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# Detection of anthelmintic resistance in gastrointestinal strongyles of cattle in Thrissur district, Kerala#

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**Abstract**

*Gastro intestinal parasite (GIP) infection is an economically important disease that affects cattle population worldwide and contributes to decreased productivity and profitability in the livestock sector. An important obstacle to the management of helminth infections in cattle is the failure of therapy owing to anthelmintic resistance. The present study is aimed to identify the predominant gastrointestinal strongyles in cattle of Thrissur district, Kerala and to determine the incidence of benzimidazole resistance against these gastrointestinal strongyles in organised cattle farms and small holder farmers' herd. Coproculture studies revealed that predominant strongyle was Mecistocirrus spp. (29.75 per cent) followed by Haemonchus spp. (21.5 per cent) and Oesophagostomum spp. (13 per cent). The in vivo method of benzimidazole resistance detection by faecal egg count reduction test (FECRT) with albendazole was done in three organised cattle farms and three small holder farmers' herd. The per cent FEC reduction in three organised farms were 38, 55 and 85 per cent and that of small holder farmers' herd ranged between 75 - 100. This study forms the first report of anthelmintic resistance in cattle in south India and the results indicated that benzimidazole resistance was highly progressed in organised cattle farms and in a progression state in small holder farmers' herd that necessitates the judicial utilisation of anthelmintics for the parasite control.*

**Key words**: *Benzimidazole resistance, FECRT, coproculture, cattle*

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Parasitic diseases are one of the major production limiting diseases of grazing ruminants globally. Among these, gastrointestinal helminths play a major role in reduction of productivity by lowering milk yield, body weight and growth and by increasing the cost of treatment and mortality (Lashari *et al.,* 2011). Epidemiological reports of the Animal Husbandry Department of Kerala revealed strongylosis to be a major infection accounting for 33.36 per cent of the parasitic infections (AHD, 2008).

Benzimidazoles represent a major class of broad spectrum anthelmintic that have been used widely since 1960s for strongylosis control in ruminants and companion animals. However, the indiscriminate use of these anthelmintics has resulted in development of resistance to one or more number of anthelmintics in many countries (Maingi *et al*., 1998; Silvestre and Cabaret, 2002; Paraud *et al*., 2009). In India, there are several reports of anthelmintic resistance from different states in goats which have been reviewed by several researchers (Dhanalakshmi *et al*., 2003; Jeyathilakan *et al*., 2003; Deepa and Devada, 2007 and Rajagopal *et al*., 2018) whereas only a few reports exists in cattle. The apparent lack of AR nematodes in cattle probably may be due to management systems used with most cattle and the lack of surveys for resistance (Coles, 2002). In the current scenario anthelmintic resistance has become a serious threat making the majority of anthelmintic groups inefficient to control the gastrointestinal parasitism.

Coproculture technique is widely used technique for recovering different species of strongyle larvae and hence specific species identification of the strongyle is possible with coproculture method (Dalbin *et al*., 2018). This information is invaluable for the diagnosis and specific treatment of strongyle infections in grazing cattle. Anthelmintic chemotherapy remains the mainstay of helminth control programmes. So, it is crucial to maintain the efficacy of currently available broad-spectrum anthelmintics and to monitor the effectiveness of these compounds using reliable methods in the field. Faecal egg count reduction test (FECRT) is the most widely used method for detecting and monitoring the presence of anthelmintic resistance in nematodes which is suitable for all types of anthelmintics including those that undergo metabolism in the host. So, the present study aims to assess the level of incidence of benzimidazole resistance using the gold standard *in vivo* detection method, FECRT among organised cattle farms and small holder farmers' herd in and around Thrissur.

## Materials and methods

### *Sample collection and processing for coproculture*

The faecal samples from cattle farms of Thrissur district, Kerala were collected per rectum and were kept for coproculture before storing in refrigerator at 4°C. Coproculture was done on pooled faecal samples from each farm by Modified Veglia's method.

The procedure described by Sathianesan and Peter (1979) was followed for coproculture. The pooled faecal samples were homogenised using mortar and pestle after adding sufficient water to provide optimum moisture for culture. The samples were then transferred to clean and dry transparent plastic bottles without soiling the sides. The culture bottles were closed and kept in darkness at room temperature for seven to 10 days. The infective $L_3$ were collected from the condensation droplets adhering to the sides of the culture bottles after adding one or two drops of water. The bottles were held horizontally and rotated and the fluid aspirated using a Pasteur pipette without touching the faecal pad. The larval suspension was then stored at 4°C for species identification.

### *Morphological identification of $L_3$ larvae*

A drop of larval suspension was mixed with a drop of 1 per cent iodine solution on a glass slide. A cover slip was placed over it and the larvae examined under the 40 or 100X magnification. The infective $L_3$ were identified morphologically based on the keys provided by Van Wyk and Mayhew (2013). The length of the tail sheath extension (distance which the tail sheath extends beyond the caudal tip of the larva) as related to that of *T. colubriformis*

and the proportion of the whip like filament to the total sheath length were the two criteria considered for identification. The length of the tail sheath extension in *T. colubriformis* (30µm) was considered to be 1.0 'X', *H. contortus* to be 2.0 -2.7'X', *H. placei* was 2.7 - 4.0'X' and *O. radiatum* was 5.4 'X'. The proportion of the filament to the total sheath length was 10-15 per cent in *H. contortus,* ±20 per cent in *H. placei*, and ±40-45 per cent in *O. radiatum*.

***Faecal egg count reduction test (FECRT)***

The *in vivo* detection method, FECRT was used for determination of anthelmintic resistance against albendazole. Animals that were not exposed to any benzimidazole group of anthelmintics for a period of eight to twelve weeks and were positive for strongyle infection with epg > 200 were selected from three organised cattle farms and three small holder farmers' herd in Thrissur district.

At day zero, faecal samples were collected directly from rectum of each animal. The samples were examined for strongyle infection. The strongyle positive animals were given albendazole at the dose rate of 10mg/ kg body weight orally. Post treatment samples were collected after 10 days. Faecal egg counts of the pre and post treatment samples were determined by McMaster technique (Coles *et al*., 2006) with some modifications.

In organised farms, the per cent faecal egg count reduction is calculated by the formula:

$$\text{FECRT per cent} = \frac{(\text{Pre-treatment FEC} - \text{Post treatment FEC}) \times 100}{\text{Pre-treatment FEC}}$$

The per cent egg count reduction and 95 per cent confidence limits was calculated using statistical programme, RESO (Version 2) (Martin and Wursthom, 1991). Resistance was concluded to be present if, the per cent reduction in egg count was less than 95 per cent and lower 95 per cent confidence limit was less than 90 per cent. If any one of these criteria alone was met, then resistance was suspected.

In small holder farmers' herd, faecal egg count reduction test was carried out without the control group and the faecal egg count reduction percentage was calculated from the mean pre-treatment and post-treatment egg counts as per the formula.

$$\text{FECR per cent} = 100\ [1- (\tfrac{T2}{T1})]$$

(Kochapakdee *et al*., 1995) where T1 and T2 were the mean pre-treatment and post-treatment egg counts respectively.

**Results and discussion**

***Coproculture***

The faecal samples were subjected to coproculture for recovering strongyle larvae. *Mecistocirrus* spp. (29.75 per cent) was the most predominant strongyle larvae present followed by *Haemonchus* spp. (21.5 per cent) and *Oesophagostomum* spp. (13 per cent). The species of *Haemonchus* were identified by the presence of sharp kink in the tail sheath just posterior to the end of the tail and the tail sheath enclosed a fine whip like filament. The buccal capsule of *Haemonchus* spp. was found to be globular (Fig. 1). The $L_3$ of *Mecistocirrus* spp. could be differentiated by an inverted 'U' shaped structure at the anterior and its simple tail with absence of kink at the posterior end (Fig. 2). The characteristic feature of the infective larvae of *Oesophagostomum* spp. is the relatively long STEs (Fig. 3). Coproculture examination in cattle of India was reported by Panwar *et al*. (2018), Renwal *et al.* (2017), Nath *et al*. (2015) where the predominant strongyle species observed was *Haemonchus* spp. Previous reports from Kerala (Dalbin *et al.*, 2018) also suggested that predominant strongyle species in cattle were *Haemonchus* spp. (13.66 %) followed by

**Fig. 1.** Infective $L_3$ of *Haemonchus* spp.

**Fig. 2.** Infective $L_3$ of *Mecistocirrus* spp.

**Fig. 3.** Infective $L_3$ of *Oesophagostomum* spp.

*Mecistocirrus* spp. (8%) *Trichostrongylus* spp. (3.33%), *Bunostomum* spp. (2%) and *Cooperia* spp. (1.33%). The baseline data on coproculture studies for prevalence of strongyle species in dairy cattle generated in this study would be of immense help in formulating control strategies against strongyle infections in cattle of the state.

***Benzimidazole resistance in organised cattle farms***

Faecal egg count reduction test was carried out in three organised cattle farms with EPG greater than 200 to determine benzimidazole resistance. The FECR per cent in three organised farms were 38, 55 and 85 per cent and the lower 95 per cent CL were 0, 40 and 10, respectively (Table 1). All the farms were found to be resistant with FECR per cent less than 95 per cent and lower 95 per cent CL less than 90 per cent. Similar observations of benzimidazole resistance in organised cattle farms in Argentina was reported by Mejia *et al*. (2003), in Brazil by das Neves *et al*. (2014) and in Bangladesh by Rahman *et al.* (2018). Similarly in India, Choudhury *et al*. (2018) reported FECR per cent ranging from 90 to 100 which indicated resistance to benzimidazole group of drugs in organised farms in Guwahati, Assam and attributed it to the regular usage of same class of anthelmintics, underdosing and prophylactic mass treatment of animals.

There were only a few reports of benzimidazole resistance detected by FECRT in cattle and most of the data available were pertaining to small ruminants. There were several reports on high prevalence of benzimidazole resistance in organised goat farms in India (Easwaran *et al*., 2009, Maharshi *et al*., 2011, Rialch *et al.,* 2013, Chandra *et al.,* 2015, Singh *et al*., 2016.). Previous reports of benzimidazole resistance in small ruminants in Kerala include that of Deepa and Devada (2007) who conducted FECRT in organised goat farm, Thrissur, observed resistance to albendazole and attributed it to prolonged and continuous use of anthelmintics. Rajagopal *et al*. (2018) also reported benzimidazole resistance in organised farm, Thiruvananthapuram, Kerala and attributed it to the prolonged and intensive use of anthelmintic drugs.

***Benzimidazole resistance in small holder farmers' herd***

Faecal egg count reduction test was done in three small holder farmers' herd by assessing the faecal egg count before and after treatment. The FECR per cent of the three small holder farmers' herd were 75, 100 and 100 and lower 95 per cent confidence limit were more than 90. Among the three small holder farmers' herd, small holder farmers' herd, Nadathara 2 and 3 were susceptible with FECR per cent more than 95 and lower 95 per cent CL more than 90. Resistance was detected in small holder farmers' herd, Nadathara 1 where lower 95 per cent CL was less than 90 but FECR per cent was 75 (Table 1.).

In the present study, benzimidazole resistance was observed in one out of the three (33.33 per cent) small holder farmers' herd whereas two out of three (66.67 per cent) were susceptible to benzimidazoles with cent per cent reduction in FEC. There are only a few reports on detection of benzimidazole resistance by FECRT in small holder farmers' herd. Benzimidazole resistance in small holder farmers' herd in Assam was reported by Choudhury *et al.* (2018) with a FECR per cent

**Table 1**. FECR per cent and confidence limits of cattle farms in Thrissur

| Sl No | Farm | FECR % | 95 per cent confidence limit | | Remarks |
|---|---|---|---|---|---|
| | | | Lower | Upper | |
| 1 | Organised farm, Kuttoor | 38 | 0 | 86 | Resistant |
| 2 | Organised farm, Thalikode | 55 | 40 | 66 | Resistant |
| 3 | Organised farm, Nadathara | 85 | 10 | 98 | Resistant |
| 4 | Small holder farmers' herd, Nadathara 1 | 75 | 0 | 97 | Resistant |
| 5 | Small holder farmer's herd, Nadathara 2 | 100 | - | - | Susceptible |
| 6 | Small holder farmers' herd, Nadathara 3 | 100 | - | - | Susceptible |

ranging from 92 to 100. Suspected resistance to albendazole was reported by Kumar *et al.* (2014) in cattle of Chattisgarh whereas Vohra *et al*. (2019) reported susceptibility to fenbendazole in small holder farmers' herd of Haryana. Benzimidazole resistance in small holder goat farms in various regions of Tamil Nadu were reported by Varadharajan and Vijayalakshmi (2015) and Vijayasarathi *et al*. (2016). Resistance in small holder flocks of goat against fenbendazole was recorded by Singh *et al*. (2016) in Ludhiana and Amritsar districts of Punjab while Meena *et al*. (2022) observed resistance to fenbendazole and levamisole in goat flocks in semi-arid Rajasthan. Previous reports from goat flocks in Kerala included that of Rajagopal *et al.* (2013) who recorded resistance against albendazole in small holder goat flocks in Thrissur district, Kerala and they opined that it could be due to under dosing without proper veterinarian's advice.

As per the results of FECRT, benzimidazole resistance was detected in all the organised farms screened, whereas only 33.33 per cent resistance was observed in small holder farmers' herd. The results from present study showed that benzimidazole resistance is predominant and much progressed in organised farms, whereas it is still in the early stages in small holder farmers' herds. Similarly, Asha (2017) recorded benzimidazole resistance in all organised goat farms screened and 43.75 per cent resistance in small-scale flocks. The resistance in organised goat farms were attributed to routine deworming with benzimidazoles, treatment as a whole flock and dosing based on visual estimation of body weight which led to increased selection pressure. Compared to organised farms, the flock of small-scale farmers were less treated, results in reduced development of resistance.

The high prevalence of benzimidazole resistance in organised farms and comparatively lower prevalence in small holder farmers' herd observed in this study might be due to the increased frequency of deworming in organised farms and mass treatment of the animals in a farm without proper calculation of drug dose.

## Conclusion

Anthelmintic resistance was detected in cattle in Kerala by FECRT. The most prevalent strongyle in cattle was *Mecistocirrus* spp. followed by *Haemonchus* spp. and *Oesophagostomum* spp. Accurate identification of the strongyle species will add baseline data to the epidemiological study of parasitism. The results of FECRT from the present study showed that benzimidazole resistance is present and much progressed in organised farms and in a progression state in small holder farmers' herd. This might be attributed to the high frequency of deworming in organised farms than small holder farmers' herd. Further investigation for confirmation by *in vitro* assay and molecular genotyping can be done to determine the frequency of resistance in each farm.

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## Conflict of interest

The authors declare that they have no conflict of interest.

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