

VETERINARSKI ARHIV 83 (3), 275-280, 2013

Emergence of ivermectin resistance in gastrointestinal nematodes of goats in a semi-organized farm of Mathura district - India

Amit Kumar Jaiswal, Vikrant Sudan*, Daya Shanker,
and Pradeep Kumar

Department of Parasitology, College of Veterinary Science and Animal Husbandry, Uttar Pradesh Pandit Deen Dayal Upadhyaya Pashu Chikitsa Vigyan Vishwavidyalaya Evam Go Anusandhan Sansthan (DUVASU), Mathura, India

JAISWAL, A. K., V. SUDAN, D. SHANKER, P. KUMAR: Emergence of ivermectin resistance in gastrointestinal nematodes of goats in a semi-organized farm of Mathura district - India. Vet. arhiv 83, 275-280, 2013.

Frequent and indiscriminate use of anthelmintic drugs has led to anthelmintic resistance in animals. Different *in vivo* and *in vitro* tests have been employed to detect anthelmintic resistance. Among the *in vivo* tests, faecal egg count reduction test (FECRT) can best be used to evaluate anthelmintic efficacy in commercial flocks and herds. In the present study, a total 40 animals, aged 12-24 months, were randomly divided into four groups of 10 goats each. All the 40 goats selected had egg counts of more than 500 eggs per gram (epg). Among these, Group I was kept as untreated control, while Groups II, III and IV were treated with fenbendazole at a dosage rate of 5mg/kg body weight orally, levamisole at a dosage rate of 7.5 mg/kg body weight orally and ivermectin at a dosage rate of 0.2 mg/kg body weight by subcutaneous injection, respectively. Faecal samples from the selected animals were collected on day 0 and day 14 post treatment. EPG was determined from each collected faecal sample and data were analyzed statistically. In Groups II, III and IV, a 71.08 percentage reduction in FEC was recorded with a 95% CI of (32.04-81.12), 97.59 with a 95% CI of (98.32-97.96) and 93.97 with a 95% CI of (91.15-96.80) respectively. Coprocultures of each group identified *Haemonchus* spp. as the predominant parasite, with an occurrence rate of 85%, followed by *Trichostrongylus* spp.(7%), *Oesophagostomum* spp. (5%), *Bunostomum* spp. (2%) and *Strongyloides* spp. (1%). The results revealed that gastro intestinal nematodes were found to be resistant to fenbendazole (Group II), but susceptible to Levamisole (Group III), while they were suspected to be resistant to ivermectin (Group IV). This seems to be the first documentation of ivermectin induced anthelmintic resistance against gastro intestinal helminths in goats in the Indian subcontinent.

Key words: anthelmintic resistance, ivermectin, goats

*Corresponding author:

Vikrant Sudan, Department of Parasitology, College of Veterinary Science and Animal Husbandry, Uttar Pradesh Pandit Deen Dayal Upadhyaya Pashu Chikitsa Vigyan Vishwavidyalaya Evam Go Anusandhan Sansthan (DUVASU), Mathura - 281001, India, E-mail: viks.sudan@gmail.com

ISSN 0372-5480
Printed in Croatia

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Introduction

Parasitic infections, especially metazoan parasites, have been a persistent and major constraint on the growth and development of goats. Most diseases caused by them are chronic and debilitating in terms of their effects upon the hosts but are rarely fatal. A number of anthelmintics, such as benzimidazoles, levamisole and ivermectin have been used to eliminate gastro intestinal nematodes in goats. Frequent and indiscriminate use of anthelmintics has led to resistance in gastro intestinal nematodes against a wide range of drugs (YADAV and UPPAL, 1992; YADAV et al., 1993; RAM et al., 2007). Reduced efficacy of fenbendazole against gastro intestinal nematode parasites in goats has been well documented in literature (YADAV and UPPAL, 1992; YADAV et al., 1995 and RAM et al., 2007). The reports of resistance against ivermectin are comparatively fewer in domestic animals (VIEIRA et al., 1992; MILLER and BARRAS, 1994; RANJAN et al., 2002). However, there seems to be no earlier documentation of ivermectin resistance in caprine gastro intestinal nematodes in India. For evaluation of anthelmintic efficacy, different *in vivo* and *in vitro* tests are recommended (COLES et al., 1992). The faecal egg count reduction test (FECRT) is the first method used for *in vivo* detection of anthelmintic resistance, it is still the most commonest method used (PRESIEDENTE, 1985) and can be easily conducted in a herd for detection of anthelmintic resistance (COLES et al., 1992). The present study was carried out to detect the anthelmintic resistance (AR) to commonly used anthelmintics in a small, semi-organized goat farm in Mathura, India.

Materials and methods

Experimental animals. The study was carried out on a small semi-organized goat farm in the Mathura District of Uttar Pradesh, India, with about 50 goats. Barbari goats were selected for the experiment and were maintained in tin sheds with non-concrete floors. The goats were allowed to browse biomass naturally growing in the open land around the premises, in addition to being fed with standard concentrate, dehydrated groundnut agro-waste and greens, as per their age and body weight, and water was provided *ad libitum*. The farm had a history of irregular deworming and no fixed regimes of deworming were followed. At the time of the start of the experiment animals, there had been no history of deworming in the previous 12 weeks.

Experimental design. The study was conducted on a total of 40 barbari goats. The animals were assigned into four groups (Groups I, II, III and IV) of ten goats each, irrespective of age, sex and weight. All selected goats had faecal egg counts of more than 500 eggs per gram of faeces (epg). Group I was kept as the untreated control, while Groups II, III and IV were treated with fenbendazole (Fentas, Intas Pharmaceuticals) at a dosage rate of 5.0 mg/kg body weight orally, levamisole at a dosage rate of 7.5 mg/kg body weight orally and ivermectin (Neomac, Intas Pharmaceuticals) at a dosage rate 0.2 mg/kg body weight subcutaneous injection, respectively.

Faecal egg count and faecal culture. Per rectal copro samples were collected from each experimental goat individually in separate plastic containers on day 0 and day 14 post-treatment. The samples were stored at 4 °C and were brought to the laboratory and processed for egg determination using a modified McMasters technique (COLES et al., 1992), within 2 hours of collection. The data were analyzed statistically as described by COLES et al., 1992.

Larva culture and identification. Pooled faecal culture was carried out for each individual group, on days 0 and 14 post treatment, using the standard Baermans technique. Briefly, the samples were incubated at 27 °C for 5 days and later put into the Baermans apparatus for 12 hours. The larvae were collected after centrifugation at 1500 rpm for 5 minutes and identified using standard keys (TAYLOR et al., 2007).

Statistical analysis: The percentage reduction in faecal egg count and approximate 95% confidence limits were calculated (COLES et al., 1992) by formula: $R = 100(1 - X_t/X_c)$ and

$$\text{Upper confidence limit} = 100[1 - X_t/X_c \exp(-2.048\sqrt{Y^2})]$$

$$\text{Lower confidence limit} = 100[1 - X_t/X_c \exp(+2.048\sqrt{Y^2})]$$

where R is percentage reduction in faecal egg count, $X_{t\text{is}}$ average epg of treatment group, X_c is average epg of control group and Y^2 is variance in reduction (log scale).

Results

In the present study, the comparative efficacy was evaluated of the three most commonly available and frequently used anthelmintics for goats on the Indian market. As per COLES et al., 1992, resistance is present if (i) the percentage reduction in egg count is less than 95% and (ii) the 95% confidence level is less than 90%. If only one of the two criteria is met, resistance is suspected. Among Groups II, III and IV, the percentage reduction in FEC recorded was 71.08, with a 95% CI of (32.04-81.12), 97.59 with a 95% CI of (98.32-97.96) and 93.97 with a 95% CI of (91.15-96.80) respectively. Before treatment, the faecal culture revealed *Haemonchus* spp. as the predominant parasite with an occurrence rate of 85% followed by *Trichostrongylus* spp. (7%), *Oesophagostomum* spp. (5%), *Bunostomum* spp. (2%) and *Strongyloides* spp. (1%). The faecal culture of each group after treatment again showed *Haemonchus* spp. (90%) as the predominant parasite with the highest occurrence rate, followed by *Trichostrongylus* spp. (7%) and *Oesophagostomum* spp. (3%) where as *Bunostomum* spp. and *Strongyloides* spp. were not detected. Hence, anthelmintic resistance against ivermectin was observed in *Haemonchus* spp., *Trichostrongylus* spp. and *Oesophagostomum* spp. whereas *Bunostomum* spp. and *Strongyloides* spp. were found to be fully susceptible to ivermectin.

Table 1. Results of the present study in tabulated form as described by COLES et al., 1992

Particulars	Group-I Untreated control	Group-II Treated with fenbendazole	Group-III Treated with levamisol	Group-IV Treated with ivermectin
No. of animals in each group	10	10	10	10
Arithmetic mean	830	240	20	50
Variance counts	47333.3	21000	666.67	2222.22
Percent reduction in FCERT	0	71.08	97.59	93.97
Approximate 95% Upper confidence limit	-	81.12	97.96	96.80
Approximate 95% Lower confidence limit	-	32.04	98.32	91.15
Interpretation of data	No significance	Resistance	No resistance	Suspected to resistance

Discussion

The role of management practices, and the frequent and indiscriminate use of anthelmintics in the development of resistance have been reported by various workers (MARTIN et al., 1989; YADAV et al., 1995). The reduced efficacy of fenbendazole and levamisole drugs against gastro intestinal nematode parasites in goats has been well documented by YADAV and UPPAL, 1992; YADAV et al., 1995 and RAM et al., 2007. Contrary to these, levamisole was found to be most effective against gastro intestinal nematodes in goats in the present study. This might be attributed to the less frequent use of levamisole for deworming in the studied farm. However, ivermectin was found to be less effective and suspected for resistance against gastro intestinal nematodes. Contrary to this, the highest potency of ivermectin against gastro intestinal nematode parasites in goats was recorded by GILL (1996) and RAM et al. (2007). This difference in results may be due to the past higher frequency of use of ivermectin in the studied farm for deworming, causing selection pressure among parasites and initiation of resistance in parasites. There are comparatively fewer reports of resistance against ivermectin in domestic animals from the Indian sub continent (VIEIRA et al., 1992; MILLER and BARRAS, 1994; RANJAN et al., 2002). MAKAWANA and SINGH (2009) also noticed ivermectin resistance in a sheep farm in Gujrat (India). However, there seems to be no earlier documentation of ivermectin resistance in goats against gastro intestinal nematodes from the Indian sub continent.

On the basis of the present study, it may be concluded that there is an urgent need to resort to rotational use of different groups of anthelmintics in goats. Control strategies are required to prevent natural nematodal infections and to manage anthelmintic resistance in goats. Although full resistance has not been reported, nevertheless, the present study may

act as a frontier in the field of anthelmintic resistance against ivermectin in gastrointestinal nematodes of goats from India. Recently, LESPINE et al. (2012) reported the subcutaneous efficacy of ivermectin to be 94% in comparison to cent percent in case of oral route. This has opened a new frontier in the field of ivermectin resistance, as all resistance research based on faecal egg reduction tests might be actually due to decreased availability. This would be interesting to investigate precisely, through well planned experimental studies, to elucidate the actual mechanisms behind the development of resistance and to investigate what the resistance actually is and not just the decreased availability of the drug. Hence, there is an urgent need for the use of molecular technologies particularly the use of PCR based detection of anthelmintic resistance in goats, and further studies dealing with the identification of particular alleles and the positions at which mutations occur leading to anthelmintic resistance. Moreover, the various amino acids substituted and/ or mutated can be identified in that way.

Acknowledgements

The authors express their deep sense of gratitude to the Dean, COVSc & A.H, DUVASU, Mathura for facilities provided.

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Received: 2 May 2012

Accepted: 5 December 2012

JAISWAL, A. K., V. SUDAN, D. SHANKER, P. KUMAR: Pojavnost otpornosti želučano-crijevnih oblića koza na ivermektin u uzgajalištima na području Mathura u Indiji. *Vet. arhiv* 83, 275-280, 2013.

SAŽETAK

Česta i neopravdana upotreba antihelmintika dovela je do pojave otpornosti parazita životinja. Ta se otpornost može dokazati različitim testovima *in vivo* i *in vitro*. Od testova *in vivo* za određivanje antihelmintičkog učinka u komercijalnih se stada dobrim pokazao test određivanja smanjenja broja jajašaca. U ovom je istraživanju ukupno 40 koza u dobi od 12 do 24 mjeseca bilo podijeljeno u četiri skupine po 10 koza. Svaka koza imala je više od 500 jajašaca po gramu izmeta. Koze su bile svrstane u kontrolnu skupinu (skupina 1) te skupinu 2 koja je bila peroralno liječena fenbendazolom u dozi od 5 mg/kg tjelesne mase, skupinu 3 peroralno liječenu levamisolom u dozi od 7,5 mg/kg i skupinu 4 koja je bila liječena supkutanom primjenom ivermektina u dozi od 0,2 mg/kg. Uzorci izmeta bili su prikupljeni 0. i 14. dana nakon liječenja. U svakom uzorku određen je broj jajašaca po gramu. U 2. skupini zabilježeno je smanjenje broja jajašaca od 71,08 s 95% CI (32,04-81,12). U 3. skupini zabilježeno je smanjenje od 97,59 s 95% CI (98,32-97,96) dok je u 4. skupini zabilježeno smanjenje od 93,97 s 95% CI (91,15-96,80). Koprokulturom je dokazana prisutnost parazita roda *Haemonchus* koji je ujedno bio i najčešće dokazan (85%). Osim njega bili su dokazani *Trichostrongylus* spp. (7%), *Oesophagostomum* spp. (5%), *Bunostomum* spp. (2%) i *Strongyloides* spp. (1%). Rezultati su pokazali da su želučano-crijevnih oblići koza otporni na fenbendazol (skupina 2), ali ujedno osjetljivi na levamisol (skupina 3). Postavljena je i sumnja na otpornost želučano-crijevnih oblića na ivermektin (skupina 4). Ovo je pionirsko istraživanje o otpornosti želučano-crijevnih oblića koza na ivermektin na području indijskog potkontinenta.

Ključne riječi: otpornost na antihelmintike, ivermektin, koze
