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In Vivo and *In Vitro* Detection of Anthelmintic Resistance Against Gastrointestinal Nematodes in Sheep

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Abstract

The present study was carried out to know the resistance against the drug fenbendazole by *in vivo* and *in vitro* method of detection in four sheep farms located in Bangalore and Tumkur districts of Karnataka. Sheep which were not dewormed for the past 2-3 months in a herd and with minimum of 150-200 egg per gram (EPG) count before treatment were selected for the current study. The less value of lower 95% confidence limit indicated the resistance to fenbendazole detected by *in vivo* test and the ED50 values and LD50 values are more than 0.1 µg/ml and also percentage paralysis at different time intervals indicates the existence of higher number of resistant worms to the drug fenbendazole.

Key words: Gastrointestinal nematode, sheep, anthelmintic resistance.

Parasitic gastroenteritis, dominated by haemonchosis, is one of the major constraints to profitable sheep production in most parts of the tropical and subtropical world (Sanyal, 2009). It is indisputable that *Haemonchus contortus* is the most notorious parasite in livestock due to its biotic potential and blood sucking ability (Getachew *et al*, 2007). The present work was therefore undertaken to screen the gastrointestinal nematodes in sheep to know the development of resistance against the drug fenbendazole, one of the commonly used anthelmintic.

Materials and Methods

The sheep flocks from four farms located in different parts of Karnataka *viz.*, KVAFSU sheep farm, Hebbal, Bangalore (farm I); LRIC sheep farm, Konnehalli, Tumkur (farm II); Bangalore veterinary hospital (farm III) and Tumkur veterinary hospital (farm IV) were managed in semi-intensive system of grazing on pasture. In each farm 60 infected sheep were divided into two groups of 30 animals each. Group-1 was treated with the drug fenbendazole (7.5 mg/kg BW) and group-2 served as control. 10g of faeces was collected on the day of treatment and on 12^{th} day of post-treatment and EPG was determined by modified McMaster method. The *in vitro* tests were run in triplicates. Fenbendazole with DMSO was serially diluted with water to obtain concentrations ranging from 0.05 to 20 µg/ml. Eggs were recovered from faecal sample and screened for resistance by Egg Hatch Assay (EHA) as described by Coles *et al.,* (1992). The third stage larvae were identified. Larval developmental assay (LDA) was performed as per Hubert and Kerboeuf (1992). The numbers of live larvae were calculated by estimating the percentage recovery in control wells and LD50 value was determined. For Larval paralysis assay (LPA), 25-30 infective larvae were pipetted into wells with 1ml of 1mM eserine (Sutherland *et al.*, 1990). The immobile and mobile larvalcounts were determined.

Results and Discussion

Coproculture studies revealed the mixed infection with existence of five different species of stronygle larvae viz. *Haemonchus contortus, Trichostrongylus sp., Oesophagostomum sp., Bunostomum sp.,* and *Cooperia sp.* with the higher percentage of *Haemonchus contortus* in all the four sheep farms under study.

The drug fenbendazole reduced the faecal egg count by 69%, 75%, 80% and 77% in farm I, farm II, farm III and farm IV with lower 95% confidence limit of 33, 61, 76 and

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Table I: Faecal egg count reductions calculated on pre and post anthelmintic treatment.

Farms	Mean pre treatment FEC		Mean post treatment FEC		Percent reduction		Lower 95% confidence limit		Upper 95% confidence limit		Results	
	G 1	G 2	G 1	G 2	G 1	G 2	G 1	G 2	G 1	G 2	G 1	G 2
I	900	780	550	1010	69	-	33	-	85	-	R	-
II	900	920	450	1340	75	-	61	-	92	-	R	-
III	1040	670	210	980	80	-	76	-	87	-	R	-
IV	1000	940	300	1400	77	-	65	-	84	-	R	-

G1- group treated with fenbendazole G2- control group

65% respectively (Table I) which indicates that strongyles developed the resistance against this anthelmintic. The lower efficacy and resistance to fenbendazole might be caused due to their continuous and prolonged use in controlling gastrointestinal nematodes in these farms.

The ED50 values obtained after probit analysis for fenbendazole by means of EHA ranged between 0.24 to 1.56µg/ml in all the farms demonstrating the presence of benzimidazole resistant nematodes (Table II). The LD50 values obtained after probit analysis for fenbendazole by means of LDA ranged between 0.3 to 1.10µg/ml in all the farms indicating the resistance. In the present investigation the ED50 and the LD50 values in all farms were higher than 0.1µg/ml which indicates the development of resistance to fenbendazole in all the four farms. The percentage of infective stage larvae immobile in 1mM eserine with different time interval is represented in table II, although there was a significant difference in percentage, the values indicate the existence of resistance.

Reports of *H. contortus* (Yadav *et al*., 1992; Gill, 1996; Swarnkar *et al*., 1999; Varady*et al,* 2009) resistant to benzimidazole or to levamizole in sheep have been published in India. The prevalence of nematodes resistant to benzimidazole in sheep in Karnataka was first reported by Dhanalakshmi *et al*., (2003). These investigations reveal the resistance by *H. contortus, Trichostrongylus sp., Oesophagostomum sp., Bunostomum sp.,* and *Cooperia sp.* to the drug fenbendazole in farms of Bangalore and Tumkur districts of Karnataka.

Table II: ED50 value in egg hatch assay, LD50 value in larval developmental assay and the percentage paralysis in larval paralysis assay in four farms for the drug fenbendazole

Farms	EHA (ED50) in µg/ml	LDA(LD50) in µg/ml	Percentage paralysis at different time intervals.			
			15 min	30 min	45 min	60 min
I	0.24 ± 0.03	0.3 ± 0.13	10.92	36.2	49	54.03*
II	0.95 ± 0.038	1.03 ± 0.04	5.95	28.65	42.27	60.05*
III	0.38 ± 0.23	0.9 ± 0.017	8.43	31.42	43.70	63.63*
IV	0.56 ± 0.06	1.10 ± 0.12	7.69	23.81	41.09	52.15*

* Indicates the existence of higher number of resistant worm population

Summary

The lower 95% confidence limit of < 90 indicates the resistance by *in vivo* method. The EHA values ranged from 0.24 - 1.56µg/ml, LDA was 0.3-1.10µg/ml and percentage paralysis of immobile larvae indicated resistance by *in vitro* methods, necessitating the immediate need to control resistance by advocating the alteration in usage of anthelmintics against strongyles of sheep in the places mentioned.

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The Prevalence of Brucellosis in Goat and Sheep Milk Samples in Duhok District

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Abstract

The aim of present study was to detect *Brucella* spp. in the milk samples of sheep and goat from Duhok (North Iraq) by enzyme-linked immunosorbent assay (i-ELISA). Eighty milk samples were collected from markets in different areas of the Duhok District including Akre, Zakho, Sumeil and Duhok city. By milk-ELISA, 61.25% of milk samples were found to be positive for Brucella antibody.

Key words: Brucellosis, ELISA, sheep milk, goat milk

Brucellosis, also known as "undulant fever", "Mediterranean fever" or "Malta fever" is a zoonosis and the infection is almost invariably transmitted by direct or indirect contact with infected animals or their products. It effects people of all age groups and of both sexes (Brooks *et al.*, 2010). Although there has been great progress in controlling the disease in many countries, there still remain regions where the infection persists in domestic animals and transmission to the human population frequently occurs. It is an important human disease in many parts of the world especially in the Mediterranean countries and Middle East,

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