston et al. (1991) found very little genetic variation in mixed *H. contortus* and *Trichostrongylus colubriformis* infections in goats.

Thai native (TN) goats, which are raised for meat, are small in size. The exotic Anglo-Nubian (AN) breed has been introduced for cross breeding with a view to producing a genotype with larger body size and increased productivity. Cross-breds with different degrees of AN blood, have been produced. Although some studies on Thai native and their AN crosses have been conducted on goats on pastures (Kochapakdee et al., 1993; Pralomkarn et al., 1994), there is no information available on the comparative susceptibility of these genotypes under controlled experimental conditions. Under the framework of a larger study on genetic resistance of goats to gastro-intestinal nematodes, the present work was carried out to study and to compare the response of TN,
75% TN × 25% AN and 50% TN × 50% AN goats to repeated (trickle) infection with *H. contortus*.

2. **Materials and methods**

2.1. **Animals and their management**

   The study was conducted at the Small Ruminant Research and Development Centre farm, Hat Yai, Southern Thailand. Female kids of three genotypes, namely TN, 50% TN × 50% AN, 75% TN × 25% AN, were used in the experiment. The cross-bred kids were from the third generation cross (F3). From birth till weaning at 12 weeks of age, the kids ran on pastures with their mothers. At weaning all kids were moved to a large raised slatted-floor pen. They were treated with ivermectin to remove nematode infections. As a few animals were still positive for gastro-intestinal nematodes on faecal examination 10 days following ivermectin administration, they were treated again with levamisole. Further faecal examinations showed the absence of gastro-intestinal nematode eggs. All the kids were vaccinated against foot and mouth disease (Type A.O. and Asia 1) and haemorrhagic septicaemia. The animals were fed clean grass (*Pennisetum purpureum*) or grass and legume (*Brachiaria mutica, Centrosema pubescens, Stylosanthes hamata, cv Verano*) cut from a fenced area not grazed by animals and presumed to be free from nematode infection. They were also offered a concentrate diet at 1.5% body weight once a day. The concentrate was composed of corn, palm kernel cake, soyabean meal, broken rice, ground oyster shell salt, calcium phosphate and had the crude protein content of 16.25% DM. Animals were provided with access to water ad libitum. The pen was cleaned daily to avoid accidental auto infection.

2.2. **Parasite**

   Third stage infective larvae (L3) of *Haemonchus contortus* were obtained from the Institute of Animal Production, Sei Putih, North Sumatra, Indonesia. This strain was isolated from sheep at Sei Putih in 1993 and has been passaged once through clean lambs. The larvae were stored at 10°C until used. Viability of larvae was ascertained by microscopic examination and at each dosing of larvae, the number of living larvae was counted. The larvae were administered orally, using a syringe.

2.3. **Experimental design**

   The design was a 3 × 2 factorial in completely randomized design. Treatments were genotypes (TN, 75% TN × 25% AN, 50% TN × 50% AN) and parasite infection (infected and uninfected controls). The number of animals in each treatment group is shown in Table 1. The kids were infected with 750 L3 of *H. contortus* three times a week for 3 weeks with a total of 6750 L3 given to each animal of infected group. The experiment lasted for 9 weeks. At Day 63 a total of 27 kids (two control animals of each genotype and eight, five and eight of infected animals of TN, 75% TN × 25% AN and 50% TN × 50% AN respectively) were slaughtered for worm counts.
Table 1
Experimental protocol and number of goats of three genotypes used in the study. Infected group was given 750 larvae of *Haemonchus contortus* three times a week for 3 weeks.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Treated animals</th>
<th>Necropsied</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Infected</td>
<td>Control</td>
</tr>
<tr>
<td></td>
<td>Infected</td>
<td>Control</td>
</tr>
<tr>
<td>TN</td>
<td>12</td>
<td>6</td>
</tr>
<tr>
<td>75% TN× 25% AN</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td>50% TN× 50% AN</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td>Total</td>
<td>28</td>
<td>18</td>
</tr>
</tbody>
</table>

TN, Thai native; AN, Anglo-Nubian.

Each week throughout the experiment, animals were weighed, faecal samples collected for counting worm eggs and blood samples taken for haematological and biochemical examination.

2.4. Blood examination

Every week all kids were bled from the jugular vein and blood collected in tubes with EDTA as anticoagulant and in tubes without anticoagulant. Packed cell volume (PCV) was measured by microhaematocrit method. The concentration of haemoglobin and total leucocyte counts were measured by an automatic cell counter (Baker 8000, US Summit, USA). The differential leucocyte counts were done on Wright-stained blood smears. The total serum protein and albumin were measured by an auto-analyser (Hitachi-704, Boehringer Mannheim Ltd, Germany).

2.5. Faecal examination

Faecal samples from all kids were collected once a week at the time of weighing. Faecal egg counts were determined by the McMaster method with the precision of each egg counted representing 50 EPG of faeces.

Table 2
Live weight gain of goats according to genotype and infection with *Haemonchus contortus*.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Least square means ± SE (kg)</th>
<th>(g kg$^{0.75}$ day$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Infected</td>
<td>Control</td>
</tr>
<tr>
<td>TN</td>
<td>3.99 ± 0.79</td>
<td>7.50 ± 1.11</td>
</tr>
<tr>
<td>75% TN× 25% AN</td>
<td>3.85 ± 0.96</td>
<td>6.07 ± 1.11</td>
</tr>
<tr>
<td>50% TN× 50% AN</td>
<td>5.99 ± 0.96</td>
<td>8.73 ± 1.11</td>
</tr>
</tbody>
</table>

TN, Thai native; AN, Anglo-Nubian.

$^{ab}$ Means within rows and columns with differing superscripts differ significantly ($P < 0.05$).
Table 3
Least square means and standard error of faecal egg counts (log_{10} + 1) of three genotypes of kids over a period of 4–9 weeks

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Least square mean</th>
<th>Standard error</th>
</tr>
</thead>
<tbody>
<tr>
<td>TN</td>
<td>1.94 a</td>
<td>0.16</td>
</tr>
<tr>
<td>75% TN × 25% AN</td>
<td>1.67 a</td>
<td>0.20</td>
</tr>
<tr>
<td>50% TN × 50% AN</td>
<td>2.98 b</td>
<td>0.20</td>
</tr>
</tbody>
</table>

TN, Thai native; AN, Anglo-Nubian.

a,b Means within column with differing superscripts differ significantly (P < 0.0005).

2.6. Worm counts at necropsy

After slaughter of kids, the abomasum was separated, opened and its contents emptied into a container. The mucosa was thoroughly washed and the washings added to the contents. The whole sample was preserved in 5% formaline. The abomasum was then digested in warm normal saline at 37°C for 6 h and thoroughly washed. The washings were preserved in 5% formaline. A 10% aliquot sample of contents, washings and digestion was searched for nematodes. If no worms were found, then a second 10% sample was examined.

2.7. Statistical analysis

Values of EPG and worm counts were normalized by log_{10} (EPG or worm count + 1) transformation before statistical analysis. Data were analyzed using least-squares procedures (Statistical Analysis Systems Institute Inc., 1987) and treatments were compared using analysis of variance.

3. Results

3.1. Body weight

Weight gain was influenced significantly by the genotype (P < 0.05) and infection (P < 0.01). Infected animals in all genotypes gained less weight than their controls.

Table 4
Worm burden of goats at necropsy on Day 63 of infection

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Least square mean ± SE</th>
<th>Arithmetic mean</th>
<th>Establishment of L3 (range) %</th>
</tr>
</thead>
<tbody>
<tr>
<td>TN (n = 8)</td>
<td>3.167 ± 0.294 a</td>
<td>488</td>
<td>8.2 (2.8--19.9)</td>
</tr>
<tr>
<td>50% TN × 50% AN (n = 5)</td>
<td>4.207 ± 0.294 b</td>
<td>1146</td>
<td>16.97 (8--24)</td>
</tr>
<tr>
<td>75% TN × 25% AN (n = 8)</td>
<td>4.072 ± 0.372 a,b</td>
<td>1209</td>
<td>17.91 (13.5--22.6)</td>
</tr>
</tbody>
</table>

TN, Thai native; AN, Anglo-Nubian.
a,b Means within column with differing superscripts differ significantly (P < 0.5).
Table 5
Least square means ± standard error of packed cell volume, haemoglobin, total protein and albumin of *Haemonchus contortus* infected and control goats.

<table>
<thead>
<tr>
<th>Week</th>
<th>PCV (%)</th>
<th>Haemoglobin (g dl⁻¹)</th>
<th>Total protein (g dl⁻¹)</th>
<th>Albumin (g dl⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Infected</td>
<td>Control</td>
<td>Infected</td>
</tr>
<tr>
<td>0</td>
<td>27.28±1.37</td>
<td>28.41±1.13</td>
<td>8.46±0.39</td>
<td>8.85±0.32</td>
</tr>
<tr>
<td>1</td>
<td>25.06±1.14</td>
<td>26.89±0.93</td>
<td>7.69±0.34</td>
<td>8.25±0.27</td>
</tr>
<tr>
<td>2</td>
<td>25.56±1.12</td>
<td>26.81±0.91</td>
<td>8.19±0.30</td>
<td>8.44±0.24</td>
</tr>
<tr>
<td>3</td>
<td>24.92±1.05</td>
<td>23.69±0.86</td>
<td>7.96±0.36</td>
<td>7.56±0.30</td>
</tr>
<tr>
<td>4</td>
<td>26.78±1.04</td>
<td>23.52±0.85</td>
<td>8.03±0.34</td>
<td>7.31±0.28</td>
</tr>
<tr>
<td>5</td>
<td>27.39±0.95</td>
<td>24.72±0.78</td>
<td>8.64±0.30</td>
<td>7.71±0.25</td>
</tr>
<tr>
<td>6</td>
<td>26.67±1.81</td>
<td>26.06±1.49</td>
<td>8.88±0.34</td>
<td>8.17±0.28</td>
</tr>
<tr>
<td>7</td>
<td>27.94±0.94</td>
<td>26.43±0.77</td>
<td>9.17±0.30</td>
<td>8.54±0.25</td>
</tr>
<tr>
<td>8</td>
<td>27.72±0.92</td>
<td>24.79±0.75</td>
<td>8.76±0.26</td>
<td>7.88±0.21</td>
</tr>
<tr>
<td>9</td>
<td>29.73±1.28</td>
<td>26.67±1.03</td>
<td>9.30±0.41</td>
<td>8.27±0.33</td>
</tr>
</tbody>
</table>

PCV, packed cell volume.
*, P < 0.05; **, P < 0.01.
(P < 0.005) (Table 2). TN and 75% TN × 25% AN kids had similar weight gains but 50% TN × 50% AN kids had higher weight gains than these two genotypes.

3.2. Faecal egg counts

First eggs in the faeces were detected at Week 4 of experiment in eight of 28 infected animals. Peak egg counts were found between Weeks 7 and 9. The peak of weekly mean EPG was 250 at Week 9 for TN, 413 at Week 8 for 75% TN × 25% AN and 2550 at Week 7 for 50% TN × 50% AN goats. Mean EPG of individual animals over six samplings during the patent period of infection varied in the range 33–800 for TN, 0–258 for 75% TN × 25% AN and 408–2192 for 50% TN × 50% AN goats. Analysis of egg counts showed no difference between TN and 75% TN × 25% AN goats, but the egg counts of 50% TN × 50% AN were significantly higher than the TN (P < 0.0005) and 75% TN × 25% AN (P < 0.0001) (Table 3).

![Graph showing changes in packed cell volume (PCV), haemoglobin (Hgb), total protein and albumin over time for different genotypes](image-url)

Fig. 1. Change in packed cell volume (PCV), haemoglobin (Hgb), total protein and albumin on time of Thai native (TN), 75% Thai native × 25% Anglo-Nubian (AN25%), and 50% Thai native × 50% Anglo-Nubian (AN50%) goats following experimental infection with repeated doses of *Haemonchus contortus*. Data shown are percent change in infected groups compared to the uninfected controls within a genotype.
3.3. Worm counts

Results of the worm counts are presented in Table 4. Five out of six necropsied control animals had negligible numbers (1–20 worms) of *H. contortus*. TN had a worm burden lower than 50% TN × 50% AN (*P* < 0.05) and 75% TN × 25% AN (*P* = 0.07) goats. The percentage of larvae recovered as adult worms at necropsy was lower (8.2%) in TN compared with 50% TN × 50% AN (16.9%) and 75% TN × 25% AN (17.91%) goats.

3.4. Blood findings

Infection had a significant effect on PCV, haemoglobin, total protein and albumin at some weeks of the experiments (Table 5). Compared to uninfected controls, infected animals had significantly lower PCV on Weeks 4, 5 and 8 (*P* < 0.05), lower haemoglobin on Weeks 5 and 8 (*P* < 0.05), lower protein on Weeks 4, 5 (*P* < 0.01), 6, 7 and 8 (*P* < 0.05) and lower albumin on Weeks 5, 6, 7, 8 and 9 (*P* < 0.01).

As the normal values of PCV, haemoglobin, protein and albumin before infection were different in three genotypes, to evaluate the effect of infection, the infected animals of each genotype were compared to their respective controls at each sampling time and results expressed as a percentage change from controls (Fig. 1). In general, the changes in all four parameters due to *H. contortus* infection were similar in 50% TN × 50% AN and 75% TN × 25% AN; TN goats had invariably less reduction due to infection than the other two genotypes. Fig. 2 presents the overall change in infected animals of all three genotypes taken together, compared to all uninfected controls. There was no significant difference between genotypes in the values of leucocyte, polymorphonuclears, lymphocytes and mean corpuscular volume (MCV).

4. Discussion

The results of this study have shown a genotype effect to the trickle infection by *H. contortus*. Another feature observed was a large variation among goats within and
between genotypes in the parasitological variables, even though the animals were matched for genotype, sex and age.

The body weight of animals in all groups increased during the experimental period, which indicates that the dose of 6750 larvae of *H. contortus* given as a trickle infection was not high enough to stop the weight gain, but was able only to induce a reduction in the weight gain of infected animals compared to their controls matched for genotypes. AN is a heavier breed than TN and has a higher growth potential. Kochapakdee et al. (1995) examined the effect of genotype and internal parasites on growth rates of goats under Thai village conditions and found that TN goats had significantly lower growth rates than the 50% TN × 50% AN goats. Results of the current study confirm their findings. However, in another study on weaned kids on pastures with similar level of supplementation as in the present study, Pralomkarn et al. (1994) found that TN goats gained more weight than 50% TN × 50% AN between 12-24 weeks of age.

In a comparative study of goat derived and sheep derived strains of *H. contortus*, Rahman and Collins (1991) found that the weight loss was significantly important compared to uninfected controls only in goats infected with goat derived strain but not in those infected with sheep derived strain. The strain of *H. contortus* used in the present study was isolated from sheep where goats were not grazed with sheep. In the present study, the sheep derived strain of *H. contortus* induced 36–64% reduction in weight gain of weaner kids (Table 2) at a dose level of infective larvae comparable to Rahman and Collins (1991), which indicates that sheep derived strain of *H. contortus* is pathogenic to goats.

Low faecal egg counts (Table 3), low worm counts at necropsy and low establishment rate of L3 (Table 4) confirm the higher resistance of TN over 50% TN × 50% AN goats. These results also showed that 25% AN blood does not induce significant advantage over pure TN goats. Breed or genotype effect on egg count and worm burden of goats has been shown by Preston and Allonby (1978) and Shavulimo et al. (1988) for East African, Galla and Saanen breeds in Kenya and by Richard et al. (1990) for French breeds. More extensive studies on the effect of genotype/breed have been done in sheep. (Courtney et al., 1985, Gamble and Zajac, 1992; Romjlali et al., 1996a,b).

Besides variation between the genotypes, individual variations in parasitological variables within the genotypes were well pronounced. The mean EPG of animals was overdispersed. Overdispersion of parasitological variables in nematode infections has been observed in several host-parasite systems and much of this variation is under genetic control (Wakelin, 1985; Wassom et al., 1986; Wakelin and Blackwell, 1988; Sreter et al., 1994). Using the same strain of *H. contortus*, the current study has found extensive variations in egg counts in four genotypes of sheep given trickle infection (Romjlali et al., 1996a). Such variations have two main implications. Firstly, the animals can be identified as resistant or susceptible to *H. contortus* for a selective breeding programme. Secondly, selective treatment of individuals with high egg counts may be done without resorting to mass treatment of a whole flock. This may lead to less pressure on worms, leading to reduction of risk of development of anthelmintic resistant worms.

Decrease in PCV, haemoglobin, total protein and albumin, was observed in infected animals, compared to the controls between Weeks 4 and 9 of infection (Fig. 2). The
period of significant change in blood parameters is related to the maturity of worms following infection during the first 3 weeks of the experiment. Anaemia and hypoproteinaemia are known to be the features of *H. contortus* infection (Rahman and Collins, 1990; Rahman and Collins, 1991). During the course of infection, TN goats exhibited less change in blood parameters than their crosses with AN.

In conclusion, TN goats were found to be more resistant to *H. contortus* for parasitological and blood parameters as they had lower EPG, lower worm counts and low reduction in blood values compared to their AN crosses. This may be due to the evolution of TN goats in the environment where *H. contortus* is an important parasite. AN are a recent introduction to Thailand and have evolved in the region where *H. contortus* is not a major problem. Taken together, the weight gain and parasitological and blood parameters, it appears that although 50% TN × 50% AN have higher worm burdens, they are not less resilient than TN. In fact, their weight gain was higher than TN and 75% TN × 25% AN goats. For improvement of local goats, 25% AN blood does not provide any advantage. If other constraints, such as nutrition and health can be controlled, it may be useful to improve the TN goats by cross breeding with AN or selective breeding within TN population. More information and economic analysis would be needed before deciding upon the option for village farmers.

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