References


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Benzimidazole Resistance in Gastrointestinal Nematodes of Sheep and Goats

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Abstract
The occurrence of anthelmintic resistance in gastrointestinal nematodes of sheep and goat flocks of organized farms and small holder flocks was investigated using *in vitro* egg hatch assay for TBZ anthelmintic. Resistance to TBZ was detected in many of the sheep and goat flocks of both institutional farms and small holder flocks. *Haemonchus contortus* was the predominant nematode species in both pre and post treatment faecal cultures. In both institutional farms and small holder flocks, where, TBZ resistance was detected, the ED₅₀ values for BZ in the EHA were higher than 0.1 μg BZ/ml indicating resistance. It was concluded that anthelmintic

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resistance was reported among sheep and goats of institutional farms and small holder flocks.

**Key words:** Anthelmintic resistance, Egg hatch assay (EHA), Sheep and goats, Benzimidazole (TBZ)

The growing importance of anthelmintic resistance in gastrointestinal nematodes has led to an increased demand for reliable and standardized detection methods (Coles et al., 1992). The most widely used method for detecting and monitoring the presence of anthelmintic resistance in nematodes is the faecal egg count reduction test (FECRT) which is suitable for all types of anthelmintics including those undergo metabolism in the host. In addition, the *in vitro* egg hatch assay (EHA) is employed for detecting anthelmintic resistance. Hence, this technique may prove useful for correlating the results obtained in *in vivo* and *in vitro* assay for declaration of resistance in nematodes.

**Materials and Methods**

The study was carried out on sheep and goats in three institutional farms viz., Veterinary College and Research Institute (VC&RI), Namakkal, Mecheri Sheep Research Station (MSRS), Pottaneri and Sheep Breeding Research Station (SBRS), Sandynallah in Nilgiris district and smallholders flock in four taluks of Namakkal district. Regular and rotational deworming of sheep and goats was undertaken once in 3 months in institutional farms. The smallholder’s flocks were reported to be regularly drenched for deworming once in 4-6 months.

Egg hatch assay was performed in 24 well plates as per the method described by Jackson et al., (2001). Pooled faecal sample from respective farms was collected and egg suspension was made. A 100 μl of egg suspension (with approximately 100 eggs) was added to the wells in the test plate. The thiabendazole working stock solution of 10 μl (0.05, 0.1, 0.3, 0.7 and 0.9 μg/ml) was added to 5 wells in each drug concentration followed by the addition of 1890 μl of distilled water to make a total volume of 2 ml in each well. The sixth well which was free of thiabendazole was served as the control. The plate was then incubated at 26 °C for 48 hr. Following incubation, one drop of helminthological iodine was added to each well and the larvae and unhatched eggs were counted using an inverted tissue culture microscope.

The mean number of eggs and larvae at each concentration was calculated and percentage hatch was derived using the following formula.

\[
\text{Percentage hatch} = \frac{\text{Number of eggs + Number of larvae}}{\text{Number of larvae}} \times 100
\]

The percentage of hatch for each concentration was calculated and the results were subjected to Probit analysis to obtain ED\(_{50}\) values. Those ED\(_{50}\) values above 0.1 μg TBZ/ml were considered to be resistant.

**Results and Discussion**

The percentage of egg hatch and the ED\(_{50}\) values for samples collected from sheep and goats in institutional farms and smallholder flocks are given in Table I. Results of EHA indicate that BZ resistant nematodes were present in sheep in all the three institutional farms and in goats in two institutional farms. Smallholder flock declared resistant by FECRT showed ED\(_{50}\) values above 0.1 μg TBZ/ml and similarly flocks declared susceptible by FECRT showed ED\(_{50}\) values below 0.1 μg TBZ/ml. Hence, the results obtained in FECRT correlate well with the results of EHA, excepting for two sheep flocks declared as suspected resistance. In these flocks, the ED\(_{50}\) values were very low viz., 0.0076 and -0.1501 clearly indicating susceptibility to TBZ. Similarly, a goat flock at Namakkal taluk, declared to be suspected resistant by FECRT showed ED\(_{50}\) values of 0.0357 μg TBZ/ml (<0.1 μg TBZ/ml) indicating susceptibility to BZ. There is no corresponding ED\(_{50}\) value for suspected resistant animals as in the case of FECRT. Hence, FECRT and EHA showed disagreements in this study. In Northwestern Tamil Nadu, Easwaran (2004) detected BZ resistant nematodes in sheep at SBRS and in sheep and goats at VC&RI which is in accordance with the results obtained in the present study. In Northern Tamil Nadu, BZ resistant nematodes were detected using EHA by earlier workers (Meenakshisundaram, 1999; Lourderaj, 2005). In other parts of India,
<table>
<thead>
<tr>
<th>Sl. No</th>
<th>Farms/smallholder flocks</th>
<th>Concentration of thiacetazzone (µg/ml)</th>
<th>Sheep flock</th>
<th>Goats flock</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0.05</td>
<td>0.1</td>
<td>0.3</td>
</tr>
<tr>
<td>1.</td>
<td>VC&amp;RI</td>
<td>83.0</td>
<td>68.0</td>
<td>63.0</td>
</tr>
<tr>
<td>2.</td>
<td>MSRS</td>
<td>75.0</td>
<td>68.0</td>
<td>60.0</td>
</tr>
<tr>
<td>3.</td>
<td>SBRS</td>
<td>83.5</td>
<td>83.0</td>
<td>66.0</td>
</tr>
<tr>
<td>4.</td>
<td>Namakkal taluk</td>
<td>29.0</td>
<td>11.0</td>
<td>3.5</td>
</tr>
<tr>
<td>5.</td>
<td>Rasipuram taluk</td>
<td>91.5</td>
<td>91.0</td>
<td>85.0</td>
</tr>
<tr>
<td>6.</td>
<td>Tiruchengode taluk</td>
<td>28.0</td>
<td>11.0</td>
<td>5.5</td>
</tr>
<tr>
<td>7.</td>
<td>Paramathi taluk</td>
<td>34.5</td>
<td>15.0</td>
<td>8.5</td>
</tr>
</tbody>
</table>

BZ resistant nematodes have been identified (Swarankar et al., 1999; Dhanalakshmi et al., 2003) using EHA and FECRT and with good degree of correlation.

**Summary**

The TBZ anthelmintic resistance among gastrointestinal nematodes of sheep and goat flocks of organized farms and small holder flocks was investigated using in vitro egg hatch assay. TBZ resistance was detected in many of the sheep and goat flocks of institutional farms and small holder flocks. In both institutional farms and small holder flocks, where, TBZ resistance was detected, the ED₅₀ values for TBZ resistant sheep and goats were above 0.1 µg BZ/ml and ranging from 0.5300 to 1.1734 in sheep and 0.5967 to 1.0737 µg BZ/ml in goats. *Haemonchus contortus* was the predominant nematode species in both pre and post treatment faecal cultures. It was concluded that anthelmintic resistance was reported among sheep and goats of institutional farms and small holder flocks.

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**References**


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Studies on Isolation and Identification of Microbial Agents from Cases of Mastitis in Cattle*

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Abstract

The present study was aimed to isolate and identify causative microbial agents of mastitis in cows. Total 220 milk samples (100 from subclinical cases and 120 from clinically affected cows) were collected from Durg and Rajnandgaon district of Chhattisgarh State and processed further to assess the incidence of mastitis. Prevalence of clinical and subclinical mastitis was 30% and 25% respectively in dairy cows. Of 60 isolates obtained from 220 milk samples, Staphylococcus aureus occupied prime position both in clinical (76.67%) and subclinical cases (80%) of mastitis followed by E.coli. Other pathogens isolated from mastitis cases were Candida spp. (6.67%), Streptococcus spp. (3.33%) and Aspergillus spp. (3.33%).

Key Words: Mastitis, Prevalence, Cow, Staphylococcus aureus.

*Part of M.V.Sc. Thesis by the first author

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Bovine mastitis is an important disease that affects animal health and production together with public health. It has sub-stantial economical implications on the dairy industry and is still a major challenge despite the widespread implementation of mastitis control strategies. Many bacterial and fungal species were incriminated as causative agents of bovine mastitis (Moroni et al., 2006). The clinical mastitis can be easily diagnosed by obvious clinical symptoms however, diagnosis of subclinical mastitis (SCM) requires indirect tests like Modified California Mastitis Test (MCMT) and somatic cell count (SCC) along with the microbiological examination that determines the mastitic pathogens (Dhakal, 2006). So keeping into view, the present study was aimed to isolate and identify microbial agents from cases of mastitis in cows.

Materials and Methods

The lactating cows from different dairy...