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REVIEW ARTICLE

Anthelmintic Resistance in *Haemonchus contortus* of Sheep and Goats from Asia–A Review of In Vitro and In Vivo Studies

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A B S T R A C T

The potential of *Haemonchus contortus* is quite impressive to acquire resistance to anthelmintic drugs. Asian field populations of *H. contortus* are reported to exhibit resistance to most of the anthelmintic drug families, including closantel, imidazothiazoles, macrocyclic lactones and benzimidazoles. This review paper comes up with a comprehensive overview of haemonchosis with anthelmintic resistance (AR), with particular attention to studies conducted in regions of Asia. It also confers the prevalence and mechanisms in *H. contortus* for anthelmintic resistance, indicating the necessity for alternative approaches to control the pathogen. Several SNPs identified in gene isotype-1 β-tubulin gene have been discussed that are associated with benzimidazole resistance and are prevalent in *H. contortus* populations in Asia. The methods used by *H. contortus* to withstand the effects of these medications are examined together with the history of how resistance to different anthelmintics in this species has developed. The approaches for diagnosing resistance are also compiled in this review. Such diagnosis currently largely relies on the fecal egg count reduction test, which has cost and sensitivity issues. Past and present efforts to use less expensive and time-consuming phenotypic assays with free-living life stages are also described with the developmental progress of molecular assays to provide sensitive resistance-detection tests. Overall, this paper provides valuable perception into the current understanding of AR in *Haemonchus spp.* and the challenges faced in the management of this disease in Asia.

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INTRODUCTION

Haemonchus (H.) contortus is an extremely harmful blood-feeding nematode of small ruminants, leading to Haemonchosis (Wang *et al.*, 2017; Imran and Alsayeqh, 2022). This parasite is also known as the "barber's pole worm" a major global source of mortalities (Preston *et al.*, 2016). It is transmitted through the ingestion of infective larvae present in the contaminated pasture. Grazing on pastures with high worm loads, little rainfall, high stocking density and young or immune-compromised breeds/animals are risk factors for the disease (Imran *et al*., 2018; Khanyari *et al.*, 2021). This parasite is a major issue for cattle, leading to a variety of symptoms including anemia, weight loss, reduced production and in extreme cases, death (Nazish *et al.*, 2021; Şahin *et al.*, 2021). It is a significant threat to the profitability of the livestock industry and therefore has a major economic impact (Besier *et al.*, 2016b; Qamar *et al.*, 2022). Haemonchosis also has implications for food security, as it reduces the amount of meat and dairy produced by infecting animals (Hoste and Chartier, 2002; Sargison, 2020). The worldwide estimated losses due to *H. contortus* can be substantial. The economic impact of *Haemonchus* infection in small ruminants is primarily due to reduced weight gain, decreased feed efficiency, decreased wool production, reduced reproduction and decreased market value (Zen *et al.*, 2014). The exact monetary losses vary depending on the region, the production system and the intensity of infection, but some estimates suggest losses of hundreds of millions of dollars globally (Mohammedsalih *et al.*, 2020; Kuiseu *et al.*, 2021). To reduce such huge economic losses control of the disease involves strategic deworming, pasture

management and the use of anthelmintic drugs. For instance, benzimidazole, levamisole, closantel and ivermectin have been extensively used in Asia and worldwide to treat worm infections. However, the extensive use of antiparasitic medications has led to significant issues with drug resistance in domestic animals around the globe (Abbas *et al*., 2011; Kotze *et al.*, 2014; Muhammad *et al.*, 2015; Pawar *et al.*, 2019; Yuan *et al.*, 2019; Gainza *et al.*, 2021; Pinilla *et al.*, 2022; Alvi *et al.*, 2023). The emergence of resistant strains of *H. contortus* in Asia is a growing concern in livestock production. As the worm negatively affects the production of the animal, its resistance to commonly used anthelmintic drugs can lead to adverse impacts on livestock production (Saddiqi *et al.*, 2011). Effective management strategies, including targeted use of dewormers, breed resistance may help to mitigate the development of resistant strains (Imran *et al*., 2020; Gandasegui *et al.*, 2022). Therefore, new intervention techniques must be developed in response to the rise of anthelmintic resistance in *H. contortus*. One possibility is the logical approach of developing and growing anti-parasitic medications and vaccines, based on a profound comprehension of the essential molecules in the processes of reproduction and development (Selzer and Epe, 2021). A clear understanding of the biological process at molecular level for *H. contortus* may help to identify critical molecules as potential novel therapeutic targets. This review discusses the prevalence of *H. contortus* and the current status of anthelmintic resistance in Asia. The study of key molecules in signaling pathways controlling development, the interaction between host cells and parasites, population genetics and the detection of anthelmintic resistance using conventional and molecular methods are some important areas of this study. The way these viewpoints are being brought out will help to pinpoint areas for more study and open up opportunities for new or improved control strategies.

Importance of the livestock sector in Asia: The majority of people in South Asia reside in rural regions and depend heavily on agriculture for both jobs and income. While the percentage of agriculture in total GDP varies from 12.7% in Sri Lanka to 34.7% in Nepal, the percentage of rural people in the total population spans from roughly 67% in Pakistan to over 86% in Nepal. More than 50% of the workforce in India and Nepal works in agriculture, which is a key source of employment for the enormous population in the region. Other South Asian nations have a heavy reliance on agriculture. Small farms dominate the region's agricultural production. The average size of farms in Bangladesh is 0.60, in Nepal 0.79, in Sri Lanka 0.69 and in India 1.23 hectares, respectively. In comparison to other nations in the region, Pakistan has considerably larger farms sizing 3.1 hectares (Abbas *et al.*, 2020). More than half of the world's 1 billion goats reside in Asia, which is also the region where the domestication of wild goats first started. China, India, Pakistan, Bangladesh and Mongolia have Asia's largest populations of goats and 512 million sheep and the nations with the most sheep were Turkey, Iran, Mongolia, China, India and Iran. In many low-income Asian nations, goats and sheep including dairy breeds—play important roles as producers of nourishment, food security and socioeconomic status for their human owners. Goats are suitable for a broad variety of harsh environments (Liang and Paengkoum, 2019; Mazinani and Rude, 2020).

Infection is the dramatic drop in an infected animal's output, which includes a decline in the young animals production, a decline in output of milk in nursing animals and a decline in the production of fiber (Mehmood *et al*., 2017; Fthenakis and Papadopoulos, 2018; Mahmoud *et al.*, 2022). All of them ultimately cause the farmer to suffer significant financial losses (Ilangopathy *et al.*, 2019). *H. contortus* damage might cost the breeding business, billions of dollars in lost revenue. The cost of *H. contortus* treatments alone is anticipated to be over 26 million USD annually in Kenya, 46 million USD annually in the Republic of South Africa and 103 million USD annually in India, demonstrating the importance of this parasite (McRae *et al*., 2014).

Prevalence and distribution of *Haemonchus contortus* in Asia: Sequential assessments of worm burdens in grazing animals reveal connections between nematode growth and environmental variables and offer an epidemiological background. These studies consist of three types of research organized studies utilizing "tracer" animals grazing tiny pasture patches continuously contaminated with worm eggs, worm counts from flocks or herds grazing continuously contaminated pastures and slaughterhouse surveys (Besier *et al.*, 2016a; Shamim *et al*., 2018). In the tropical and subtropical regions of Asia, studies of worm count in sheep have demonstrated their close link with rainfall patterns, where temperatures are consistently appropriate for *H. contortus* development (Chandrawathani *et al*., 2004; Yang *et al*., 2016). A huge population of sheep, cattle and goats also offers a favorable atmosphere for the growth and transmission of various bacteria, viruses and parasites, including *H. contortus*, a significant parasitic nematode that can cause significant financial losses to the cattle, sheep and goat breeding sector. To avoid and manage this species, it is crucial to comprehend the epidemiology of *H. contortus* (Wang *et al*., 2017; Alvi *et al.*, 2020; Arsenopoulos *et al.*, 2021; Adduci *et al.*, 2022). The examination of *H. contortus* infection in cattle, sheep and goats in Asia has been covered in numerous reports. Diagnosis through the conventional method of Fecal Egg Count Reduction Test (FECRT) was the most frequently employed technique in the investigations (Rialch *et al.*, 2013; Chandra *et al.*, 2015; Shakya *et al.*, 2018; Yuan *et al.*, 2019) and DNA-based techniques were also applied in more recent years to investigate *H. contortus* infection in ruminants in Asia (Mohanraj *et al.*, 2017; Bihaqi *et al.*, 2020; Dey *et al.*, 2020; Parvin *et al.*, 2022). In a study observed from Pakistan, the infection in goats was observed at 50-89.20% and in the sheep at 55-94% which indicates that *H. contortus* is dominant in Sheep of Pakistan (Bibi *et al.*, 2017; Khan *et al.*, 2021). In China, the opposite trend was observed, in the examined goat farms (Wang *et al.*, 2006; Ma *et al.*, 2014; Yang *et al*., 2016), the infection rate ranged from 28 to 100% and from 0 to 92% in the analyzed sheep farms (Wang *et al.*, 2006), which show goats are more dominant to *H. contortus*. Additionally, several other studies had previously noted the high prevalence rates of gastrointestinal (GI) parasites in goats,

particularly *H. contortus* from Indonesia, India (Uttarakhand, Nagpur) was recorded as 89.4, 96 and 88.23%, respectively (Maske *et al.*, 1990; Pant *et al.*, 2009; Widiarso *et al.*, 2018). These studies have reported a high prevalence of *H. contortus* in sheep and goats which may be influenced by various factors, including the genetic makeup of both the parasite and the host and the development of resistance to commonly used anthelmintics drugs.

Anthelmintic resistance: The control of parasites relies heavily on the use of anthelmintic drugs, which are widely used in the management of GI nematodes. Anthelmintic drugs from various classes, such as benzimidazoles (albendazole), macrocyclic lactones (ivermectin), imidazothiazoles (levamisole), amino-acetonitrile derivatives (monepantel), salicylanides (closantel) and sporoindoles (derquantel) are effective against *H. contortus* (Kaminsky *et al.*, 2008a; Sager *et al.*, 2009; Malik *et al*., 2020; Ahmad *et al*., 2021). These chemicals may be used alone or in combination in commercially available formulations. Unfortunately, the widespread use of anthelmintic drugs has led to a significant and rapid growth of AR, particularly in the nematodes of sheep, cattle, goats and horses. (Potârniche *et al.*, 2021). An anthelmintic-susceptible parasite population may become resistant by acquiring AR, which is a heritable decrease of sensitivity. (Fissiha and Kinde, 2021).

This AR has become a growing concern in many regions of the world, including Asia. Several studies have reported the presence of AR in *H. contortus* populations in various countries of Asia, including China, India, Pakistan and Iran. Resistance to multiple classes of anthelmintic, such as benzimidazoles, levamisole and macrocyclic lactones, has been reported in these populations as mentioned in Tables 1 and 2.

Benzimidazole resistance: The mode of action of benzimidazole (BZ) inhibits microtubule polymerization (Rufener *et al*., 2009), thus preventing bacterial cells from binding to tubulin monomers and causing microtubule destabilization (Mohanraj *et al*., 2017). The cost of resistance to BZ is particularly high due to the widespread use of this drug since the 1970s and this is at least partially understood at the molecular level (Elard *et al*., 1996). In 1986, researchers identified a link between the development of BZ resistance in *H. contortus* and the BZ target domain β-tubulin. Using the same experimental design (Lubega and Prichard, 1990), he found resistant *H. contortus* infection is associated with reduced binding of BZ to β-tubulin. (Kwa *et al*., 1994; Kwa *et al*., 1995) used *Caenorhabditis elegans* to explain why BZ capacity is reduced. Some mutations in the isotype-1-tubulin gene, which encodes therapeutic targets, have been shown to be associated with drug resistance. In Asia, *Haemonchus* species, isoform 1-tubulin polypeptide and *Haemophilus* sp. were found to change phenylalanine to tyrosine at codon positions 167 and 200 (F167Y and F200Y), *H. contortus* was also found to replace alanine at position 198 (E198A) with glutamate (Önder *et al*., 2016; Ekta *et al*., 2019; Amana and Alkhaled, 2023). Mutations in F200Y were examined in depth and all three mutations were found to be associated with the resistance phenotype.

Closantel resistance: The salicylanilide drug class includes the anthelmintic Closantel. To precisely target blood-eating parasites like *Haemonchus* species, salicylanilide binds tightly to plasma proteins. Moreover, Closantel may interfere with the parasite's pH homeostasis maintenance systems (Dixit *et al.*, 2019). The action of closantel is restricted in the larval stages of *Haemonchus* sp. Because immature and hypobiotic larval stages do not consume blood (Swan, 1999). There have been many documented cases of closantel resistance in GI nematodes including *Haemonchus* in different regions of the world, similar to other medication classes that have been accessible for several decades (Chandrawathani *et al.*, 2004; Bihaqi *et al.*, 2020). In the literature, it has also been reported that resistance to new anthelmintic can arise in a short time (Mederos *et al.*, 2014; Van den Brom *et al.*, 2015). The poor knowledge of closantel-resistant parasite strains and the pertinent mechanisms of resistance can be explained by the drug's narrow spectrum of activity in comparison to other antiparasitic drugs. Also, it was determined in many investigations that closantel resistance was caused by decreased drug intake by resistant helminths, significant albumin binding of the drug in helminth's gut and enhanced drug excretion from resistant worms (Rothwell and Sangster, 1997). The reduced concentration of drugs in Closantel resistant isolates of *Haemonchus* has not been attributed to changes in P-glycoprotein genes, according to subsequent investigation. (Kwa *et al.*, 1998; Kaminsky *et al*., 2008b).

Imidizothiazole resistance: Imidazothiazole drugs, such as levamisole and morantel, have been used extensively for the control of *H. contortus* infections, but their efficacy has been challenged by the emergence of resistance (Muhammad *et al.*, 2015; Islam *et al.*, 2018) Imidazothiazole resistance in *H. contortus* is believed to be due to genetic mutations that alter the target site of these drugs, which is the nicotinic acetylcholine receptor (nAChR) in the parasite's neuromuscular system. The mutations can reduce the binding affinity of the drugs to the nAChR, making them less effective in killing the parasite (Fauvin *et al.*, 2010; Neveu *et al.*, 2010). With the association with imidazothiazole resistance, several mutations have been identified in the nAChR genes of *H. contortus.* For instance, different *H. contortus* populations with various degrees of resistance to morantel and levamisole have been identified to harbor the F167Y mutation in the nAChR beta subunit gene (Barrère *et al.*, 2014; Sarai *et al.*, 2015). The imidazothiazole resistance in *H. contortus* has also been linked to other mutations in the nAChR alpha subunit gene, such as the E198A and F200Y mutations. Although the precise processes by which these mutations confer resistance are not yet completely known, it is believed that they interfere with imidazothiazoles' ability to bind to the nAChR (Turnbull *et al.*, 2019; Kotze *et al.*, 2020). Nevertheless, *Haemonchus* species have evolved some mechanisms that lessen nicotinic acetylcholine receptors' sensitivity to levamisole, making it challenging to create molecular techniques for identifying anthelmintic resistance to imidazothiazoles.

Table 1: Status of anthelmintic resistant haemonchosis in Asia detected through In-Vivo/Fecal Egg Count Reduction Test

Country	Anthelmintic involved	Dosage as per Body weight	Animal species	Age of the animal in months	No of animals	FECR%	Testing Day	Reference
Pakistan	Oxfendazole	1 ml/8 kg (2.265% w/v)	Dera din panah Goat	15-25	10	61	FECRT at 14TH day	(Jabbar *et al.*, 2008)
			Angora Goat	15-25	10	83		
			Beetal Goat	15-20	10	82		
	Ivermectin	1 ml/50 kg (1% w/v)	Dera din panah Goat	15-25	10	99	FECRT on the 14th day	
			Angora Goat	15-25	10	97		
			Beetal Goat	15-20	10	100		
	Levamisole	1 ml/2 kg (HCl 1.5% w/v)	Dera din panah Goat	15-25	10	95	FECRT on the 14th day	
			Angora Goat	15-25	10	94		
			Beetal Goat	15-20	10	88`		
	Oxfendazole	2.83 mg/kg (2.265% w/v)	Beetal Goat	6-9	40	94	FECRT on the 14th day	(Saeed *et al.*, 2010)
	Levamisole	7.5 mg/kg (1.5% w/v)	Beetal Goat	6-9	40	90		
	Ivermectin	0.2 mg/kg (1% w/v)	Beetal Goat	6-9	40	98		
	Oxfendazole	2.83 mg/kg (2.265% w/v)	Beetal Goat	9-12	40	87	FECRT on the 14th day	
	Levamisole	7.5 mg/kg (1.5% w/v)	Beetal Goat	9-12	40	95		
	Ivermectin	0.2 mg/kg (1% w/v)	Beetal Goat	9-12	40	96		
	Oxfendazole	2.83 mg/kg (2.265% w/v)	Beetal Goat	12-15	40	89	FECRT on the 14th day	
	Levamisole	1.5% w/v, dose 7.5 mg/kg	Beetal Goat	12-15	40	75		
	Ivermectin	1% w/v, dose 0.2 mg/kg		12-15	40	99		
	Oxfendazole	2.265% w/v, dose 2.83 mg/kg	Beetal Goat	12-24	40	82	FECRT on the 14th day	
	Levamisole	1.5% w/v, dose 7.5 mg/kg	Beetal Goat	12-24	40	52		
	Ivermectin	1% w/v, dose 0.2 mg/kg		12-24	40	99		
	Albendazole	N/A	Salt range Sheep	3-4	10	88	FECRT on the 14th day	(Muhammad *et al.*, 2015)
	Levamisole				10	89		
	Ivermectin				10	99		
	Albendazole	N/A	Pak karakul Sheep	3-4	10	91		
	Levamisole				10	90		
	Ivermectin				10	97		
	Albendazole	N/A	Jattal Goat	3-4	10	91		
	Levamisole				10	91		
	Ivermectin				10	98		
	Albendazole	1 ml/5kg	Goat	N/a	20	93.4	FECRT on the 30th day	(Rehman *et al.*, 2020)
	Oxfendazole	1 ml/5kg	Goat	N/a	20	94.7		
Bangladesh	Albendazole	7.5 mg/kg	Sheep	N/A	10	90	FECRT on the 14th day	(Islam *et al.*, 2018)
	Levamisole	7.5 mg/kg			10	98		
	Ivermectin	2.5 mg/kg			10	96		
	Albendazole	7.5 mg/kg	Goat	N/A	10	100		
	Levamisole	7.5 mg/kg			10	97		
	Ivermectin	2.5 mg/kg			10	100		
	Albendazole	7.5 mg/kg	Goat	N/A	10	93.46	FECRT on the 14th day	(Dey *et al.*, 2020)
	Levamisole	7.5 mg/kg			10	90.44		
	Ivermectin	0.2 mg/kg			10	85.62		
	Albendazole	7.5 mg/kg	Sheep	N/A	10	94.05		
	Levamisole	7.5 mg/kg			10	100		
	Ivermectin	0.2 mg/kg			10	87.44		
	Albendazole	7.5 mg/kg	Goat	N/A	10	90.57		
	Levamisole	7.5 mg/kg			10	93.12		
	Ivermectin	0.2 mg/kg			10	96.39		
	Albendazole	7.5 mg/kg	Goat	N/A	10	99.11		
	Levamisole	7.5 mg/kg			10	99.11		
	Ivermectin	0.2 mg/kg			10	74.11		
	Albendazole	7.5 mg/kg	Goat	N/A	10	67.55		
	Levamisole	7.5 mg/kg			10	93.81		
	Ivermectin	0.2 mg/kg			10	99.32		
	Albendazole	7.5 mg/kg	Goat	N/A	10	94.06		
	Levamisole	7.5 mg/kg			10	91.17		
	Ivermectin	0.2 mg/kg			10	93.86		
Kashmir	Fenbendazole	7.5 mg/kg	Boer and	N/A	31	62	FECRT on the 14th day	(Bihaqi *et al.*, 2020)
	Closantel	7.5 mg/kg	Bakerwal cross-		31	86		

Country	Anthelmintic involved	Dosage as per Body weight	Animal species	Age of the animal in months	No of animals	FECR%	Testing Day	Reference
	Ivermectin	0.2 mg/kg	bred Goats		31	90		
China	Ivermectin	0.2 mg/kg	Goat	N/A	N/A	48	FECRT on the 14th day	(Yuan *et al.*, 2019)
	Ivermectin +Albendazole	7.5 mg/kg	Goat	N/A	N/A	86.96		
India	Benzimidazole group	N/A	Sheep and goat	N/A	22 flocks (3 farm and 19 field flocks)	genotype (96.58%) in flocks exhibiting <50% efficacy	FECRT on the 14th day	(Maharshi *et al.*, 2011)
	Fenbendazole	5mg/kg	Sheep	N/A	4 flocks (20 animals from each)	54-100% (2 Resistant 2 susceptible)	FECRT on 10th day	(Rialch *et al.*, 2013)
	Fenbendazole	10mg/kg	Goat	N/A	10 flocks (20 animals from each)	59-100% (6 resistant 4 Susceptible)		
	Albendazole	5mg/kg	Goat	N/A	N/A	60-100% in different regions	FECRT on the 14th day	(Chandra *et al.*, 2015)
	Albendazole	10 mg/kg	Goat	15 months	N/A	83.94	FECRT on the 14th day	(Shakya *et al.*, 2018)
	Levamisole	7.5 mg/kg		15 months		89.09		
	Ivermectin	200µg/kg		15 months		100		
	Fenbendazole	2.25% w/v	Goat	8-30 months	10	84	FECRT on the 14th day	(Pawar *et al.*, 2019)
	Ivermectin	0.08% w/v			10	85		
	Levamisole	30%w/w			10	88		
	Fenbendazole	2.25% w/v	Sheep	10-36 months	10	51		
	Ivermectin	0.08% w/v			10	26		
	Levamisole	30%w/w			10	87		
Malaysia	Oxfendazole	5 mg/kg	Sheep and Goat	4-12 months of age	10 animals	22	September 2002-February 2003	(Chandrawathani *et al.*, 2004)
	Levamisole	7.5 mg/kg				75		
	Ivermectin	200 µg/kg				72		
	Closantel	5 mg/kg				76		
	Oxfendazole	5 mg/kg	Sheep and Goat	4-12 months of age	10 animals	68		
	Levamisole	7.5 mg/kg				66		
	Ivermectin	200 µg/kg				40		
	Closantel	5 mg/kg				62		
	Oxfendazole	5 mg/kg	Sheep and Goat	4-12 months of age	10 animals	61		
	Levamisole	7.5 mg/kg				91		
	Ivermectin	200 µg/kg				77		
	Closantel	5 mg/kg				78		
	Oxfendazole	5 mg/kg	Sheep and Goat	4-12 months of age	6 Animals	72		
	Levamisole	7.5 mg/kg				96		
	Ivermectin	200 µg/kg				85		
	Closantel	5mg/kg				78		
	Oxfendazole	5mg/kg	Sheep and Goat	4-12 months of age	10 Animals	68		
	Levamisole	7.5mg/kg				66		
	Ivermectin	200µg/kg				40		
	Closantel	5mg/kg				62		

FECR % = Fecal Egg Count Reduction Percentage, FECRT= Fecal Egg Count Reduction Test.

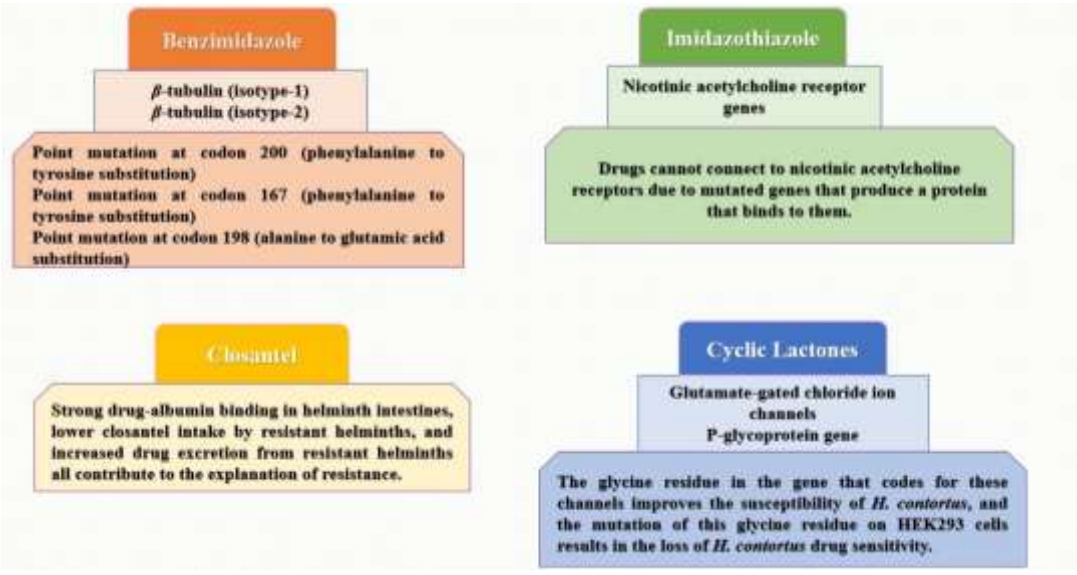

Fig. 1: Summary of resistance by different anthelmintic groups.

Table 2: Status of anthelmintic resistance in Asia specific to Benzimidazole resistance using Molecular Methods

Country	Region	No. of Haemonchus Worms/Larvae	Test Used	Drug Used	Polymorphism in the β-tubulin isotype 1 gene.	Results	Source of Sample Collection	Reference
Iraq	Bareilly	40 adult worms	AS- PCR	BZ	F200Y	8% RR, 30% RS and 62% SS	Sheep	(Amana and Alkhaled, 2023)
Bangladesh	Mymensingh, Tangail, Sirajgonj, Dinajpur, Barishal, Khulna, Sylhet and Rangamati	160 Adult Worms	AS- PCR	BZ	F200Y	9.4% RR (TAC), 61.2% RS (TAC/TTC) and 29.4% SS (TTC)	Slaughtered Sheep and goat	(Parvin *et al*., 2022)
Thailand	Chiang Mai	20 adult worms	AS- PCR	BZ	F200Y	0% RR, 60% RS and 40% SS	Goat	(Pitaksakulrat *et al*., 2021)
	Tak	17 adult worms	AS- PCR	BZ	F200Y	11.8% RR, 52.9% RS and 35.3% SS	Goat	
	Nakhon Ratchasima	20 adult worms	AS- PCR	BZ	F200Y	25% RR, 50% RS and 25% SS	Goat	
	Lopburi	20 adult worms	AS- PCR	BZ	F200Y	15% RR, 30% RS and 55% SS	Goat	
	Nakhon Sawan	3 adult worms	AS- PCR	BZ	F200Y	33% RR, 0% RS and 66% SS	Goat	
	Surat Thani	9 adult worms	AS- PCR	BZ	F200Y	88.9% RR, 11.1% RS and 0% SS	Goat	
	Nakhon Si Thammarat	15 adult worms	AS- PCR	BZ	F200Y	33.3% RR, 46.7% RS and 20% SS	Goat	
	Krabi	17 adult worms	AS- PCR	BZ	F200Y	43.9% RR, 41.5% RS and 14.6% SS	Goat	
Bangladesh	7 topographical zones	195 adult worms	AS- PCR	BZ	F200Y	6% RR (TAC), 59% RS (TAC/TTC) and 35% SS (TTC)	Slaughtered sheep and goat	(Dey *et al*., 2020)
Kashmir	Srinagar	200 Larvae	AS-PCR followed by nested PCR	BZ	N/A	52% RR on day 0 (Pre-treatment) and 100% RR (TAC/TAC) On day 7th (Post Treatment), 31% SS (TTC/TTC) and 17% RS (TAC/TTC)	Fecal sample and larval culture	(Bihaqi *et al*., 2020)
India	Ludhiana	50 Adult worms	AS-PCR	BZ	F200Y	(RR) showing 100% prevalence of resistant allele (r)	Slaughtered sheep	(Ekta *et al*., 2019)
	The western zone of Punjab	138 larvae	AS PCR	BZ	F200Y	49.28% RR, 3.62% SS and 46.37% RS population	Slaughtered sheep	
	Sattur, Pottaneri, Ooty and Hosur	673 larvae	AS- PCR	BZ	N/A	80.08 RR and 19.91 RS	Sheep	(Mohanraj *et al*., 2017)
	Northern Punjab	(23 population) 830 worms	Pyrosequencing followed by PCR	BZ	F200Y	Detected in 18 populations with allele frequency between 9–84%	Sheep and goat	(Chaudhry *et al*., 2015)
	Northern Punjab	(23 population) 830 worms	Pyrosequencing followed by PCR	BZ	E198A	Detected in 8 populations with allele frequency between 8–18%	Sheep and goat	
	Uttar Pradesh	300 larvae	AS- PCR	BZ	N/A	10-21% SS, 5-24% RS and 55–85% RR	Goat	(Chandra *et al*., 2015)
	Uttar Pradesh	300 larvae	AS- PCR	BZ	N/A	67–87.5% RR and 12.5–33% SS	Goat	
	Rohilkhand	30 larvae	AS-PCR followed by RFLP PCR	BZ	N/A	63% RR, 20% SS and 17% RS	Sheep	(Chandra *et al*., 2014)
	Bundelkhand	40 larvae	AS-PCR followed by RFLP PCR	BZ	N/A	85% RR, 10% SS and 5% RS	Sheep	
	Jaunpur	30 larvae	AS-PCR followed by RFLP PCR	BZ	N/A	53% RR, 27% SS and 20% RS	Sheep	
	Varanasi	30 larvae	AS-PCR followed by RFLP PCR	BZ	N/A	57% RR, 17% SS and 26% RS.	Sheep	
	North-eastern region and Eastern region farm areas	691 Larvae	AS- PCR	BZ	F200Y	RR 72.22% (north-eastern region) to RR 96.87% (eastern region) in farm flocks	Sheep and goat	(Maharshi *et al*., 2011)

Country	Region	No. of Haemonchus Worms/Larvae	Test Used	Drug Used	Polymorphism in the β-tubulin isotype 1 gene.	Results	Source of Sample Collection	Reference
	North-eastern region and Eastern region field areas	691 Larvae	AS- PCR	BZ	F200Y	61.79% RR (north-eastern region) to 95.83% RR (eastern region) in field flocks	Sheep and goat	
	Mukteshwar	40 adult worms	AS- PCR	BZ	F200Y	3% RR, 10% RS and 87% SS	Sheep	
	Pantnagar	40 adult worms	AS- PCR	BZ	F200Y	23% RR, 67% RS and 10% SS	Sheep	(Garg and Yadav, 2009)
	Rajasthan, India	160 adult worms	AS- PCR	BZ	F200Y	81.25% RR, 13.75% RS and 6.25% SS	Sheep	(Tiwari *et al.*, 2007)
	Rajasthan, India	160 adult worms	AS- PCR	BZ	F200Y	81.25% RR, 13.75% RS and 6.25% SS	Sheep	
	Rajasthan, India	160 adult worms	AS- PCR	BZ	F200Y	81.25% RR, 13.75% RS and 6.25% SS	Sheep	
	Bikaner region	54 Adult worms	RFLP-PCR	BZ	F200Y	54% RR, 33% RS and 17% SS	Sheep	(Tiwari *et al.*, 2006)
	Avikanagar	54 Adult worms	RFLP-PCR	BZ	F200Y	93% RR, 2% RS and 5% SS	Sheep	
	Mukteshwar	40 adult worms	AS- PCR	BZ	F200Y	3% RR, 10% RS and 87% SS	Sheep	
	Bikaner region	54 Adult worms	RFLP-PCR	BZ	F200Y	54% RR, 33% RS and 17% SS	Sheep	
	Avikanagar	54 Adult worms	RFLP-PCR	BZ	F200Y	93% RR, 2% RS and 5% SS	Sheep	
China	Qingyang Ravine and Xiazi Ravine	27 adult worms	Nested PCR	BZ	E198A	RR 11.11%, and SS 88.89%	Wild blue sheep	(Shen *et al.*, 2019)
	Helan Mountain	20 adult worms	Nested PCR	BZ	E198A	RR 7.50% and SS 92.50%	Domestic sheep	
	Hubei	24 adult worms	PCR coupled sequencing	BZ	E198A	SS 83% and RR 17%	Sheep	(Zhang *et al.*, 2016)
	Hubei	24 adult worms	PCR coupled sequencing	BZ	F200Y	SS 96% and RR 4%	Sheep	
	Inner Mongolia	24 adult worms	PCR coupled sequencing	BZ	E198A	SS 30% and RR 70%	Sheep	
	Inner Mongolia	24 adult worms	PCR coupled sequencing	BZ	F200Y	SS 81% and RR 19%	Sheep	
	Liangning	24 adult worms	PCR coupled sequencing	BZ	E198A	SS 60% and RR 40%	Sheep	
	Liangning	24 adult worms	PCR coupled sequencing	BZ	F200Y	SS 87.5% and RR 12.5%	Sheep	
	Heilongjiang	24 adult worms	PCR coupled sequencing	BZ	E198A	SS 96% and RR 4%	Sheep	
	Heilongjiang	24 adult worms	PCR coupled sequencing	BZ	F200Y	SS 92% and RR 8%	Sheep	
	Hebei	24 adult worms	PCR coupled sequencing	BZ	E198A	SS 90% and RR 10%	Goat	
	Hebei	24 adult worms	PCR coupled sequencing	BZ	F200Y	SS 98% and RR 2%	Goat	
	Yunnan	24 adult worms	PCR coupled sequencing	BZ	E198A	SS 54% and RR 46%	Goat	
	Yunnan	24 adult worms	PCR coupled sequencing	BZ	F200Y	SS 69% and RR 31%	Goat	
	Shaanxi	24 adult worms	PCR coupled sequencing	BZ	E198A	SS 100% and RR 0%	Goat	
	Shaanxi	24 adult worms	PCR coupled sequencing	BZ	F200Y	SS 100% and RR 0%	Goat	
	Guangxi	24 adult worms	PCR coupled sequencing	BZ	E198A	SS 65% and RR 35%	Goat	
	Guangxi	24 adult worms	PCR coupled sequencing	BZ	F200Y	SS 75% and RR 25%	Goat	
Pakistan	North-east Punjab	50 Adult worms	Purification and Sequencing	BZ	F200Y	74% SS and 26% RR	Sheep	(Irum *et al.*, 2014)

Homozygous resistant =RR, Homozygous susceptible = SS, Heterozygous = RS, ALLELE-SPECIFIC=AS, PCR= Polymerase Chain Reaction, RFLP= Restriction Fragment Length Polymorphism, BZ= Benzimidazole

Macrocyclic lactones resistance: Genetic modifications that induce a lower sensitivity of the parasite to the treatment are the main source of resistance to macrocyclic lactones (MLs), which include moxidectin, doramectin and ivermectin in *H. contortus*. Due to the recurrent use of MLs, which gives parasites with resistant alleles a survival advantage, these genetic alterations may be brought on by selection pressure (Lespine *et al.*, 2012;

Prichard *et al.*, 2012). Numerous researchers have inspected the mechanisms that lead to the development of resistance against macrocyclic lactones in *H. contortus*. Some reports concluded that the increased drug efflux, reduced drug uptake, metabolic degradation of the drug and altered drug targets are the reasons behind resistance (McKellar and Gokbulut, 2012). Target site mutation, which results in genetic alterations in the parasite's glutamate-gated chloride channel (GluCl), is the most often described cause of MLs resistance (Kerboeuf *et al.*, 2003).

While some studies have failed to find a correlation between these mutations and resistance (Williamson *et al.*, 2011). Additionally, mutations in the genes encoding γ-aminobutyric acid receptors and polymorphisms in the P-glycoprotein genes have also been associated with resistance. P-glycoprotein inhibitors have been shown to increase the efficacy of macrocyclic lactones against resistant *H. contortus* strains both in vitro and in vivo (Xu *et al.*, 1998; Sangster *et al.*, 1999; Mani, 2016). Overall, there is evidence of multiple mechanisms for the development of resistance to these drugs. Hence, to develop and manage appropriate antiparasitic control programs, all parties concerned should be aware of the possibility of interactions leading to multi-drug resistance. A summary of AR has been shown in Fig.1.

To address this issue, AR in *H. contortus* has been studied and reported in many parts of the world. The examination of resistant *Haemonchus* in the studies observed was through FECRT (Jabbar *et al.*, 2008; Saeed *et al.*, 2010; Islam *et al.*, 2018). Molecular techniques were also used to identify benzimidazole resistance and single nucleotide polymorphism (SNP) analysis in isotype-1 β tubulin-resistant *H. contortus* in addition to conventional methods. As a consequence, we examined the research findings on anthelmintic resistance in *H. contortus* in Asia.

In-Vivo/ Fecal egg count reduction test: By comparing the animal's pre- and post-treatment worm egg counts, one may assess a chemical's anthelmintic effectiveness. This test has undergone extensive standardization, enabling its widespread usage. FECRT states that resistance is present when both of the following conditions are satisfied: the reduction in egg count is less than 95% and the lower limit of the 95% confidence interval is equal to or less than 90% (Levecke *et al.*, 2018; Salgado *et al.*, 2019). Many studies have been reported from Asia in which the AR in sheep and goats was observed using the test fecal egg count reduction as mentioned in Table 1. In a study from Pakistan the anthelmintic efficacy of three commonly used anthelmintic, viz., oxfendazole, levamisole and ivermectin was observed in three breeds of dairy goats, i.e. Dera Din Panah, Pak Angora and Beetal kept at Government Livestock Farm. The result showed resistance to oxfendazole formulation. Whereas levamisole in two breeds and ivermectin in all breeds resulted in a decrease (P 0.05) in *H. contortus* (Jabbar *et al.*, 2008). Whereas, From Bangladesh (Dey *et al.*, 2020) reported *H. contortus* to be resistant to albendazole, levamisole and ivermectin in FECRT, as shown by the percentage decrease and 95% confidence intervals. Small ruminant farms in Malaysia were also examined to check the status of anthelmintic drug efficacy but unfortunately, all the anthelmintic groups showed a total failure to control *H. contortus* (Chandrawathani *et al.*, 2004).

In-Vivo/ Molecular test to detect benzimidazole resistance: Several benzimidazoles (BZ) resistance SNPs in parasitic worms have been detected utilizing molecular diagnostic testing employing a variety of techniques, including allele-specific (AS)-PCR, restriction fragment length polymorphism (RFLP) and pyrosequencing (Winterrowd *et al.*, 2003; von Samson-Himmelstjerna *et al.*, 2009; Mohanraj *et al.*, 2017). Additionally, alternative molecular techniques, such as restriction fragment length polymorphism-PCR, have been published for the identification and/or quantification of this SNP as well as the other two linked to BZ resistance (F167Y, E198A) (Tiwari *et al.*, 2006; Chandra *et al.*, 2014), Real-time PCR and Pyrosequencing (Chaudhry *et al.*, 2015). The molecular test was not always connected with the level of resistance, but it was able to distinguish well between resistant and susceptible isolates, according to a comparison of molecular data with other tests. As shown in Table 2. Several countries in Asia were observed to detect resistance through molecular means. Tiwari *et al.* (2007) diagnosed benzimidazole (BZ) resistance in *H. contortus* using Allele-specific PCR and observed 87 % of *Haemonchus* had the BZ resistant allele (R), compared to 13% who had the BZ susceptible allele (S). In another study from Kashmir, Allele-specific PCR was used resulting in 52% homozygous resistance on day 0 (Pre-treatment) and 100% Homozygous resistance (RR, TAC/TAC) On day 7th (Post Treatment), 31% homozygous susceptible (SS, TTC/TTC) and 17% heterozygous (RS, TAC/TTC) (Bihaqi *et al.*, 2020). In Turkey, qPCR along with sequencing was used and Point mutation (replacement of phenylalanine (TTC) to tyrosine (TAC) only at codon 200 was observed (Önder *et al.*, 2016). It has been demonstrated that molecular techniques might be utilized as a diagnostic tool to find BZ resistance in a population, even if they are unable to quantify the resistance to the same extent as FECRT (Kotze and Prichard, 2016). In reality, a mix of molecular and biological assays should be employed in the field. The use of genetic assays to identify BZ resistance in field samples made up of numerous worm species has not yet been thoroughly evaluated. In addition, fecal samples directly containing nematode eggs have not been subjected to molecular analyses, which might save time (Roeber *et al.*, 2013).

Strategies to delay resistance: Many management measures, including pasture management and refugia, are used to either avoid parasite infection or to maintain low infection pressure. This would lessen the need for anthelmintic, which might help to postpone the onset of AR (Shalaby, 2013). A farm can prevent the spread of resistance by treating newly purchased stock upon arrival and putting it into a quarantine period (Hosie and Clark, 2007). It can also prevent the spread of resistance by maintaining a population of worms that are susceptible to anthelmintic and by regularly testing for AR (Sarai *et al.*, 2014; Mehnaz *et al.*, 2023). Here are some strategies that can help delay anthelmintic resistance in *H. contortus*.

Targeted treatments: When helminth parasite populations are exposed to an anthelmintic medication, there is thought to be a risk of resistance development. Surprisingly, when using anthelmintic belonging to the same class often and at low doses, the chance of resistance development increases (Sargison, 2011). The development of resistance may be slowed by switching up the anthelmintic classes. The strategic use of anthelmintic should be based on the results of fecal egg count monitoring and targeted towards individual animals that have a high worm burden, rather than blanket treatment of the entire flock/herd. This helps to reduce the selection pressure for AR (Berk *et al.*, 2016).

Combination therapy: Using combination of anthelmintic with different modes of action can help prevent the development of resistance. This approach is particularly effective when combined with targeted treatment, as it helps to minimize the selection pressure for resistance (Leathwick *et al.*, 2012). Combinations lower the number of resistant genotypes that survive treatment and hence prevent the development of a resistant parasite population since several alleles conferring resistance to each of the various anthelmintic classes must be present in the same parasite for survival. Less people carry several resistance alleles than just one resistant allele (Van Wyk, 2001). Its increased effectiveness causes the unselected parasites in refugia to contaminate more resistant genotypes, decreasing the number of resistant parasites that can mate with other resistant adults that have endured treatment (Bartram *et al.*, 2012).

Pasture management and rotational grazing

The purpose of rotational grazing is to establish safe pastures for grazing. In order for a pasture to be considered safe, sheep or goats must not have been permitted to graze there for three months during hot, dry weather or six months during cool or cold weather. In order to lessen the possibility that delicate animals may come into touch with numerous infectious larvae, it is recommended to wean sheep and goats at two months of age and transfer them among pastures before the adults (Barger, 1999). The division of pastures into smaller lots will enable longer intervals between regrazing. Mismanaged pastures that have become severely polluted can be tilled and reseeded. As it impacts exposure to infectious larvae and pasture contamination, the stocking rate is a crucial factor in the management of parasites. Regularly rotating animals to different pastures can help to break the parasite life cycle and reduce the overall worm burden (Barger, 1999; Athanasiadou *et al.*, 2001; Niezen *et al.*, 2002). In addition, multispecies grazing also results in reducing the parasite burden, but there is limited information available on equine parasitology and most of the guidelines are acquired from ruminant studies (Nielsen *et al.*, 2018). This strategy can also help to reduce the selection pressure for resistance, as it reduces the exposure of parasites to the same anthelmintic repeatedly.

Using biological control is another excellent way to cut back on anthelmintic. The fundamental idea behind biological control is to reduce the amount of infection in pastures by employing natural enemies that may consume or destroy parasites (Larsen, 2006). Entomopathogenic fungi, such as the species in the genus *Hirsutella*, can infect and kill *Haemonchus* larvae. These fungi are used as a biological control agent to reduce parasite burdens in pastures. These therapies aim to reduce free-living larval stages to a level where their clinical or subclinical effects are minor while enhancing an acquired immune response, rather than to completely eradicate them (Waller *et al.*, 2004). It's important to note that while these strategies can help delay AR, they are not a guarantee. A comprehensive parasite management plan should be developed in consultation with a veterinarian or animal health professional.

Conclusions: *Haemonchus contortus* infection in small ruminants leads to substantial economic losses. This parasite is well-known to rapidly develop resistance towards routinely used classes of anthelmintics. The anticipated rise in prevalence in various countries and the severity of resistance in *H. contortus* to the available drugs make it hard for small ruminant farmers to control *H. contortus*. Keeping in view the rising trend of AR in *H. contortus*, in conclusion, our comprehensive review highlights the prevalence of resistance to major classes of anthelmintic with special emphasis on benzimidazole resistance-associated SNPs in the isotype-1 β-tubulin gene of *H. contortus* populations in small ruminants in Asia. This information can help inform the development of more effective strategies for the control of parasitic infections in small ruminants, to mitigate the impact of anthelmintic resistance on animal health and welfare, as well as on food security and livelihoods in rural communities. It is the need of the hour to develop better diagnostic methods, which will enable the producers to choose drugs or drug combinations that remain efficacious to prevent Haemonchosis. Furthermore, to curtail the resistance development, it is suggested to gain a deep insight into the mechanisms and genetics of AR.

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REFERENCES

Abbas RZ, Iqbal Z, Blake D, *et al.,* 2011a. Anticoccidial drug resistance in fowl coccidia:the state of play revisited. World Poult Sci J 67:337-50.

Abbas K, Li S, Xu D, *et al.*, 2020. Do socioeconomic factors determine household multidimensional energy poverty? Empirical evidence from South Asia. Energy Policy 146:111754.

Adduci I, Sajovitz F, Hinney B, *et al.*, 2022. Haemonchosis in Sheep and Goats, Control Strategies and Development of Vaccines against *Haemonchus contortus*. Anim 12:2339.

Ahmad S, Sajid MS, Tabassum R, et al., 2021. Comparative in vivo efficacy of oral formulations of ivermectin and levamisole against natural helminth infection in small ruminants. J Anim Plant Sci 31:77-85.

Alvi MA, Athar Ali RM, Asghar F, *et al*., 2023. Drug Resistance in Parasitic Infections, Parasitism and Parasitic Control in Animals:Strategies for the Developing World. CABI GB111-123.

Alvi MA, Ohiolei JA, Saqib M, *et al*., 2020. Echinococcus granulosus (sensu stricto)(G1, G3) and E. ortleppi (G5) in Pakistan:phylogeny, genetic diversity and population structural analysis based on mitochondrial DNA. Parasit Vectors 13:1-10.

Amana AM and Alkhaled MJ, 2023. Molecular evaluation of E198A SNP in the iso-type I β–tubulin gene of *Haemonchus contortus* isolated from sheep in Al-Diwanyiah, Iraq. Iraqi J Vet Sci 37:89-94.

Arsenopoulos KV, Fthenakis GC, Katsarou EI, *et al*., 2021. Haemonchosis:A challenging parasitic infection of sheep and goats. Animals 11:363.

Athanasiadou S, Kyriazakis I, Jackson F, *et al*., 2001. Direct anthelmintic effects of condensed tannins towards different gastrointestinal nematodes of sheep:in vitro and in vivo studies. Vet Parasitol 99:205-19.

Barger I, 1999. The role of epidemiological knowledge and grazing management for helminth control in small ruminants. Int J Parasitol 29:41-7.

Barrère V, Beech RN, Charvet CL, *et al*., 2014. Novel assay for the detection and monitoring of levamisole resistance in *Haemonchus contortus*. Int J Parasitol 44:235-41.

Bartram DJ, Leathwick DM, Taylor MA, *et al*., 2012. The role of combination anthelmintic formulations in the sustainable control of sheep nematodes. Vet Parasitol 186:151-8.

Berk Z, Laurenson YC, Forbes AB, *et al*., 2016. Modelling the consequences of targeted selective treatment strategies on performance and emergence of anthelmintic resistance amongst grazing calves. Int J Parasitol Drugs Drug Resist 6:258-71.

Besier R, Kahn L, Sargison N, *et al*., 2016a. Diagnosis, treatment and management of *Haemonchus contortus* in small ruminants. Adv Parasitol 93:181-238.

Besier R, Kahn L, Sargison N, *et al*., 2016b. The pathophysiology, ecology and epidemiology of *Haemonchus contortus* infection in small ruminants. Adv Parasitol 93:95-143.

Bibi R, Afshan K, Khan IA, *et al*., 2017. Phenotyping and Prevalence of *Haemonchus contortus* (Nematoda:Trichostongylidae) in Ruminants from Endemic Areas of Pakistan:Influence of Host Species and Geographical Area on Phenotypic Traits of Worms. Pak Vet J 37:170-4.

Bihaqi S, Allaie I, Banday M, *et al*., 2020. Multiple anthelmintic resistance in gastrointestinal nematodes of Caprines on Mountain Research Centre for Sheep and Goat at Kashmir Valley, India. Parasite Epidemiol Control 11:e00163.

Chandra S, Prasad A, Sankar M, *et al*., 2014. Molecular diagnosis of benzimidazole resistance in *Haemonchus contortus* in sheep from different geographic regions of North India. Vet World 7:337-41.

Chandra S, Prasad A, Yadav N, *et al*., 2015. Status of benzimidazole resistance in *Haemonchus contortus* of goats from different geographic regions of Uttar Pradesh, India. Vet Parasitol 208:263-7.

Chandrawathani P, Yusoff N, Wan L, *et al*., 2004. Total anthelmintic failure to control nematode parasites of small ruminants on government breeding farms in Sabah, East Malaysia. Vet Res Comun 28:479-89.

Chaudhry U, Redman EM, Raman M, *et al*., 2015. Genetic evidence for the spread of a benzimidazole resistance mutation across southern India from a single origin in the parasitic nematode *Haemonchus contortus*. Int J Parasitol. 45:721-8.

Dey AR, Begum N, Alim MA, *et al*., 2020. Multiple anthelmintic resistance in gastrointestinal nematodes of small ruminants in Bangladesh. Parasitol Int 77:102105.

Dixit A, Das G, Dixit P, *et al*., 2019. Efficacy of herbal extracts and closantel against fenbendazole-resistant *Haemonchus contortus*. J Helminthol 93:529-32.

Ekta S, Paramjit K, Singla L, *et al*., 2019. Molecular detection of benzimidazole resistance in *Haemonchus contortus* of sheep in Punjab. Ind J Anim Sci 89:1322-6.

Elard L, Comes AM and Humbert JF, 1996. Sequences of β-tubulin cDNA from benzimidazole-susceptible and-resistant strains of Teladorsagia circumcincta, a nematode parasite of small ruminants. Mol Biochem Parasitol 79:249-53.

Fauvin A, Charvet C, Issouf M, *et al*., 2010. cDNA-AFLP analysis in levamisole-resistant *Haemonchus contortus* reveals alternative splicing in a nicotinic acetylcholine receptor subunit. Mol Biochem Parasitol 170:105-7.

Fissiha W and Kinde MZ, 2021. Anthelmintic resistance and its mechanism:A review. Inf Drug Resist pp:5403-10.

Fthenakis G and Papadopoulos E, 2018. Impact of parasitism in goat production. Small Rum Res 163:21-3.

Gainza YA, Santos IBd, Figueiredo A, *et al*., 2021. Anthelmintic resistance of *Haemonchus contortus* from sheep flocks in Brazil:concordance of in vivo and in vitro (RESISTA-Test©) methods. Rev Bras de Parasitol Vet 30:e025120.

Gandasegui J, Onwuchekwa C, Krolewiecki AJ, *et al*., 2022. Ivermectin and albendazole coadministration:opportunities for strongyloidiasis control. Lancet Infect Dis 22:e341-7.

Garg R and Yadav C, 2009. Genotyping of benzimidazole susceptible and resistant alleles in different populations of *Haemonchus contortus* from Himalayan and sub-Himalayan regions of North-West India. Tropl Anim Health Prod 41:1127-31.

Hosie B and Clark S, 2007. Sheep flock health security. In Practice 29:246-54.

Hoste H and Chartier C, 2002. Control of gastrointestinal parasitism with nematodes in dairy goats treating the host category at risk. Vet Res 33:531-45.

Ilangopathy M, Palavesam A, Amaresan S, *et al*., 2019. Economic impact of gastrointestinal nematodes on meat production from sheep. Int J Livest Res 9:44-8.

Imran M, Alsayeqh AF, 2022. Antiparasitic Activity of Chemically Synthesized Magnesium Oxide Nanoparticles against Small Ruminant Haemonchosis. Adv. Life Sci. 9:348-54.

Imran M, Khan MN, Sajid MS et al., 2018. Comparative evaluation of natural resistance of dera din panah and nachi goat breeds towards artificial infection with *Haemonchus contortus*. Pak Vet J 38:389-93.

Imran M, Sajid MS, Abbas RZ *et al*., 2020. Immunological response of selected indigenous goat breeds of Pakistan towards artificial infection with *Haemonchus contortus*. Pak J Agric Sci, 57:879-86.

Irum S, Qayyum M and Lysoniewska K, 2014. Genetic variability in β-tubulin-1 in benzimidazole resistant *Haemonchus contortus* from sheep in North-East Punjab, Pakistan. Pak J Zool 46:431-5.

Islam S, Dey AR, Akter S, *et al*., 2018. Status of anthelmintic resistance of gastrointestinal nematodes in organized sheep and goat farms. Asian J Med Biol Res 4:378-82.

Jabbar A, Iqbal Z, Saddiqi HA, *et al*., 2008. Prevalence of multiple anthelmintic resistant gastrointestinal nematodes in dairy goats in a desolated tract (Pakistan). Parasitol Res 103:29-35.

Kaminsky R, Ducray P, Jung M, *et al*., 2008a. A new class of anthelmintics effective against drug-resistant nematodes. Nature 452:176-80.

Kaminsky R, Gauvry N, Schorderet Weber S, *et al*., 2008b. Identification of the amino-acetonitrile derivative monepantel (AAD 1566) as a new anthelmintic drug development candidate. Parasitol Res 103:931-9.

Kerboeuf D, Blackhall W, Kaminsky R, *et al*., 2003. P-glycoprotein in helminths:function and perspectives for anthelmintic treatment and reversal of resistance. Int J Antimicrob Agent 22:332-46.

Khan W, Al-Jabr O, Khan T, *et al*., 2021. Prevalence of gastrointestinal parasite in small ruminants of District Dir Upper Khyber Pakhtunkhwa Province of Pakistan. Braz J Biol 83.

Khanyari M, Suryawanshi KR, Milner-Gulland E, *et al*., 2021. Predicting parasite dynamics in mixed-use Trans-Himalayan pastures to underpin management of cross-transmission between livestock and bharal. Front Vet Sci 8:714241.

Kotze A and Prichard R, 2016. Anthelmintic resistance in *Haemonchus contortus*:history, mechanisms and diagnosis. Adv Parasitol 93:397-428.

Kotze AC, Gilleard JS, Doyle SR, *et al*., 2020. Challenges and opportunities for the adoption of molecular diagnostics for anthelmintic resistance. Int J Parasitol Drugs Drug Resist 14:264-73.

Kotze AC, Hunt PW, Skuce P, *et al*., 2014. Recent advances in candidate-gene and whole-genome approaches to the discovery of anthelmintic resistance markers and the description of drug/receptor interactions. Int J Parasitol Drugs Drug Resist 4:164-84.

Kuiseu J, Zinsou F, TA e, *et al*., 2021. Prevalence, effects and alternative control methods of *Haemonchus contortus* in small ruminants:A review. J Vet Med Anim Health 13:84-97.

Kwa M, Okoli M, Schulz-Key H, *et al*., 1998. Research note Use of P-glycoprotein gene probes to investigate anthelmintic resistance in *Haemonchus contortus* and comparison with Onchocerca volvulus. Int J Parasitol 28:1235-40.

Kwa MS, Veenstra JG and Roos MH, 1994. Benzimidazole resistance in *Haemonchus contortus* is correlated with a conserved mutation at amino acid 200 in β-tubulin isotype 1. Mol Biochem Parasitol 63:299-303.

Kwa MS, Veenstra JG, Van Dijk M, *et al*., 1995. β-Tubulin genes from the parasitic Nematode *Haemonchus contortus* Modulate drug resistance in Caenorhabditis elegans. J Mol Biol 246:500-10.

Larsen M, 2006. Biological control of nematode parasites in sheep. J Anim Sci 84:E133-E139.

Leathwick D, Waghorn T, Miller C, *et al*., 2012. Managing anthelmintic resistance–use of a combination anthelmintic and leaving some lambs untreated to slow the development of resistance to ivermectin. Vet Parasitol 187:285-94.

Lespine A, Ménez C, Bourguinat C, *et al*., 2012. P-glycoproteins and other multidrug resistance transporters in the pharmacology of anthelmintics:prospects for reversing transport-dependent anthelmintic resistance. Int J Parasitol Drugs Drug Resist 2:58-75.

Levecke B, Kaplan RM, Thamsborg SM, *et al*., 2018. How to improve the standardization and the diagnostic performance of the fecal egg count reduction test? Vet Parasitol 253:71-8.

Liang JB and Paengkoum P, 2019. Current status, challenges and the way forward for dairy goat production in Asia–conference summary of dairy goats in Asia. Asian-Australas J Anim Sci 32:1233-43.

Lubega GW and Prichard RK, 1990. Specific interaction of benzimidazole anthelmintics with tubulin:high-affinity binding and benzimidazole resistance in *Haemonchus contortus*. Mol Biochem Parasitol 38:221-32.

Ma J, He S, Li H, *et al*., 2014. First survey of helminths in adult goats in Hunan Province, China. Trop Biomed 31:261-9.

Maharshi A, Swarnkar C, Singh D, *et al*., 2011. Correlation between status of benzimidazole resistance in *Haemonchus contortus* on bio and molecular assays. Indian J Anim Sci 81:110.

Mahmoud H, Ali A, Khalil A, *et al*., 2022. The infection rate of Fasciola and Anaplasma in cattle and buffaloes in Qena, Egypt. Int J Vet Sci 11:308-14.

Malik MA, Sajid MS, Shamim A, et al., 2020. Comparative efficacy of oral formulations of Ivermectin and Levamisole under In Vitro conditions against *Haemonchus contortus*. Pak J Sci 72:48-53.

Mani T, 2016. Macrocyclic Lactone Interactions with a P-Glycoprotein and Polymorphism in ABC Transporter and Ion Channel Genes in Dirofilaria immitis. McGill University (Canada).

Maske D, Bhilegaonkar N and Sardey M, 1990. Prevalence of parasitic infections in domestic animals at Nagpur (Maharashtra). J Vet Parasitol 4:23-5.

Mazinani M and Rude B, 2020. Population, world production and quality of sheep and goat products. Am J Anim Vet 15:291-9.

McKellar QA and Gokbulut C, 2012. Pharmacokinetic features of the antiparasitic macrocyclic lactones. Curr Pharm Biotechnol 13:888-911.

McRae KM, McEwan JC, Dodds KG, *et al*., 2014. Signatures of selection in sheep bred for resistance or susceptibility to gastrointestinal nematodes. BMC Genoms 15:1-13.

Mederos AE, Ramos Z and Banchero GE, 2014. First report of monepantel *Haemonchus contortus* resistance on sheep farms in Uruguay. Parasit Vectors 7:1-4.

Mehnaz S, Abbas RZ, Kanchev K, *et al*., 2023. Natural control perspectives of *Dermanyssus gallinae* in poultry. Int J Agri Biosci 12:136-42.

Mehmood K, Zhang H, Sabir AJ, *et al*., 2017. A review on epidemiology, global prevalence and economical losses of fasciolosis in ruminants. Microb Path 109:253-62.

Mohammedsalih KM, Krücken J, Khalafalla A, *et al*., 2020. New codon 198 β-tubulin polymorphisms in highly benzimidazole resistant *Haemonchus contortus* from goats in three different states in Sudan. Parasit Vectors 13:1-15.

Mohanraj K, Subhadra S, Kalyanasundaram A, *et al*., 2017. Genotyping of benzimidazole resistant and susceptible isolates of *Haemonchus contortus* from sheep by allele-specific PCR. J Parasit Dis 41:282-8.

Muhammad A, Ahmed H, Iqbal MN, *et al*., 2015. Detection of multiple anthelmintic resistance of *Haemonchus contortus* and Teladorsagia circumcincta in sheep and goats of Northern Punjab, Pakistan. Kafkas Univ Vet Fak Derg 21:389-95.

Nazish A, Khattak B, Ali Khan T, *et al*., 2021. Antinematode activity of abomasum bacterial culture filtrates against *Haemonchus contortus* in small ruminants. Animals 11:1843.

Neveu C, Charvet CL, Fauvin A, *et al*., 2010. Genetic diversity of levamisole receptor subunits in parasitic nematode species and abbreviated transcripts associated with resistance. Pharmacogenet Genom 20:414-25.

Nielsen M, Branan M, Wiedenheft A, *et al*., 2018. Parasite control strategies used by equine owners in the United States:a national survey. Vet Parasitol 250:45-51.

Niezen J, Waghorn G, Graham T *et al*., 2002. The effect of diet fed to lambs on subsequent development of Trichostrongylus colubriformis larvae in vitro and on pasture. Vet Parasitol 105:269-83.

Önder Z, Yildirim A, Inci A, *et al*., 2016. Molecular prevalence, phylogenetic characterization and benzimidazole resistance of *Haemonchus contortus* from sheep. Kafkas Univ Vet Fak Derg 22:93-9.

Pant K, Rajput M, Kumar J, *et al*., 2009. Prevalence of helminthes in small ruminants in Tarai region of Uttarakhand. Vet World 2:265.

Parvin S, Dey AR, Rony SA, *et al*., 2022. Frequency of benzimidazole resistance in *Haemonchus contortus* populations isolated from sheep and goats in Bangladesh. Annals Parasitol 68:563-8.

Pawar P, Das Singla L, Kaur P, *et al*., 2019. Evaluation and correlation of multiple anthelmintic resistances to gastrointestinal nematodes using different fecal egg count reduction methods in small ruminants of Punjab, India. Acta Parasitol 64:456-63.

Pinilla JC, Gutierrez A and Florez AA, 2022. Canine visceral Leishmaniasis in Colombia resistant to the treatment of choice (Meglumine Antimoniate plus allopurinol). Int J Vet Sci 11:117-20.

Pitaksakulrat O, Chaiyasaeng M, Artchayasawat A, *et al*., 2021. The first molecular identification of benzimidazole resistance in *Haemonchus contortus* from goats in Thailand. Vet World 14:764.

Potârniche AV, Mickiewicz M, Olah D, *et al*., 2021. First report of anthelmintic resistance in gastrointestinal nematodes in goats in Romania. Anim 11:2761.

Preston S, Jiao Y, Jabbar A, *et al*., 2016. Screening of the 'Pathogen Box'identifies an approved pesticide with major anthelmintic activity against the barber's pole worm. Int J Parasitol: Drugs and Drug Resistance. 6:329-34.

Prichard R, Ménez C and Lespine A, 2012. Moxidectin and the avermectins:consanguinity but not identity. Int J Parasitol Drugs Drug Resist 2:134-53.

Qamar W, Zaman MA, Faheem M, *et al*., 2022. Molecular Confirmation and Genetic characterization of *Haemonchus contortus* Isolates at the Nuclear Ribosomal ITS2 Region:First Update from Jhang Region of Pakistan. Pak Vet J 42:251-5.

Rehman S, Afshan K, Razzaq A, *et al*., 2020. Anthelmintic Efficiency of Synthetic and herbal Compounds Against Gastrointestinal Nematodes in Naturally Infected Goats. Kafkas Univ Vet Fak Derg 26:813-6.

Rialch A, Vatsya S and Kumar RR, 2013. Detection of benzimidazole resistance in gastrointestinal nematodes of sheep and goats of sub-Himalyan region of northern India using different tests. Vet Parasitol 198:312-8.

Roeber F, Jex AR and Gasser RB, 2013. Advances in the diagnosis of key gastrointestinal nematode infections of livestock, with an emphasis on small ruminants. Biotechnol Adv 31:1135-52.

Rothwell J and Sangster N, 1997. *Haemonchus contortus*:the uptake and metabolism of closantel. Int J Parasitol 27:313-9.

Rufener L, Kaminsky R and Mäser P, 2009. In vitro selection of *Haemonchus contortus* for benzimidazole resistance reveals a mutation at amino acid 198 of β-tubulin. Mol Biochem Parasitol168:120-2.

Saddiqi HA, Jabbar A, Sarwar M, *et al*., 2011. Small ruminant resistance against gastrointestinal nematodes:a case of *Haemonchus contortus*. Parasitol Res. 09:1483-500.

Saeed M, Iqbal Z, Jabbar A, *et al*., 2010. Multiple anthelmintic resistance and the possible contributory factors in Beetal goats in an irrigated area (Pakistan). Res Vet Sci 88:267-72.

Sager H, Hosking B, Bapst B, *et al*., 2009. Efficacy of the amino-acetonitrile derivative, monepantel, against experimental and natural adult stage gastrointestinal nematode infections in sheep. Vet Parasitol159:49-54.

Şahin Ö, Aytekin İ, Boztepe S, *et al*., 2021. Relationships between FAMACHA© scores and parasite incidence in sheep and goats. Trop Anim Health Prod 53:331.

Salgado JA, Cruz LV, Rocha Lod, *et al*., 2019. Implication of the fecal egg count reduction test (FECRT) in sheep for better use of available drugs. Rev Bras DE Parasitol Vet 28:700-7.

Sangster NC, Bannan SC, Weiss AS, *et al*., 1999. *Haemonchus contortus*:Sequence heterogeneity of internucleotide binding

domains from p-glycoproteinsand an association with avermectin/milbemycin resistance. Exp Parasitol 91:250-7.

Sarai RS, Kopp SR, Coleman GT, *et al*., 2014. Drug-efflux and target-site gene expression patterns in *Haemonchus contortus* larvae able to survive increasing concentrations of levamisole in vitro. Int J Parasitol Drugs Drug Resist 4:77-84.

Sarai RS, Kopp SR, Knox MR, *et al*., 2015. In vitro levamisole selection pressure on larval stages of *Haemonchus contortus* over nine generations gives rise to drug resistance and target site gene expression changes specific to the early larval stages only. Vet Parasitol 211:45-53.

Sargison N, 2020. The critical importance of planned small ruminant livestock health and production in addressing global challenges surrounding food production and poverty alleviation. NZ Vet J 68:136-144.

Sargison ND, 2011. Pharmaceutical control of endoparasitic helminth infections in sheep. Vet Clin:Food Anim Pract 27:139-56.

Selzer PM and Epe C, 2021. Antiparasitics in animal health:Quo Vadis? Trends Parasitol 37:77-89.

Shakya P, Jayraw A, Shakya M, *et al*., 2018. Anthelmintic resistance in gastrointestinal nematodes of goats in an organized farm from Mhow, Madhya Pradesh. J Vet Parasitol 2:9-13.

Shalaby HA, 2013. Anthelmintics resistance; how to overcome it? Iran J Parasitol 8:18.

Shamim A, Sajid MS, Khan MN, et al., 2018. Peptides isolation from crude somatic antigens of *Haemonchus contortus* through SDS-PAGE. Indian J Anim Res 52:914-6.

Shen D-d, Peng Z-w, Hu M, *et al*., 2019. A detection of benzimidazole resistance-associated SNPs in the isotype-1 β-tubulin gene in *Haemonchus contortus* from wild blue sheep (Pseudois nayaur) sympatric with sheep in Helan Mountains, China. BMC Vet Res 15:1-9.

Swan G, 1999. The pharmacology of halogenated salicylanilides and their anthelmintic use in animals. J S Afr Vet Assoc 70:61-70.

Tiwari J, Kolte A, Kumar S, *et al*., 2007. Diagnosis of benzimidazole resistance in *Haemonchus contortus* of sheep by allele specific PCR. Asian-australas J Anim Sci. 20:7-11.

Tiwari J, Kumar S, Kolte A, *et al*., 2006. Detection of benzimidazole resistance in *Haemonchus contortus* using RFLP-PCR technique. Vet Parasitol 138:301-7.

Turnbull F, Devaney E, Morrison AA, *et al*., 2019. Genotypic characterisation of monepantel resistance in historical and newly derived field strains of Teladorsagia circumcincta. Int J Parasitol Drugs Drug Resist 11:59-69.

Van den Brom R, Moll L, Kappert C, *et al*., 2015. *Haemonchus contortus* resistance to monepantel in sheep. Vet Parasitol. 209:278-80.

Van Wyk JA, 2001. Refugia-overlooked as perhaps the most potent factor concerning the development of anthelmintic resistance**.** http://hdl.handle.net/2263/18482

von Samson-Himmelstjerna G, Walsh T, Donnan A, *et al*., 2009. Molecular detection of benzimidazole resistance in *Haemonchus contortus* using real-time PCR and pyrosequencing. Parasitol 136:349-58.

Waller P, Schwan O, Ljungström B-L, *et al*., 2004. Evaluation of biological control of sheep parasites using Duddingtonia flagrans under commercial farming conditions on the island of Gotland, Sweden. Vet Parasitol 126:299-315.

Wang C, Li F, Zhang Z, *et al*., 2017. Recent research progress in China on *Haemonchus contortus*. Front Microbiol 8:1509.

Wang C, Qiu J, Zhu X, *et al*., 2006. Survey of helminths in adult sheep in Heilongjiang Province, People's Republic of China. Vet Parasitol 140:378-82.

Widiarso BP, Kurniasih K, Prastowo J, *et al*., 2018. Morphology and morphometry of *Haemonchus contortus* exposed to Gigantochloa apus crude aqueous extract. Vet World 11:921.

Williamson SM, Storey B, Howell S, *et al*., 2011. Candidate anthelmintic resistance-associated gene expression and sequence polymorphisms in a triple-resistant field isolate of *Haemonchus contortus*. Mol Biochem Parasitol 180:99-105.

Winterrowd C, Pomroy W, Sangster NC*, et al*., 2003. Benzimidazole-resistant β-tubulin alleles in a population of parasitic nematodes (Cooperia oncophora) of cattle. Vet Parasitol 117:161-72.

Xu M, Molento M, Blackhall W, *et al*., 1998. Ivermectin resistance in nematodes may be caused by alteration of P-glycoprotein homolog. Mol Biochem Parasitol 91:327-35.

Yang X, Gasser RB, Fang R, *et al*., 2016. First survey of parasitic helminths of goats along the Han River in Hubei Province, China. Acta Parasitol. 61:602-6.

Yuan W, Lu K, Li H, *et al*., 2019. Seasonal dynamics of gastrointestinal nematode infections of goats and emergence of ivermectin resistance in *Haemonchus contortus* in Hubei Province, China. Acta Parasitol 64:638-44.

Zen Sd, Santos M and Monteiro C, 2014. Evolução da caprino e ovinocultura. Boletim Ativos da Pecuária de Caprino e Ovinocultura 9:1-3.

Zhang Z, Gasser RB, Yang X, *et al*., 2016. Two benzimidazole resistance-associated SNPs in the isotype-1 β-tubulin gene predominate in *Haemonchus contortus* populations from eight regions in China. Int J Parasitol Drugs Drug Resist 6:199-206.