IN VITRO DETECTION OF BENZIMIDAZOLE RESISTANCE OF GASTROINTESTINAL NEMATODES IN GOATS*

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Anthelmintic resistance is initially suspected when a flock exhibit poor clinical response to treatment. Often resistance is not diagnosed and continued use of the same anthelmintic group increases the frequency of individuals until there is a major failure of control. Le Jambre (1976) used Egg Hatch Test (EHT) as a widespread application to detect benzimidazole resistance. This was followed by Cawthorne and Cheong (1984), Taylor and Hunt (1988), Swarnkar *et al.*(1999) and several others . The EHT is well validated and provides a more quantitative estimate of benzimidazole resistance. Although it was thiabendazole resistance that was detected by the above workers, Coles *et al.* (1992) have recomme nded that resistance to other benzimidazole compounds could also be detected by EHT. The present paper confirms the level of resistance to albendazole among gastrointestinal nematodes in an organized goat farm.

The procedure adopted for Egg Hatch Test (EHT) was according to Coles *et al.* (1992) with some modification. Fecal samples from 10 randomly selected kids aged six months, maintained in the Kerala Agricultural University Goat Farm Mannuthy were collected and brought to the laboratory within three hours.

Copro culture: Fecal samples of the selected goats were pooled and cultured to identify the nematode larvae (Sathianesan and Peter, 1970)

Preparation of eggs:

1. Homogenized the faecal samples with a stirrer by placing the faeces in a measuring cylinder with 200 ml water, until all the pellets were broken.

2. Sieved and poured the filtrate into centrifuge tubes

3. Centrifuged for two minutes at 3000 rpm and gently poured off the supernatant.

4. Agitated the tubes to loosen the sediment and then added saturated sodium chloride solution until a meniscus formed above the tube. Applied a cover slip and re centrifuged for two min at 3000 rpm

5. Carefully removed the cover slips from the tubes and washed off the eggs into a conical centrifuge tube. Filled with water and centrifuged for two min again at 3000 rpm.

6. Removed the water, re suspended the eggs in water, estimated the number of eggs per milliliter and diluted to the required concentration.

Test Procedure:

Placed two ml of fresh medium containing about 25 eggs per ml from each of the 10 samples in each well of a 24 multiwell plate (Medox agencies, Chennai). Added 10µl of serial concentrations of albendazole such as 0.05µg, 0.1µg, 0.15µg, 0.18µg and 0.20µg in aqueous hydrochloric acid to each well to find out the optimum dilution required to prevent 50 per cent of the visible eggs hatching (ED50). Control wells received only solvent. Incubated at 27 ºC for 48 hours and counted the eggs and hatched out larvae at each anthelmintic concentration of each sample microscopically, after adding two drops of Lugol's iodine to stop further hatching.

Calculation:

The mean per cent hatch inhibition was calculated from the mean per cent hatch of the respective albendazole concentration as per the formula given below.

Mean % hatch inhibition =100-(Mean %hatch (respective albendazole concentration)÷ (Mean % hatch (respective water control) x100

The per cent of eggs failing to hatch

Table 1. Anthelmintic resistance by Egg Hatch Test

Animal No.	Albendazole concentrations(µg/ml)					
	Water	0.05	0.1	0.15	0.18	0.20
	% hatch	% hatch	% hatch	% hatch	% hatch	% hatch
1	80	40	46	50	30	20
2	70	60	44	38	30	20
3	64	46	42	36	34	18
4	80	62	56	50	44	38
5	76	78	56	48	50	50
6	68	80	76	58	48	42
7	78	86	78	76	58	56
8	66	80	76	74	70	60
9	76	80	78	76	70	58
10	70	88	78	70	66	58

Table 2. Calculation for ED50 value of albendazole.

Mean hatch		Mean hatch inhibition	Probit values
Water	72.8		
0.05	70	3.9	3.2376
0.1	63	13.46	3.8969
0.15	57.6	20.88	4.1901
0.18	43	40.93	4.7699
0.20	42	42.31	4.8058

at each concentration of albendazole were transferred to obtain probit values (Finney, 1971) from which the ED_{50} values were obtained using "Trend" in a statistical analysis (Bauer, 2005).

Interpretation:

Animals in which the ED_{50} value was in excess of 0.1µg albendazole were considered to carry benzimidazole resistant strains of nematodes. The larvae identified by coproculture were those of *Haemonchus, Oesophagostomum, Bunostomum and Strongyloides spp.*

The results of EHT are furnished in the tables 1 & 2. The ED_{50} value of albendazole (ìg/ml) was found to be 0.211556 which exceeded the prescribed value of 0.1 ìg/ml of thiabendazole. Hence the albendazole resistance is hereby confirmed by EHT. These findings concur with those of Cawthorne and Cheong (1984), Taylor and Hunt (1988) and Swarnkar *et al.* (1999). This albendazole resistance must be due to prolonged and continuous use of the drug in the farm. The level of anthelmintic resistance should be closely monitored and maintained in a low profile resorting to suitable detection methods. Anthelmintic resistance is best controlled in the ground level, by the use of correct type of

drugs at the correct dose against GI nematodes in goats. Breeding for worm resistance, development of vaccines and biological control using nematophagous fungi are the prospective control measures against anthelmintic resistance.

Summary

Specific resistance to benzimidazole group in gastro intestinal nematodes of goats was carried out by Egg hatch test. Albendazole was the drug used and the test revealed ED50 value (µg/ml) to be 0.211556 which was much higher than the prescribed value (0.1µg/ml). EHT gives a quantitative estimate of benzimidazole resistance and may be used in field surveys along with FECRT to monitor the development of anthelmintic resistance.

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