Research in Veterinary Science 88 (2010) 267–272

Contents lists available at ScienceDirect

Research in Veterinary Science

journal homepage: www.elsevier.com/locate/rvsc

Multiple anthelmintic resistance and the possible contributory factors in Beetal goats in an irrigated area (Pakistan)

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ARTICLE INFO

Article history:
Accepted 6 August 2009

Keywords:
Multiple anthelmintic resistance
Haemonchus contortus
Trichostrongylus colubriformis
Teladorsagia circumcincta
Beetal goats
Irrigated area

ABSTRACT

This paper presents the first report of multiple anthelmintic resistance in the gastrointestinal nematodes of goats and its possible contributory factors in an irrigated area (Pakistan). A total of 18 privately owned Beetal goat flocks were selected in order to determine the anthelmintic resistance against commonly used anthelmintics. Forty to 48 animals from each flock were selected according to their weight and egg count. The three anthelmintics viz., oxfendazole, levamisole and ivermectin, were given to three groups at manufacturer's recommended dose while one group was kept as untreated control. Anthelmintic resistance was determined through faecal egg count reduction and egg hatch tests while assessment of the contributory factors of anthelmintic resistance was measured through the rural participatory approach. Faecal egg count reduction test revealed high prevalence of anthelmintic resistance (83.3%) and it was either single (levamisole) or multiple (oxfendazole and levamisole). Egg hatch test confirmed the resistance against oxfendazole as detected with faecal egg count reduction test. None of the goat flocks was resistant to ivermectin. Copro-cultures revealed that *Haemonchus contortus*, *Trichostrongylus colubriformis* and *Teladorsagia circumcincta* were the most common species exhibiting resistance to levamisole and oxfendazole. Step-wise logistic regression of the data on worm control practices revealed significant role of under-dosing, low-protein diets, healthcare supervision by the traditional healers and mass treatments.

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1. Introduction

Gastrointestinal nematodes (GINs) are the major parasites of goats which cause substantial production losses (Chartier et al., 2000; Faizal et al., 2002) and these losses are more important in developing countries where resource-poor farmers keep goats for direct cash income from milk, meat, and skin to meet the daily-life-necessities. The goats are more prone to the GIN infection as compared to sheep due to their low ability to mount an immune response to the nematodes (reviewed by Hoste et al. (2008)).

Among different available control methods for GINs, chemotherapy is the only reliable one but this is mainly limited to the three major chemical classes of anthelmintics (benzimidazoles, imidazothiozoles and macrocyclic lactones). Additionally, resistance in the goat nematodes against these drugs has been recorded globally (e.g., van Wyk et al., 1999; Chartier et al., 2001; Chandrawathani et al., 2003, 2004; Sissay et al., 2006; Saeed et al., 2007; Cringoli et al., 2007; Jabbar et al., 2008). Consequently, it is imperative to have a regular monitoring of the efficacy of anthelmintics and worm control practices. Maingi et al. (1996a) conducted a survey to assess the worm control practices in goats and identified the factors contributing towards the dissemination of nematodes, the on going control programs and chemotherapeutic aspects. In combination of the detailed epidemiological studies, information obtained from worm control surveys could be used to make recommendations for the rational use of anthelmintics.

There has been no methodical studies on anthelmintic resistance (AR) and its possible associated factors in small ruminants in Pakistan. Additionally, there is not a single published report available on the AR in the animals grazing on temporary pastures in irrigated areas. Recently, few reports of multiple anthelmintic failure in sheep (Saddiqi et al., 2006) and single (Saeed et al.,

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doi:10.1016/j.rvsc.2009.08.004

2007) or multiple (Jabbar et al., 2008) AR in goats were published but these studies were conducted on the livestock farms owned by the state government where the access to resources is relatively easier as compared to private farms. Later on, we also received complaints of anthelmintic failure from privately owned farms of goats from the surrounding areas of district Faisalabad (Punjab). Therefore, present study was conducted to determine the presence of AR in the GINs of goats and its possible contributory factors in an irrigated area.

2. Materials and methods

2.1. Study area and its meteorological characteristics

This study was conducted in district Faisalabad (Punjab), Pakistan, which is located between 31°21′52″ north and 72°59′40″ east longitudes. The Ravi River flows on the eastern and the Chenab River on the western boundaries of the district. The climate is dry with extreme conditions having temperature varying from 4 to 50 °C and annual rainfall is 150–350 mm. The area is irrigated through the canals and tube wells, and the important crops are cotton, wheat, sugarcane, maize, rice, gram and vegetables. Owing to the overpopulation and less incentive in cash crops, there is a drift in farmers' focus from major and minor crops to raise the domestic livestock species. The small ruminants are kept to generate income to meet the daily household expenditures especially by the small or landless farmers.

2.2. Animal characteristics and selection criteria

Female Beetal goats (a milch breed) 'locally known as poor man's cow' are kept for milk while male animals are reared for mutton by the private farmers. These animals graze on the harvested crops, remnants of the fodder and vegetables, herbs, weeds and shrubs along the banks of water channels and oftenly the concentrates (wheat bran, grams etc. soaked in milk) are offered especially to males. The farmers usually do not practice any specific deworming schedule due to economic constraints and when used, often they dilute the anthelmintics before the administration (personal communication). Contrarily, some farmers (well-off) use anthelmintics to keep their stock at the optimum production level. We received a number of reports about anthelmintic inefficacy from some of these flocks. Of these farms, 18 flocks were selected which were reported for anthelmintic failures and had not been dewormed for the last 10 weeks (Table 1). Selection of the goats from these flocks was made following the written consent from the flock owners in accordance with the animal ethics committee guidelines of the university.

2.3. Experimental design

2.3.1. Pre-treatment faecal egg counts

Before the commencement of actual trial, pre-treatment faecal egg counts (FEC) were performed on whole selected flocks to ensure that sufficiently high faecal eggs per gram (EPG) of faeces were present in the animals to warrant their inclusion in the trial. Based on the guidelines published by the World Association for the Advancement of Veterinary Parasitology (WAAVP) (Coles et al., 1992), a value of >150 EPG was used as a cut off for inclusion in the AR survey. Individual faecal samples were collected from the rectum of all animals on each farm and FECs were performed by the modified McMaster technique (Urquhart et al., 2003).

2.3.2. Anthelmintic treatments

A total of 40–48 animals were selected from each flock and randomly divided into four groups with equal number of animals. The animals were given anthelmintics at a recommended dose (product labels suggest same dose for goat as well as sheep) according to the heaviest animals of the flock with oxfendazole (OXF, Oxazole – Star Labs Pvt. Ltd.; oxfendazole 2.265% w/v, dose 2.83 mg/kg BW); levamisole (LEV, Vernil – Star Labs Pvt. Ltd.; levamisole HCl 1.5% w/v, dose 7.5 mg/kg BW) and ivermectin (IVM, Ivoteck – Star Labs Pvt. Ltd.; 1% ivermectin w/v, dose 0.2 mg/kg BW) while the fourth group was kept as an untreated control. Oxfendazole and LEV were drenched while IVM was administered as subcutaneous injection.

2.3.3. Evaluation of drug efficacy

Faecal egg counts (FEC) and copro-culture were performed on day 0 before the administration of anthelmintics to assess the level of nematode infection and species/genra, respectively, within the flocks. The presence of AR was assessed by determining the efficacy of anthelmintics by FEC on day 14 post-treatment (PT). Faecal egg count reduction test (FECRT) was carried out according to the standard methods (Coles et al., 1992; Wood et al., 1995).

Pooled collections (~5 g of faeces from each animal making a total of 50–60 g) from each experimental group were used for copro-cultures on day 0 (pre-treatment) and 14 PT. Larvae were recovered through Baermann apparatus (MAFF, 1986) and the identification of third stage larvae (L_3) was done using the standard keys (MAFF, 1986; van Wyk et al., 2004).

2.3.3.1. Egg hatch test. Egg hatch test was employed for the detection of OXF resistance against GINs (Lyndal-Murphy, 1992). The stock solution (8 μg/ml) was serially diluted to OXF concentrations ranging from 3.0 to 0.0058 μg/ml. Eggs (200 eggs/10 μl distilled water) were added to 1 ml OXF solution and the control well and incubated at 27 °C for 48 h. A drop of diluted lugol's iodine solution was used to stop the development of larvae. The test was performed in triplicate for each of the suspected flocks. Eggs and first stage larvae were counted.

2.4. Assessment of AR contributory factors

Information on the worm control practices was collected on different management aspects of the same goat flocks for the assessment of their role in the development of AR. For this purpose, a score card was designed and the score ranged from one to three (Table 2) and the Rural Participatory Appraisal (RPA) procedures were followed to collect the information from the respondents (Etkin, 1993; Catley and Mohammed, 1996). The purposive sample of the key respondents for data collection included: veterinary officers, veterinary assistants and traditional healers looking after the goat flocks. The survey team comprised of (i) a veterinarian well versed with the parasitic diseases of livestock and the local language, i.e. *Punjabi* (ii) two trained field assistants recruited from the local community and (iii) a community leader. Information was collected from the key respondents to score the various categories (Table 2). Interviews, focused group discussions and field visits were used as the tools of PRA technique.

2.5. Statistical analyses

A complete randomized experimental design was used for the experiment conducted for the determination of AR. The FECRT and the 95% confidence intervals for the reduction estimates were calculated according to the methods described in the WAAVP recommendations for the detection of AR and efficacy (Coles et al., 1992, 2006; Wood et al., 1995). For FECRT, a reduction in FEC less

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