Oxfendazole Resistance in Gastrointestinal Nematodes of Beetal Goats at Livestock Farms of Punjab (Pakistan)

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Abstract

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This study was carried out to screen goat farms for anthelmintic resistance (AR) against oxfendazole (OXF) and to determine contributory factors for its development.

For this purpose, Beetal goat farms (n = 18) were randomly selected, with natural mixed gastrointestinal nematodosis infection. *In vivo* (faecal egg count reduction test) and *in vitro* (egg hatch assay) tests were used to ascertain the presence of AR while a scorecard was used to determine the role of possible contributory factors for oxfendazole resistance. For *in vivo* test, the experimental animals were divided into two groups of 10 animals each; one group received OXF treatment, while the other served as control. Pre- and post-treatment coproculture was performed to identify the species and genera of nematodes. Egg hatch assay (EHA) was used to confirm the results of FECRT.

Fecal egg count reduction test (FECRT) revealed the development of resistance on six farms and post-treatment larval cultures indicated *Haemonchus contortus*, *Trichostrongylus colubriformis, Cooperia curticei, Teladorsagia circumcincta* and *Oesophagostomum* spp. as dominant species with resistance. Furthermore, EHA confirmed the results of FECRT. Among the presumptive factors for AR, the highest composite score was for rotation of anthelmintics followed by treatment frequency, dose rate and nature of medication.

The scorecard for the development of AR, used in this study, may be helpful for the assessment of contributory factors of AR.

Oxfendazole, resistance, Beetal goat, Pakistan, in vivo test

Gastrointestinal nematodes adversely affect animal production causing huge economic losses all over the world (Chandrawathani et al. 1999; Vázquez 2000), and the livestock in Pakistan is no exception to it. Use of chemicals for the treatment and control of gastrointestinal parasites is most widely practiced throughout the world (Ancheta et al. 2004). Development of AR to commercially available drugs has, however, become a serious problem (Mascie-Taylor and Karim 2003; Kaplan 2004; Coles et al. 2006; Jabbar et al. 2006). In many parts of the world, therefore, anthelmintics are loosing their efficacy; e.g. in Europe (Requejo-Fernandez et al. 1997), Africa (Mwamachi et al. 1995), Australia (Waller 1986), New Zealand (McKenna et al. 1990), Pakistan (Saddiqi et al. 2006), Texas (Miller and Craig 1996) and Virginia (Zajac and Gipson 2000).

In Pakistan, one of the important factors of high prevalence of nematodes in goats (Iqbal et al. 1986; Azad et al. 1997; Iqbal et al. 2005; Lateef et al. 2005; Raza et al. 2006) may be the treatment failure with the commonly used anthelmintics (Afaq et al. unpublished data). The present study was, therefore, carried out to screen the gastrointestinal nematodes of Beetal goats for the development of resistance against oxfendazole, one of the commonly used anthelmintics. In addition, a scorecard was developed to ascertain the role of some of the contributory factors in the development of AR.

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Materials and Methods

Eighteen Beetal goat farms (Table 1) in the province of Punjab (Pakistan) were screened at random for AR using faecal egg count reduction and egg hatch tests.

Table 1. Flock composition and percentage reduction of faecal egg counts with lower confidence limits of 95% confidence interval in goats treated with oxfendazole

Flock No.	Age (months)	Sex	% Reduction	Confidence Limits	Resistant/Suspected/ Susceptible
1 (n = 40)	6-9	Male	94	81-98	Resistant
2 (n = 48)	6-10	Female	100	98-100	Susceptible
3 (n = 40)	9-12	Female	87	25-98	Resistant
4 (n = 40)	12-15	Female	89	55-97	Resistant
5 (n = 40)	12-18	Female	93	85-97	Resistant
6 (n = 40)	12-24	Male	82	42-95	Resistant
7 (n = 40)	12-24	Female	99	92-100	Susceptible
8 (n = 48)	15-25	Female	98	95-99	Susceptible
9 (n = 40)	15-25	Female	96	72-99	Suspected
10 (n = 40)	15-25	Female	96	83-99	Suspected
11 (n = 48)	15-25	Female	99	97-100	Susceptible
12 (n = 48)	15-25	Female	98	92-99	Susceptible
13 (n = 40)	15-25	Female	95	70-99	Suspected
14 (n = 40)	15-25	Female	97	79-99	Suspected
15 (n = 40)	15-25	Female	98	92-100	Susceptible
16 (n = 40)	15-25	Female	80	18-95	Resistant
17 (n = 40)	15-25	Female	97	92-97	Susceptible
18 (n = 40)	15-25	Female	99	87-100	Suspected

n = Number of animals

The FECRT was conducted according to the World Association for the Advancement of Veterinary Parasitology (WAAVP) guidelines for detection of anthelmintic resistance (Coles et al. 1992). Briefly, faecal samples (3 - 5 g) were collected and examined before medication from each animal directly from the rectum. The eggs per gram of faeces (EPG) were recorded using the McMaster technique (Soulsby 1982). On the basis of EPG, animals were randomly divided into two groups of an equal number of animals each. The treated group was given 2.265% w/v oxfendazole (Oxazole, Manufactured by Star laboratories, 23-Km, Multan Road, Lahore, Pakistan) as per manufacturer's recommendations while the other served as untreated control. Coproculture was carried out to assess the composition of infection before and after medication. For this purpose, pooled collections (~ 5 g from each animal making a total of 50 g) from each experimental group were used for coprocultures on days 0 (pre-treatment) and 14 (post-treatment). Larvae were recovered through Baermann apparatus (MAFF 1986) and identification of third stage larvae (L_3) was done using the key described by MAFF (1986) and van Wyk et al. (2004).

The percentage reduction was calculated by the formula 100 (1-Xt/Xc) where "t" was treated egg count and "c" control group egg count on day 14 post treatment. The nematodes were declared having developed resistance if (i) the percentage reduction in the egg count was less than 95% and (ii) the lower limit of 95% confidence level was less than 90% as described by Coles et al. (1992). If only one of the two criteria was met then resistance was suspected.

Animals with an evidence of resistant nematodes with FECRT were subjected to EHT following the method of Le Jambre (1976) with minor modifications (Coles et al. 1992). Briefly, 15 concentrations of oxfendazole (0.001 to 22.65 $\mu g \cdot ml^{-1}$) were prepared by twofold serial dilution using 0.1% NaCl (diluent) to enable the calculation of the dose required to prevent 50% of the viable eggs hatching (LC_{50}). One ml (150 $eggs \cdot ml^{-1}$) of egg suspension was taken in each well of a 24 well multiwell plate (Flow Laboratories). 500 µl of different concentrations of oxfendazole were added to each experimental well while the control well received only the diluent (0.1% NaCl). The plate was incubated at 27 °C for 48 h. After incubation, two drops of Lugol's iodine were added. At least 100 of the remaining eggs (dead and embryonated) and hatched larvae were counted.

Logarithmic concentration (LC_{50}) value was calculated for the eggs by log probit analysis (Finney 1971). Eggs having LC_{50} value in excess of 0.1µg oxfendazole per ml were indicative of anthelmintic resistance (Le Jambre 1976).

For evaluation of flocks for the presence of anthelmintic resistance against oxfendazole, a scorecard was designed before the study to assess the role of some factors reported to contribute towards the development of anthelmintic resistance. Each factor contained three scores. For each parameter, it was assumed that the contributory role in the development of anthelmintic resistance increased in favor of anthelmintic resistance as score increased from one to three as follows:

Treatment frequency
1 = Yearly or more than a year, 2 = After every six months, 3 = After every three months or less

Dose rate
1 = Correct dose and to all the animals in a flock/herd, 2 = Correct dose and to the animals suspected for worms in a flock/herd, 3 = Underdose

Rotation of anthelmintic compounds
1 = Strictly rotate, 2 = Rotates after two years, 3 = No rotation

Nature of anthelmintics
1 = Modern, 2 = Mixed modern and traditional, 3 = Traditional (ethnobotanicals)

Results

Percentage reduction in fecal egg counts and their confidence limits showed that resistance against oxfendazole was present at six farms (n = 6/18). Five farms (n = 5/18) were suspected for resistance and there was no evidence of resistance at seven farms (Table 1). Percentage reduction of fecal egg counts ranged from 80 to 100 percent (Table 1). The results of FECRT were also confirmed by egg hatch test (Table 2).

Table 2. LC50 values at flocks of goats with an evidence of development of resistance against Oxfendazole

Flock No.	Oxfendazole (LC50 µg/mL)
1	0.14
3	0.32
4	0.61
5	0.18
6	0.96
16	3.52

The distribution of nematode genera or species revealed that *Haemonchus contortus* was resistant at seven farms, *Trichostrongylus colubriformis* at six farms, *C. curticei* at six farms, *Oesophagostomum* spp. at four farms and no resistance was found in *Teladorsagia circumcincta* at all the three farms from which it was isolated. Anthelmintic resistance was suspected at six farms in *H. contortus*, at three farms in *T. colubriformis,* at four farms, in *C. curticei,* at two farms in *Oesophagostomum* spp. and at one farm against *T. circumcincta* (Table 3).

The data (Table 4) revealed that all the factors included in the study contributed to the development of resistance. Non-rotation of anthelmintic compounds was, however, found as the most significant contributory factor followed by treatment frequency, lower doses or selective treatments, and use of traditional medicines.

Discussion

As evident from the results, the parasites of great concern in this study were *H. contortus*, *T. colubriformis, C. curticei, T. circumcincta* and *Oesophagostomum* spp. as they cause the most severe clinical problems in this region (Malik et al. 1995). The frequent occurrence of trichostrongyluosis as a chronic rather than acute infection may allow the firm establishment of anthelmintic resistance without obvious clinical signs (Jackson et al. 1992). The present findings are not different from those reported from other countries as far as benzimidazole resistance is concerned (Uppal et al. 1992; Maingi et al. 1996; Requejo-Fernandez et al. 1997; Waruiru et al. 1998; Čorba et al. 1998; Čerňanská et al. 2006).

Table 3. Percentage of larval cultures at different flocks of goats treated with oxfendazole

Flock No.	*Haemonchus contortus*			*Trichostrongylus colubriformis*			*Cooperia curticei*			*Teladorsagia circumcinta*			*Oesophagostomum* spp.		
	%	CL	R/S / Sc	%	CL	R/S / Sc	%	CL	R/S / Sc	%	CL	R/S / Sc	%	CL	R/S / Sc
1	85	55-95	R	99	96-100	Sc	93	77-98	R	-	-	-	-	-	-
2	100	-	Sc	100	-	Sc	100	-	Sc	-	-	-	-	-	-
3	73	0-96	R	74	0-96	R	97	81-99	S	68	0-94	R	-	-	-
4	89	55-97	R	93	73-98	R	95	78-99	S	69	0-92	R	-	-	-
5	94	87-97	R	87	72-94	R	74	41-88	R	98	96-99	Sc	-	-	-
6	70	1-91	R	87	57-96	R	91	71-97	R	87	57-96	R	96	86-99	S
7	99	92-100	Sc	99	96-100	Sc	98	85-100	S	100	-	Sc	100	-	Sc
8	97	90-99	Sc	97	90-99	Sc	-	-	-	100	-	Sc	-	-	-
9	95	68-99	S	96	76-99	S	91	38-99	R	98	85-100	S	-	-	-
10	97	87-99	S	93	66-98	R	-	-	-	99	97-100	Sc	-	-	-
11	99	98-100	N	99	96-100	Sc	100	-	Sc	99	97-100	Sc	-	-	-
12	98	93-99	S	96	87-99	S	97	90-99	Sc	-	-	-	-	-	-
13	94	62-99	R	98	86-100	S	97	81-100	S	-	-	-	-	-	-
14	95	67-99	S	99	91-100	Sc	-	-	-	-	-	-	99	91-100	Sc
15	97	87-99	S	99	95-100	Sc	96	84-99	S	100	-	Sc	-	-	-
16	79	16-95	R	91	63-98	R	39	0-85	R	54	0-89	R	-	-	-
17	97	93-99	Sc	99	97-100	Sc	68	16-88	R	95	87-98	S	-	-	-
18	98	84-100	S	99	91-100	Sc	99	93-100	Sc	99	90-100	Sc	-	-	-

% = Per cent reduction, CL = Confidence limits, R = Resistant, S = Suspected, Sc = Susceptible

Table 4. Composite scores (Mean ± SE) based on contributory factors on different Beetal goat farms

Status	Treatment frequency	Dose rate	Rotation of anthelmintic	Nature of anthelmintic
Resistant (n = 06)	2.67 ± 0.21Aa	2.50 ± 0.23Aa	3.00 ± 0Ab	1.50 ± 0.23Ac
Suspected (n = 05)	2 ± 0Ba	1.6 ± 0.25Bb	2.8 ± 0.20Ac	1.4 ± 0.25Ab
Susceptible (n = 07)	1.29 ± 0.19Ca	1.00 ± 0Cb	1.86 ± 0.14Bc	1.0 ± 0Bb

Different large alphabets in a column and small alphabets in a row indicate difference in mean scores for each parameter.

This study is, however, the first report of anthelmintic resistance in Beetal goats, which are among the most popular animals kept by the farmers in the area of study to meet their day-to-day needs.

Several factors affect the development of anthelmintic resistance in nematodes. The selection pressure exerted by the anthelmintic depends on the timing of use, underdosing and the drenching frequency (Sykes et al. 1992; Coles et al. 2006). Differences in bioavailability and efficacy for benzimidazole exist between sheep and goats. The repeated and exclusive use of benzimidazole products obviously led to the development of resistant nematode strains (Sykes et al. 1992); whereas, the impact of a continuous suboptimal dosage is more controversial (Borgsteede et al. 1996). Studies of the efficacy of benzimidazole in goats (Pomory 1996) suggest that it may be better to treat goats with drugs in the benzimidazole family at one-and-half or twice the recommended dosage. For instance, Bogan et al. (1987) found only a 42% bioavailability of oxfendazole in goats when compared with sheep after oral administration. In the present study, maximum contribution was found to be associated with the rotation of anthelmintics followed by

treatment frequency, dose arte and nature of medication. Different contributory factors for AR have been reported by different workers (Jackson 1993; Varady et al. 1994; Pal and Qayyum 1996; Shakoor et al. 1997; Van Wyk et al. 1997; Monteiro et al. 1998; Coles et al. 2006, Jabbar et al. 2006).

The reduction of the frequency of anthelmintic treatment together with an annual rotation in the type of anthelmintic used should also be a major goal for farmers to maintain the efficacy of available anthelmintics. Farmers should be educated in the importance of a correct use of anthelmintics in order to maintain their efficacy (Chandrawwathani et al. 1994). The reduction in the anthelmintic treatment will be feasible by keeping in mind the local epidemiology of trichostrongylosis and if some additional pasture management strategies are also developed (Coles and Roush 1992). Egg hatch assay was used to confirm the results of FECRT, which indicated development of resistance in gastrointestinal nematodes against OXF on all farms, in contrast to Martin et al. (1989) and Dorny et al. (1994).

The resistance management strategies may, therefore, be prioritized in view of the factors identified in the area of study. Designing a balanced approach may help the farmers in the management of resistance without compromising the production of animals.

Rezistence střevních nematod na oxfendazol u koz plemene Beetal na farmách v Pandžábu (Pákistán)

Účelem této studie bylo testování kozích farem na rezistenci vůči anthelmintikům (AR) s oxfendazolem (OXF) a určení pomocných faktorů přispívajících k jejímu vzniku.

Za tímto účelem byly náhodně vybrány farmy chovající kozy plemene Beetal (n = 18) s přirozenou smíšenou infekcí gastrointestinálními nematody. Ke zjištění přítomnosti AR byl *in vivo* použit test redukce počtu vajíček v trusu a *in vitro* test líhnutí vajíček. Výsledky byly využity k určení funkce možných pomocných faktorů pro rezistenci na oxfendazol. Pro test *in vivo* byla zvířata rozdělena do dvou skupin po deseti kusech. Jedna skupina byla ošetřena OXF, zatímco druhá sloužila jako kontrola. K identifikaci druhu nematod byla provedena kultivace fécés před a po ošetření, k potvrzení výsledku určení rodu testem FECRT byl použit test líhnutí vajíček (EHA).

Test redukce počtu vajíček v trusu (FECRT) odhalil vznik rezistence na šesti farmách a kultivací larev po ošetření byly jako dominantní druhy s rezistencí identifikovány *Haemonchus contortus*, *Trichostrongylus colubriformis, Cooperia curticei, Teladorsagia circumcincta* and *Oesophagostomum* spp. Mimoto EHA potvrdil výsledky FECRT. Z předpokládaných faktorů AR, mělo největší vliv střídání anthelmintik následované frekvencí ošetření, počtem dávek a způsobem podání. Výsledky této studie vývoje rezistence vůči anthelmintikům mohou být nápomocné při určování pomocných faktorů AR.

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