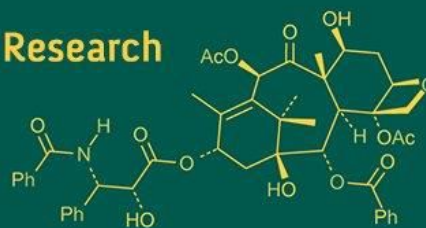
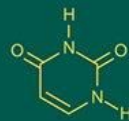
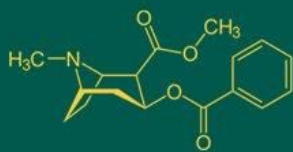


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Field evaluation of anthelmintic resistance in *Haemonchus contortus* from naturally infected goats of Nagpur, Maharashtra

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Abstract

Among the gastrointestinal parasites of goats, *Haemonchus contortus* (*H. contortus*) is one of the most harmful parasite which has been traditionally controlled using benzimidazole group of anthelmintics. However, recent reports from goat farmers of the Nagpur district of Maharashtra indicating a reduced effectiveness of this drug raising concerns about the emergence of drug resistance. Accordingly, the present study was conducted to assess benzimidazole resistance in *H. contortus* in naturally infected goats of Nagpur region by Faecal Egg Count Reduction Test (FECRT). The results showed low egg count reduction percentages after treatment with benzimidazole in selected talukas of Nagpur district indicating resistance in Nagpur (33.83%) followed by Kamptee (36.50%), Ramtek (43.16%) and least in Kalmeshwar (60.83%) talukas confirming resistance to benzimidazole in these areas based on threshold reduction of egg count below 90%. In contrast, goats from Umred taluka remained susceptible, with FECR values exceeding the recommended threshold. Overall, the study highlights the reduced efficacy of benzimidazole in 80% goat population of Nagpur region. The study has provided essential baseline data on benzimidazole resistance in goats of Nagpur district and suggests the implementation of potential alternative approaches for managing *H. contortus* infections in the future.

Keywords: Goats, gastrointestinal, parasites, Anthelmintics, resistance

Introduction

Haemonchus contortus, a barber pole worm, wire worm or stomach worm located in abomasums of small ruminants predominant in tropical as well as subtropical countries (Naeem *et al.*, 2021) [12]. The major impact of this nematode has a blood feeding activity responsible for huge blood loss, wherein each worm sucks about 0.05 ml of blood per a day (Urquhart *et al.*, 1996) [22]. It can cause protein deficiency, anaemia, bottle jaw oedema and gastrointestinal disturbances leading to death. (Fetterer and Rhoads, 1998) [5].

The anthelmintic chemotherapy has been the primary method for controlling this infection. Among the various classes of anthelmintics, benzimidazoles (BZ) a broad spectrum anthelmintic widely used due to their efficacy, ease of administration and cost-effectiveness. Nevertheless, resistance to these compounds has been increasingly reported in sheep and goat populations across different agro-climatic regions (Jackson and Coop, 2000) [8] leading to persistent infections, economic losses and compromised animal health. Small ruminant production in developing regions is largely sustained by marginal and landless farmers, for whom gastrointestinal parasitism, particularly due to the *Haemonchus contortus* with the repeated, frequent, and often indiscriminate use of BZ compounds has resulted in intense anthelmintic resistance (AR) thereby reducing the therapeutic efficacy of commonly used anthelmintics and posing a significant challenge to sustainable parasite control in small ruminants. (Stuchlikova *et al.*, 2018) [20]. The reports from the goat farmers regarding the reduced efficacy of the anthelmintic make it essential to focus on the study to assess the benzimidazole resistance status for haemonchosis in naturally infected goats from Nagpur district of Maharashtra state.

Materials and Methods

The survey of anthelmintic resistance particularly for albendazole (benzimidazole group) in

naturally infected goats was conducted in four villages each of five talukas of the Nagpur district of Maharashtra state namely; Nagpur, Umred, Kalmeshwar, Ramtek and Kamptee by selecting the animals from organized as well as from unorganized goat farms with a history of treatment of goats with albendazole. The faecal samples from goats were collected per rectal and examined using centrifugal sedimentation and floatation methods. The eggs of nematodes were identified based on their morphological characters and egg micrometry (Soulsby, 1982) [19]. The animals positive for haemonchosis were grouped and used for further studies. *in vivo* anthelmintic resistance was assessed using the Faecal Egg Count Reduction Test as per W.A.A.V.P. guidelines (Coles *et al.*, 1992) [3], which estimates drug efficacy by comparing egg counts before and after treatment. Animals under study were treated with commercially available albendazole (ABZ) according to the manufacturer's recommended dose in milligrams per kilogram of body weight orally as a single dose.

Faecal egg counts

Faecal egg counts per gram of sample was carried by modified McMaster method. (Coles *et al.*, 1992) [3]. About 3 g of faeces was homogenized in 42 ml of water, from which 15 ml liquid was centrifuged, supernatant was discarded and saturated sodium chloride solution (NaCl) was added in homogenized pallet to give the same volume as before (15 ml). Both the chambers of a McMaster slide were charged with the sample. The number of the eggs under the two ruled grids (total volume 0.3 ml) were counted at 10 X magnification. The number of eggs counted were multiplied by 50 to give the eggs per gram feces (EPG).

Resistance was confirmed when percent egg reduction after treatment was < 95% and the 95% confidence level was <90%; if only one criterion was met, resistance was suspected. FECR was interpreted by comparing the EPG before and after treatment. The percent efficacy of drug was calculated using the following formula according to Coles *et al.* (1992) [3].

$$\text{FECRT (\%)} = [(T_1 - T_2)/T_1] \times 100$$

where

T₁ is pre-treatment egg count and T₂ is post-treatment egg count.

Results and Discussion

The detection of BZ resistance in goats was evaluated on the basis of faecal egg count reduction (FECR) percentage after treatment. In Nagpur taluka, the mean egg count of *Haemonchus contortus* in goats on 0th day of treatment was 3810.8±21.30 however on the 14th day it was noted as 2491.6±22.40 with percent FECR of 33.83%. The low FECR value with percent reduction in egg count less than 95% indicated the presence of anthelmintic resistance in Nagpur taluka. The mean *Haemonchus contortus* EPG in goats of Umred taluka treated with ABZ on 0th day exhibited a marked reduction from 5144.16±39.21 to 229.16±17.34 on day 14, corresponding to a FECR of 95.33% showing the significant susceptibility to drug declared as susceptible to the anthelmintic used. The *Haemonchus contortus* worm burden in goats of Kalmeshwar taluka was declined from 4512.5±35.12 to 1433.3±18.22 by day 14, yielding a FECR of 60.83%, indicated ABZ resistance in this taluka. The

reduction in mean EPG of *Haemonchus contortus* eggs in goats of Ramtek taluka, showed a reduction in mean EPG from 4520.8±35.33 to 2529.1±24.80 on day 0 and day 14 respectively with a FECR of 43.16% confirmed anthelmintic resistance. Similarly, in Kamptee taluka, the treatment group demonstrated a decrease in mean EPG count of *Haemonchus contortus* in goats ranging from 4542.5±36.67 to 2841.3±26.38 on day 14, resulting in a non significant FECR of 36.50% indicated the presence of resistant parasite populations in Kamptee taluka. In contrast with the findings, the untreated control group showed an increase in mean EPG from 2090.83±20.21 to 4063.3±30.21 over the same period.

The overall findings of the status of benzimidazole resistance in *Haemonchus contortus*, infecting goats based on the FECRT values indicated the high grade of ABZ resistance in Nagpur taluka followed by Kamptee, Ramtek, and Kalmeshwar taluka. The percent reduction in egg count less than 95% for *in vivo* detection of anthelmintic resistance revealed widespread anthelmintic resistance in organized as well as non organized goat farms in most of the talukas under study. The percent FECR values were in range from Umred taluka being the only area showing susceptibility, as evidenced by a FECR value exceeding the recommended threshold of 95%. Overall, the FECRT results demonstrated poor efficacy of benzimidazole, with clear evidence of resistance across all treatment groups under the present study pose a serious problem in parasitic disease control. In the present study, out of the 25 goat farms with total number of 150 animals infected with *Haemonchus contortus* with frequent anthelmintic treatment with benzimidazole screened, 20 farms with 120 goats were found to have ABZ resistance. The overall percent ABZ resistance in Nagpur district was observed as 80% with threshold reduction of egg count below 90%. However only 5 organized farms with 30 animals were susceptible for benzimidazole.

Discussion

There are several reports on the high prevalence of anthelmintic resistance in organized as well as intensively managed farms in India. In North-West India out of 22 intensively managed farms, 16 farms showed moderate degree and 4 farms showed severe resistance to fenbendazole. (Kumar and Yadav; 1994) [9]. In Hisar in sheep and goat farms belonging to Universities and Government farms showed single and multiple anthelmintic resistance in sheep and goats. (Singh and Yadav; 1997) [18]. Resistance in *Haemonchus* spp to benzimidazole in Pashmina goats at Mukteshwar was observed due to its prolonged use. (Laha *et al.* 1999) [10]. In sheep flocks maintained at Central Sheep and Wool Research Institute, Avikanagar, Rajasthan with frequent administration of Benzimidazole upto eight years, had developed resistance. (Warnakar *et al.*, 2001) [21]. In Maharashtra reduced efficacy of albendazole against *Haemonchus contortus* and *Trichostrongylus colubriformis*, with egg count reduction values below the critical 95% threshold, confirming established BZ resistance. (Ghalsasi *et al.*, 2012) [7]. Investigations in small-scale goat rearing units in Kerala revealed reduced efficacy of albendazole, with faecal egg count reduction values around 71% and lower confidence limits below the recommended threshold, indicating established resistance. Sheep farms of Karnataka

corroborated fenbendazole resistance, as evidenced by low lower 95% confidence limits in both *in vivo* and *in vitro* assays (Rajagopal *et al.*, 2013; Amulya *et al.*, 2016) ^[15, 1]. In sub-Himalayan regions of northern India, where FECRT detected benzimidazole resistance in a majority of sheep and goat flocks, with egg count reduction percentages ranging from moderate to markedly low levels. Faecal egg count reduction tests in sheep revealed suboptimal efficacy of albendazole and fenbendazole, with egg count reduction percentages remaining below the recommended threshold and lower confidence limits confirming the presence of benzimidazole resistance in Kattupakkam, Tamilnadu. Benzimidazoles, levamisole and ivermectin, was documented in both organized and smallholder sheep and goat flocks in Tamil Nadu, with higher resistance levels observed in goats (Rialch *et al.*, 2013; Meenakshisundaram *et al.*, 2014) ^[16, 11]. Likewise, partial to moderate resistance in organised sheep farms of Jammu and also from semi-arid regions of Gujarat demonstrated poor efficacy of albendazole, levamisole, and ivermectin, reflecting the emergence of multi-drug resistance in field populations of gastrointestinal nematodes (Gelot *et al.*, 2016; Sharma *et al.*, 2015) ^[6, 17]. Field investigations in goats from central India further confirmed a high prevalence of BZ resistance, with a predominance of resistant genotypes detected through FECRT, EHT, and AS-PCR (Dixit *et al.*, 2017) ^[4]. The widespread occurrence of benzimidazole resistance in gastrointestinal nematodes of goats reported in Dhenkanal district of Odissa. (Nanda *et al.*, 2017) ^[13] and from the Chhattisgarh Plain zone varying levels of albendazole resistance during the monsoon period (Nath *et al.*, 2024) ^[14]. Evidence from different regions of India indicates that benzimidazole (BZ) resistance in *Haemonchus contortus* is widespread in small ruminants. Similarly in present study the findings revealed the presence of ABZ resistance in organized as well as unorganized goat farms. The results in present study are in agreement with researchers who reported BZ resistance from various parts of the country may be due to indiscriminate, frequent and repeated use of same group of anthelmintic. The present investigation clearly establishes the widespread occurrence of BZ resistance in *Haemonchus contortus* infecting goats in Nagpur district of Maharashtra. Umred taluka was the only area where susceptibility to albendazole was observed may be due to the least exposure of goats to the parasitic infection, less animal population and good management practices.

Conclusion

The present study conclusively confirms widespread benzimidazole resistance in *Haemonchus contortus* infecting goats in Nagpur, Maharashtra align with earlier reports and are likely due to indiscriminate and repeated use of anthelmintics. The study underscores the urgent need for adoption of strategic parasite control measures, rational use of anthelmintics, regular monitoring of drug efficacy, and improved farmer awareness to ensure sustainable management of gastrointestinal nematode infections in goats.

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