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Evaluation of Commonly Used Anthelmintics Resistance Against Nematodes Infection in Different Breeds of Sheep in Balochistan

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Abstract.- In present study, four sheep breeds (*i.e.,* Balochi, Rakhshani, Karakul and Cross) were evaluated for resistance with three anthelmintics (Oxfendazole. Levamisole and Ivermectin) against nematodes (*Haemonchus*, *Trichostrongylus*, *Nematodirus* and *Ostertagia*) between April 2011 to March 2012 at Maslakh Sheep Farm, Pishin district, Balochistan. The overall results showed that there was reduction of EPG between 95% to 99% indicating the susceptibility of these anthelmintics against nematodes in the study area. The results of egg hatch test showed LC 50 less than 0.1µg/ml of oxfendazole. This indicated that no resistance was found in eggs hatching to oxfendazole. In addition, the Egg hatch test also confirmed the result of Faecal Egg Count Reduction Test. The qualitative examination of faecal samples after Coproculture revealed four gastrointestinal nematode genera, *Haemonchus contortus*, *Trichostrongylus, Nematodirus* and *Ostertagia*.

Keywords: Sheep, anthelmintic resistance, Oxfendazole, Levamisole, Ivermectin, *Haemonchus contortus*, *Trichostrongylus, Nematodirus, Ostertagia.*

INTRODUCTION

The control of nematodes for the past thirty years have mostly relied on the use of anthelmintics, but with the passage of time these have lost their efficacy due to the development of resistance. Anthelmintic resistance has been reported in number of countries (Van Wyk *et al*., 1997; Waller, 1997; Afaq, 2003). Resistance of benzimidazole against *Haemonchus contortus* was observed very early after their introduction by Drudge *et al*. (1964). Similarly, levamisole/morantel resistance to *H. contortus* was detected in sheep by Le Jamber (1976). Macrocyclic lactones resistance was reported in South Africa by Carmichael *et al*. (1987). In accordance with the prevailing climate, parasitic species and treatment regimes, the rate of emergence of anthelmintic resistance were vary geographically adopted in the region (Prichard, 1990; Jackson, 1993). Anthelmintic resistance is becoming a main constraint in small ruminant production throughout the world and has serious implications due to non availability of new drugs (Papadopoulos, 2008).

The present study has been designed to evaluate the development of resistance against commonly used anthelmintics in sheep at Maslakh Sheep Farm district Pishin, Balochistan.

MATERIALS AND METHODS

Farm and anthelmintics

The Karakul Sheep Research Farm was targeted to study anthelmintic resistance against gastrointestinal nematodes in four sheep breeds namely Balochi, Rakhshani, Karakul and Cross breed. Three hundred and twenty sheep of four breeds (each breed comprising of eighty animals) were selected randomly from the farm on the basis of age under one year. Animals naturally parasitized with gastro-intestinal nematodes having at least 150 eggs per gram of faeces were selected to study the efficacy of anthelmintic. Each sheep breed was divided into four groups A, B, C and D. Group A

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and B, each of twenty animals were administered with oxfendazole (Systamex ICI Pakistan limited) @ 4.53 mg/kg body weight and Nilzan plus (Levamisole HCl + Oxyclozanid and Cobalt Sulphate ICI Pakistan limited) @ 7.5mg/kg body weight orally with a calibrated drenching gun, respectively. group C was administered with ivermectin (Evomec PDH, Lahore) @ 200 µg/kg body weight subcutaneously, where as Group D was kept as control. Medicines were administered according to the manufacturers' recommendations.

The anthelmintic resistance in gastrointestinal nematodes of sheep was detected through faecal egg count reduction test with oxfendazole, levamizole and ivermectin and egg hatch assay for oxfendazole (Coles *et al*., 1992; Lyndal-Murphy, 1992).

Faecal egg count reduction test

Five grams faecal sample were collected directly from the rectum of individual animal and put in ice box before treatment and day 14 post treatments the faecal samples were examined fresh or stored at 4°C for later examination. Faecal examination was conducted using standard procedure described by Coles *et al*. (1992). The nematodes species were identified through coproculture followed by microscopic examination of eggs and counted EPG according to modified McMaster technique (Coles, 1986).

The percentage of eggs reduction was calculated by the formula R%= 100 (1-Xt/Xc) where Xt was treated group and Xc was control group as described by Coles *et al*. (1992, 2006). The reduction in eggs per gram of faeces less than 95% and 95% confidence levels of less than 90% were taken as an indication as described by Coles *et al*. (1992) for the presence of anthelmintic resistant nematodes in treated animals. If only one of the two criteria was met, than resistance was suspected. Calculations were made according to Coles *et al*. (1992) using a spreadsheet created by Angus Cameron, Aus. Vet. Animal Health Services for the University of Sydney. Its calculation are based on those of the Reso faecal egg count reduction test analysis program (Version 2.0)

Egg hatch test

Egg hatch test were conducted using a standard procedure (Le Jamber, 1976; Coles *et al*., 1992). Five grams faecal samples were collected directly from the rectum of individual sheep. Faecal samples were mixed thoroughly by an electric mixer to give one composite sample for each flock. Each composite sample was properly labeled for identification. Faecal samples were homogenized until all pellets are broken and sieved through a wire mesh. Saturated salt solution was added to the filtered faecal sample and was poured into a shallow tray. A plastic sheet cut to the shape of the tray and was floated on the top of the tray containing faecal suspension. The floated eggs were adhered to the plastic sheet. After thirty minutes the plastic sheet was removed and egg were washed off with a wash bottle containing water into a beaker. Number of eggs was estimated by McMaster technique.

Egg hatch test was carried out following the standard procedure (Le jamber, 1976) with minor modification by a number of workers (Coles *et al*., 1992; Taylor *et al*., 2002). One ml oxfendazole was dissolved in 500 ml deionized water to get a stock solution. Stock solution was used to prepare fifteen concentrations of oxfendazole (0.001 to 22.65 µg/ml) by two fold serial dilution using 0.1% NaCl solution to enable the calculation of the dose required to prevent 50% of the viable eggs from hatching (LC_{50}). One ml of eggs suspension was taken in each well of a 24 multi-well plate. 500 µl of different concentration of oxfendazole was added to each well, while the control well was received 0.1% NaCl solution. Plate was incubated at 25°C for 48 h. A drop of lugol's iodine was added to each well at termination of incubation. Hatched larvae and remaining eggs were counted under inverted microscope.

Statistical analysis

Logarithmic concentration (LC) value was calculated for the eggs by log probit analysis (Finney, 1971). Eggs having LC value in excess of 0.1ug anthelmintic/ml was indicative of anthelmintic resistance against oxfendazole (Le Jamber, 1976; Coles *et al*., 1992).

RESULTS

Faecal egg count

Table I shows percent reduction in eggs per

gram (EPG) of faeces after treatment with different anti-helmintics. All the three helminties seem to be very effective for all breeds of sheep. Oxfendazole, levamizole and ivermectin resulted in 95-97%, 96-98% and 98-99% decrease, respectively in the egg count (Table II).

Table I.- Mean faecal egg count (eggs/g) in sheep naturally infected with mixed species of nematodes before and after treatment with different anthelmintic in Balochi, Rukhshani, Karakul and cross breed.

Anthelmintics	Day 0 (n=20)	Day 14 (n=20)
Oxfendazole		
Balochi	2195	55
Rukhshani	2325	70
Karakul	2465	100
Cross bred	2590	125
Levamisole		
Balochi	2320	55
Rukhshani	2400	70
Karakul	2385	95
Cross bred	2515	95
Ivermectin		
Balochi	1920	25
Rukhshani	2405	35
Karakul	2240	40
Cross bred	2420	50

Table II shows the effect of antihelmintics on the four nematode species namely *H. contortus*, *Trichostrongylus, Nematodirus* and *Ostertagia* in different breeds of sheep. Oxfendazole treatment for 14 days resulted in 95-97% reduction in the eggs of *H. contortus,* 95-98% in eggs of *Trichostrongylus*, 96-99% in *Nematodirus* and *Ostertagia.* Levamisole treatment likewise resulted in 95-96% decrease in *H. contortus*, 95-98% in *Trichostrongylus*, 97-99% in *Nematodirus* and *Ostertagia.* Likewise, ivermectin treatment caused 98-99% decrease in the number of eggs of *H. contortus*, and *Nematodirus* 97-99% in *Trichostrongylus* and 99% in *Ostertagia*

Egg hatching

The results of egg hatch test indicated (Fig. 1) that LC_{50} in this experiment was less than 0.1µg/ml of oxfendazole. This indicates that no resistance was found in the eggs to oxfendazole. Egg hatch test also confirmed the result of faecal egg count reduction test. The qualitative examination of faecal samples after Coproculture revealed four gastrointestinal nematode genera *i.e.*, *Haemonchus*, *Trichostrongylus, Nematodirus* and *Ostertagia.*

DISCUSSION

The result of the present study revealed that the eggs of *Haemonchus*, *Trichostrongylus*, *Nematodirus* and *Ostertagia* were highly sensitive to oxfendazole, levamisole and ivermectin, in all four breeds of sheep. These results are also in agreements with Swarnkar *et al*. (1999) who reported 100% anthelmintic efficacy of levamizole and fenbendazole in Karakuk sheep at India. LC_{50} in egg hatching assay was 0.074± 0.015 µg thiabendazole/ml. Similarly, Menkir *et al*. (2006) also reported anthelmintic efficacy of combination of these two drugs and ivermectin in sheep and goats. In contrast *Haemonchus*, *Trichostrongylus* spp. in goat flock showed resistance to combination of albendazole and tetramizole, and ivermectin. The present findings are also in agreements with Han-Bo *et al*. (1997) who found that the ivermectin was 100% effective against intestinal nematodes. Sheferaw and Asha (2010) also reported reduction faecal egg count of gastrointestinal nematodes of sheep after treatment with ivermectin. Mirhadi *et al*. (2011) also reported that ivermectin was 99.1% effective against *Nematodirus spathiger*. Similarly, Yadav *et al*. (1995) reported that ivermectin and closantel treated sheep showed 100% reduction in EPG of *H. contortus*, whereas benzimidazole, levamizole and morantel showed FEC reduction between 56% and 81% in sheep. These results indicated multiple drug resistance against *Haemonchus contortus*. Bartley *et al*. (2006) using FECR reported ivermectin resistant nematodes such as *Teladorsagia* and *Trichostrongylus*. Saddiqi *et al*. (2006) showed resistance of some nematodes (*Haemonchus* and *Trichostrongylus*) against oxfendazole which had low efficacy. Borgsteede *et al*. (1996) reported no resistance of *H. contortus*, *Trichostrongylus colubriformis*, *Teladorsagia circumcincta* and *Cooperia curticei* against

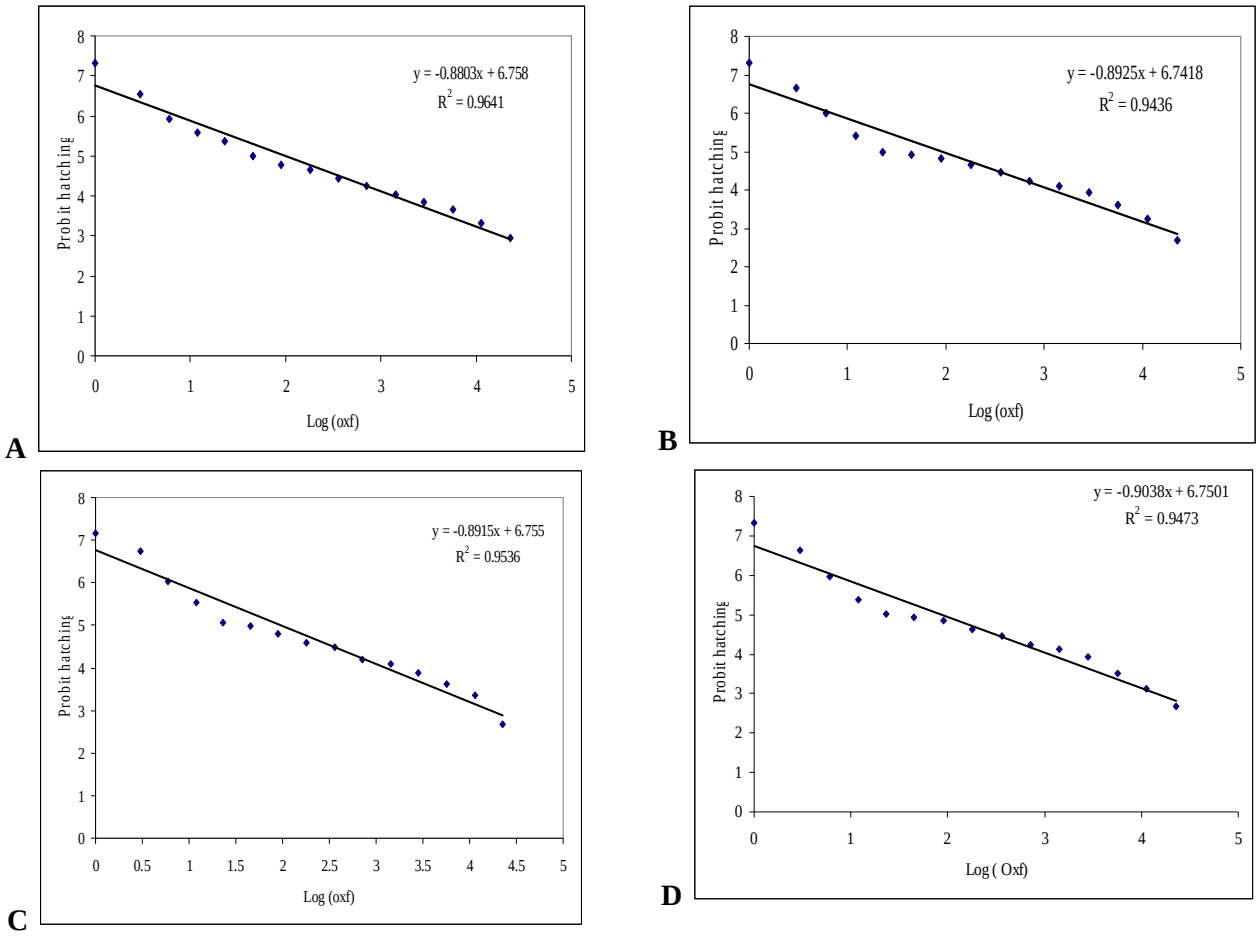

Fig. 1. Log-dose probit response line of oxfendazole in Balochi (A), Rukhshani (B), Karakul (C) and Cross (D) sheep breeds.

levamisole and ivermectin. In contrast *H. contortus*, *T. colubriformis*, *C. curticei* and *T. circumcincta* were resistant to benzimidazole. Gill (1996) observed resistance to albendazole and levamisole against nematode parasites on all the farms of sheep, contrary to the present study. However, no resistance was found against ivermectin, which is similar to the present study. Similarly, Farias *et al*. (1997) recorded resistance of *Trichostrongylus, Ostertagia* and *Haemonchus* against benzimidazole and levamisole, but no resistance of *Trichostrongylus*, *Ostertagia* and *Haemonchus* against ivermectin. Waruirua *et al*. (1998) observed the resistance of *H. contortus* against benzimidazole, levamizole/ rafoxanide and that of *T. colubriformis* and *Oesophagostomum* against levamizole, but no resistance of *H. contortus* against ivermectin. Chartiera *et al*. (1998) reported resistance to benzimidazole and levamisole against *Teladorsagia*, *Trichostrongylus* and *Cooperia* in sheep farms, but in contrast ivermectin resistant nematodes were not found in sheep farm. Present findings disagree with Chandrawathani *et al*. (1999) who observed resistance to benzimidazole, levamizole, the combination of benzimidazole/ levamizole, ivermectin and closantel against *H. contortus* in sheep farm. David *et al*. (2003) reported the resistance of *Teladorsagia* to thiabendazole, but no resistance to levamizole and ivermectin was detected against *Teladorsagia* which is similar to the present findings but was contrast to the case of thiabendazole. Eernanska *et al*. (2006) detected sheep resistant to albendazole on one farm and suspected on two. Resistance to ivermectin was

Table II.- Mean faecal egg counts of different species of nematodes in sheep before and after treatment with oxfendazole, levamisole and ivermedctin (eggs/g) in Balochi, Rukhshani, Karakul and cross breed.

Nematodes species	Oxfendazole		Levamisole		Ivermedctin	
	Day 0 (n=20)	Day 14 (n=20)	Day 0 (n=20)	Day 14 (n=20)	Day 0 (n=20)	Day 14 (n=20)
Haemonchus						
Balochi	610	20	660	30	490	25
Rukhshani	665	25	705	30	720	10
Karakul	660	30	680	30	650	15
Cross bred	705	35	695	30	670	15
Trichostrongylus						
Balochi	725	15	730	15	555	5
Rukhshani	750	25	765	20	775	15
Karakul	740	35	710	35	675	10
Cross bred	755	40	740	30	710	20
Nematodirus						
Balochi	440	5	455	5	455	5
Rukhshani	465	10	460	10	450	5
Karakul	535	20	495	15	465	5
Cross bred	550	20	530	15	515	10
Ostertagia						
Balochi	420	5	475	5	420	5
Rukhshani	445	10	470	10	460	5
Karakul	530	20	510	15	460	5
Cross bred	580	25	550	15	525	5

detected on six and suspected on eight farms against gastrointestinal nematode of sheep. Marian *et al.* (2006) used *in vitro* egg hatch test for benzimidazole resistance and compared it with FECR test. On two farms the presence of resistant population was determined through EH test in both farms; the LD_{50} was higher than 0.1 mg/ml thiabendazole indicating resistance. Torres-Acosta *et al*. (2003) declared benzimidazole resistance against *H. contortus*, when the FEC reduction % age was less than 95% and confidence level was less than 90%. Resistance was suspected when one of the two criteria was met.

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