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An the lminthic Drug Resistance in Veterinary and Human Helminths

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Summary

The majority of currently available anthelmintihcs used to control parasitic nematodes of cattle and sheep belong to only three main groups, the benzimidazoles, imidazothiazoles and the avermectins/ milbemycins. Most worm control strategies currently advocated rely heavily on the use of anthelminthics. However, the regular use of any chemical to control infective organisms poses the risk of resistance development. This development of resistance to anthelminthic drugs has caused decreased productivity of livestock and threatens the success of treatment in humans. This dramatic and rapid spread of resistance to all major classes of veterinary anthelmintics should be a warning against too strong a reliance on drugs in human helminth control programmes.

Keywords :
Helminths; Anthelminthics; Drug resistance ; Veterinary; Human

Introduction

Helminths are a diverse group of parasitic worms, encompassing nematodes, cestodes and trematodes, and are a major health problem for humans and animals in many parts of the world. Helminth infections are estimated to have a disease burden equivalent to 25% of that of HIV/AIDS and 50% of that of malaria (Grant *et al.,* 2010). Although their disease impact could be reduced dramatically by improved sanitation for humans and pasture control in domestic animals, such methods are not sufficient to eradicate these parasites. In the absence of vaccines, control of these parasites is reliant on chemotherapy to ease symptoms and reduce transmission (Catherine *et al.,* 2007).

Although drug treatment is regarded as the most efficacious of controlling parasitic infections , the reality is that drug resistance to all the anthelminthic groups is spreading all-over the world. Also, reinfection after drug therapy is now a common phenomenon in addition to anthelminthic resistance (AR) problem (Grant *et al.,* 2010).

At present, AR is the most important disease problem of the sheep farming industry in Australia, South Africa and possibly South America. High prevalence of AR, often exceeding 50 %, have now been reported in all parts of the world for gastro-intestinal helminths of sheep, goats and horses kept in industrial livestock systems. Surprisingly, up to now very little problems with AR have been noticed in cattle helminths (Geerts and Gryseels, 2000). In humans, there is now increasing reliance on mass drug administration programs, and recent reports suggest that, as with animal parasites, resistance is beginning to emerge. In addition, in recent years, several reports of apparent failures in the treatment of human schistosomes and nematodes have been published. These evidences have led to an increased awareness of the potential problem of AR in the treatment

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and control of human helminths (Catherine *et al.,* 2007).

In this paper, the reports on AR in livestock helminths is indicated. The possible factors which contribute to the development of AR in livestock and man are discussed and conclusions drawn from the mistakes in the treatment and control of livestock helminths.

Mechanism of anthelminthic drug resistance

Anthelminthic resistance has become a serious problem, worldwide, in helminth parasites of farm animals and horses. Understanding the mechanisms and genetics of anthelminthic resistance is important to efforts to overcome resistance, to efforts to slow the spread of resistance parasites, to delay the development of resistance to new anthelminthic drugs, and to better manage parasite control, including using anthelminthic combinations, with existing anthelminthics (Prichard, 2008).

There are several phases in the process of resistance development. Firstly, there is an initial phase of susceptibility where the number of resistant individuals within the parasite population is low. With continued exposure to the same drug group, an intermediate phase then follows in which the frequency of heterozygous resistant individuals within the population increases. Finally, sustained selection pressure results in a resistant phase where homozygous resistant individuals predominate within the population. (Coles *et al.,* 1994).

In principle, drug resistance can arise in a limited number of ways (i) a change in the molecular target, so that the drug no longer recognizes the target and is thus ineffective; (ii) a change in metabolism that inactivates or removes the drug, or that prevents its activation; (iii) a change in the distribution of the drug in the target organism that prevents the drug from accessing its site of action; or (iv) amplification of target genes to overcome drug action (Adrian *et al.,* 2004**).**

Factors contributing to the development of drug resistance

Treatment frequency

This is an important determinant of the speed of selection of AR: the greater the drug pressure, the faster the selection of resistant nematode strains. Treatment frequencies of 5 or more a year (up to 10/year) are not uncommon in livestock (Dorny *et al.,* 1994); in humans the frequency of treatments is limited to 1 to 3 per year for STH's (Renganathan *et al.,* 1995). However, even at these lower treatment frequencies, selection of AR has been repeatedly reported in sheep and goat nematodes (Boudsocq *et al.,* 1999).

This is especially the case when the same drug has been used over prolonged periods, as is the case with BZ's in the control of STH's, these lower treatment frequencies might be able to select for resistance. This has been clearly shown in nematodes of livestock, where farmers tend to use a single drug until it fails (Reinemeyer *et al.,* 1992).

Refugia

Refugia are the proportion of the parasite population that is not exposed to drugs and thus escapes selection for resistance. It is a very important factor whose impact on the development of AR is too often overlooked (Van Wyk, 2001). The size of refugia will be mainly determined by (1) the fraction of the population treated (i.e. mass treatments versus selective or targeted selective treatments) and (2) the proportion of the worm population present in the environment where it is not subject to drug action (e.g. in the soil) (Chitsulo *et al.,* 2000).

The size of refugia is also largely determined by factors such as the timing of the treatment and the climate immediately prior to treatment as both will influence the selection pressure.

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The generation of parasites, which develops after treatment in dry environments, may completely consist of a high proportion of resistant worms because climatic conditions will kill previously deposited eggs and larvae. However, in wetter environments, pre-parasitic stages of susceptible worms might survive on pasture and dilute the resistant genes in the next worm generation. However, the relevance of the timing of treatment on the refugia for the human STH has not been studied yet. Also, recommending treatment at certain times when a high proportion of parasites are in refugia in the environment would delay the spread of resistance but on the other hand would increase the rate of reinfection and reduce the effects of treatment, so a compromise had to be achieved (WHO, 2008).

Under dosing

Under dosing may constitute an important risk factor for the development of AR. The impact depends on the initial (before exposure to a given anthelmintic) and the resultant (after treatment) frequency of resistance alleles in the helminth population. Depending on the initial frequency of the resistance alleles, there might be a range of dose levels where under dosing promotes resistance and a range of dose levels where it actually impedes resistance (WHO, 2008).

Most of the currently applied anthelminthics are in fact sub curative in at least part of the population. This is considered acceptable for morbidity control, but in the long run such strategies may contribute to the development of AR as well. Assuming that resistance is determined by a single major gene comprising two alleles at a single autosomal locus and low initial frequency of the allele for resistance, the most dangerous dose is the one that kills all susceptible homozygotes but none of the heterozygous or homozygous resistant genotypes. In contrast, when the initial frequency of the allele for resistance is high, the dose, which promotes resistance most strongly, is that which kills all susceptible homozygotes and all heterozygotes, but none of the resistant homozygotes (Smith *et al.,* 1999).

To reduce the costs of anthelminthic treatment campaigns in developing countries, the use of lower dosages than the recommended therapeutic ones has been advocated (WHO, 2008). Drugs are commonly shared or used at half (or less) the normal doses by poor families. Furthermore, generic products of substandard quality, repacked and/or reformulated products, and expired drugs are widespread in pharmacies and general markets. Also, the presence of poor-quality drugs has been documented in human as well as in veterinary medicine (Monteiro *et al.,* 1998). Human drugs, especially antibiotics and anthelminthics, are produced by a large number of unlicensed companies all over the world. Quality control of these drugs is usually is not a common phenomena.

Anthelminthic resistance detection methods

Fecal egg count reduction test (FECRT)

The FECRT provides an estimation of anthelminthic efficacy by comparing faecal egg counts of animals before and after treatment (Presidente, 1985). If the interval between treatments is less than 10 days, egg production may be suppressed leading to an overestimation of anthelminthic efficacy with the benzimidazole anthelminthics (Hotson *et al.,* 1970). For this reason, the recommendation is to collect faecal samples 10–14 days after treatment (Coles *et al.,* 1992). When levamisole resistance is suspected, faecal samples should be taken at less than 7 days post-treatment. However, the FECRT may not provide sufficient information on its own for correct interpretation. It also appears that the test lacks the sensitivity to detect levels of resistance below 25% (Martin *et al.,* 1989**).**

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The controlled test

This test is the most reliable method of assessing anthelminthic efficacy (Presidente, 1985) but also the most costly in terms of labor requirements and animal usage (Boersema, 1983) and is now rarely used. In an attempt to reduce the costs and time taken, laboratory animal models have been used (Kelly *et al.,* 1982). To characterize the sensitivity of a field isolate, groups of worm free animals should be inoculated with infective larvae and the anthelmintic tested at 0.5, 1 and 2 times the recommended dose rate (Presidente, 1985). Inclusion in the test of a known susceptible strain has been recommended (Martin, 1982). With mixed worm populations, culturing needs to take account of the differential development of certain species. Resistance is generally confirmed when the reduction in geometric mean worm counts is less than 90%, or greater than 1000 worms survive treatment (Presidente, 1985).

Egg hatch assays (EHAs)

Benzimidazole anthelminthics prevent embryonation and hatching of nematode eggs. A number of egg hatch/embryonation assays have been developed for the detection of resistance to this group of anthelminthic. The essential aim is to incubate undeveloped eggs in serial concentrations of the anthelminthic, usually thiabendazole because it is the most soluble. The percentage of eggs that hatch (or conversely die) at each concentration is determined, corrected for natural mortality from control wells, and a dose-response line plotted against drug concentration. One effect prevented embryonation; the other prevented hatching. Variation can occur in dose-response lines and ED values if only hatched larvae are counted, as compared to hatched larvae and embryonated eggs (Scott *et al.,* 1989).

As is the case with the FECRT, the EHA lacks the sensitivity to detect levels if resistance is below 25% (Martin *et al.,* 1989). The requirement for undeveloped eggs in vitro EHAs has been a major obstacle to the application of the EHA in routine diagnosis. As development proceeds beyond the ventral indentation stage, a false positive result may be obtained because sensitivity to thiabendazole decreases as embryonation proceeds (Weston *et al.,* 1984).

Larval development tests (LDTs)

Most anthelminthics affect the metabolism of a parasite in some way and as such the effects of anthelminthics on parasite growth, offer potential ways of detecting resistance. ALDT was described in which first stage larvae were cultured to third stage larvae in the presence of heat treated lyophilized *Escherichia coli*, as a food source, and the anthelminthic under test (Coles *et al.,* 1988). Suitable controls were also run without the presence of anthelminthic. It was concluded that the test could be run with any anthelminthic to which resistance was suspected. It was considered rapid, reliable, inexpensive and suitable for use in the field investigation of anthelminthic resistance. Also, because the test required first stage larvae, there was no pre-requisite for undeveloped eggs or fresh faecal samples (Coles, 1988). The test was further used for the detection of ivermectin resistance (Giordano *et al.,* 1988). A variation of this test using yeast extract as a food source has been described (Taylor, 1990).

Adult development test

In vitro techniques for the culture of trichostrongylid nematodes have been reported. For example, *Haemonchus contortus* has been cultured through to the adult, egg laying stage (Stringfellow, 1986). In vitro adult developmental assays for detecting benzimidazole resistance in *H. contortus*, based on these in vitro culture techniques, have been described (Stringfellow, 1988). There has been little further progress in this area, however, due to the complexity of the culture techniques required.

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Biochemical tests

The mechanism of benzimidazole resistance appears to be associated with a reduced affinity of tubulin for the anthelminthic (Sangster *et al.,* 1985). Based on these studies, Lacey and Snowden (1988**)** described a diagnostic assay for the detection of benzimidazole resistant nematodes using the binding of tritiated benzimidazole carbamates to tubulin extracts of third stage larvae. The assay is claimed to be rapid, robust, highly reproducible and sensitive to minor changes in the resistance status of parasite populations, but it requires relatively large numbers of larvae making it unsuitable for routine field assays.

Biochemical assays comparing non-specific esterases and acetylcholinesterases of benzimidazole resistant and susceptible trichostrongylid nematode strains have also been described (Sutherland *et al.,* 1988). In these studies a simple colorimetric assay was developed in which samples were compared either by visual examination or through the use of a densitometer. Significantly greater esterase or acetylcholinesterase activity was found in the benzimidazole resistant strains.

Polymerase chain reaction (PCR)

The first specific primers to detect drug resistant parasite nematodes were developed by (Kwa et al., 1994). These primers discriminated between heterozygous and homozygous BZ-resistant *H. contortus* for the alleles in question (beta-tubulin isotype 1), even when these genotypes are phenotypically indistinguishable, and could also identify BZ-resistant *T. colubriformis.* According to Roos *et al* (1995), PCR detected 1% of resistant individuals within susceptible worm population, a tremendous improvement over other in vivo and in vitro tests.

Recently, Elard *et al* (1999) developed a more simplified method for the diagnosis of BZ resistant *O. (Telodorsagia) circumcincta*. Using four primers (2 allele-specific and 2 allele-non-specific ones) in the same PCR, adult worms were characterized as to the mutation of the residue 200 of the isotype 1 beta-tubulin. The technique has now been refined for use on a single worm, egg or larva. Since the frequencies of alleles associated with anthelminthic drug resistance might be quite high even in susceptible populations, it is indeed important to examine DNA from individual parasites. If DNA is prepared from pooled parasites, the association between particular alleles is likely to be obscured (Anderson *et al.,* 1998).

As the same mutation is responsible for BZ resistance in many parasitic nematodes, this method may provide a means for investigation of the frequencies of alleles bearing it in a wide range of animal and human intestinal nematodes.

Drug resistance in human helminths

Drug resistance in nematodes

The main drugs used to treat human nematodes nowadays are mebendazole, albendazole, pyrantel pamoate, and levamisole for intestinal nematodes, ivermectin (IVM) for onchocerciasis, and DEC alone or DEC-albendazole and IVM-albendazole combination treatments for filariasis (Albonico *et al.,* 1999). Depending on local epidemiology, availability, and cost, these drugs have been widely available in most health care systems for the curative treatment of clinical cases for many years. In addition, the use of anthelminthics is now being strongly advocated in a preventive, population-based as well (Bundy and De Silva, 1998).

It is estimated that some 1.3 to 2.0 billion people in the world suffer from helminth infections. Although direct mortality is low, intestinal helminth infections are believed to contribute to "general morbidity." By providing single-dose anthelminthics on a regular basis to entire populations or high-risk

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groups such as: schoolchildren and pregnant women; it is hoped to reduce both morbidity and transmission. It has even been proposed to combine albendazole, IVM, and praziquantel (PZQ) at a low dose in a single tablet and to distribute it to virtually all school-age children in the developing world **(**Warrens, 1990 and 1993**).** Hence, anthelminthic drugs remain the principal means of intervention for therapy and prophylaxis of nematode parasitic diseases in humans and animals.

There are many chemotherapeutic trials in humans that show differing efficacies of the same drug. This is a cause for concern because it could indicate that natural tolerance to anthelminthic agents exists in some populations of nematodes and, with mass therapy, full resistance could develop rapidly. Two well-conducted trials on hookworms have given good evidence for benzimidazole resistance in *Necator americanus* in West Africa (De clercq *et al.,* 1997) and pyrantel resistance in *Ancylostoma duodenale* in Australia **(**Reynoldson *et al.,* 1997). Without new anthelminthics hookworms could become difficult to treat. Also there have been two reports of failure in the treatment of human hookworm infection, involving mebendazole in Mali and Pyrantel in north-west Australia. In both studies, the anthelminthics were observed to be of low efficacy (WHO, 2002**).**

Drug resistance in schistosomes

The eradication of schistosomiasis or its elimination by multiple, integrated intervention techniques is beyond the human and financial resources of most endemic countries. However, a reduction in disease or morbidity due to schistosomiasis is now feasible and can be attained within the limited resources of many endemic countries. The simplicity of diagnostic techniques and the safety and ease of administering effective anti-schistosomal drugs such as praziquantel makes disease or morbidity control affordable, particularly if it is integrated with other programmes. Morbidity control should be coupled with transmission control such as sanitation, health education, provision of safe water supplies and snail control (http://www.wpro.who.int/NR/rdonlyres).

The three drugs currently recommended by the WHO for the use against schistosomes are praziquantel which is effective against all species and to a lesser extent metrifonate and oxamniquine which are effective against *S. haematobium* and *S. mansoni*, respectively. So far no evidence of drug resistance to metrifonate has been reported. In the case of oxamniquine (and the closely related hycanthone), drug resistance has been encountered both in the field and laboratory since 1970s. Cross resistance has not been found between praziquantel, oxamniquine and metrifonate. Any infection uncured by one drug may still be successfully treated with an appropriate alternative drug (WHO, 1996**).**

Systematic mass therapy of infection with human schistosomes is not widely used and resistance has not become a practical issue, although individual cases of resistance to oxamniquine have been known for several years. Because of its excellent activity praziquantel is now the preferred drug and has been used heavily in China and Egypt. The first signs of resistance to praziquantel are emerging in Egypt, where a small percentage of patients do not respond to therapy and isolates tested in mice also show resistance (Ismail *et al.,* 1996).

Drug resistance associated with the treatment of human schistosomiasis appears to be an emerging problem requiring more attention from scientific community than the subject currently receives. According to the studies, some of isolated strains of *S. mansoni* lack susceptibility for antischistosomal drugs. Strains of *S. mansoni* have now been identified from Brazil which is resistance to oxamniquine**,** hycanthone and niridazol; from

Puerto Rico which are resistance to hycanthone and oxamniquine; and from Kenya which are resistance to niridazol and probably oxamniquine (John *et al.,* 1987).

The recent reports on possible emerging drug resistance in human nematodes and schistosomes do not provide conclusive evidence, neither for the increase of innately tolerant strains nor for the appearance of newly mutated resistant strains. However, they strongly suggest that such tolerant or resistant strains can and do exist, and that these strains may emerge more prominently under drug pressure (hookworm in Australia, schistosomes in Egypt), or under specific circumstances (schistosomes in Senegal) (Geerts and Gryseels, 2000).

Conclusion and Recommendations

Under intensive systems of animal management, most worm control strategies currently depend on the use of anthelmintics. However, the regular use of any chemical to control infective organisms leads for the development of resistant populations in livestock's. But, there is as yet no unequivocal evidence that resistance to commonly used anthelminthics in humans is an emerging problem, either through new mutations or by the selection of innately tolerant strains. However, experiences with other infectious agents, particularly with the quick and dramatic spread of AR in livestock, should warn the medical world against the widespread use of anthelminthics for the control of helminths.

If drug-based strategies are implemented, the following recommendations may delay the development of resistance. (i) The intervention should be targeted and justified. (ii) Other control measures should be incorporated. (iii) The number of treatments should be reduced. (iv) Exposure of the whole parasite population to the drug should be avoided. (v) The correct dosage should be used. (vi) Simultaneous or rotational use of different drugs should be implemented. (vii) The development of drug resistance should be monitored. The most appropriate strategy would therefore seem not to embark on control strategies based on the widespread and frequent use of anthelminthics and to restrict their use to curative medicine and possibly targeted interventions in very-high-risk groups or areas, which can be identified through rapid appraisal methods or through the regular health information system. Meanwhile, the most important scientific challenge is to develop the appropriate tools, methods, and protocols to reliably and quickly detect the appearance of drug resistance in helminths

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References

[1.] Adrian, J., Wolstenholme, I. F., Roger, P., Georg von S., and Nicholas C. S. (2004). Drug resistance in veterinary helminthes. *Trends Parasitol.* **20(10):**469-476.

[2.] Albonico, M., Crompton, D. W. T., and Savioli, L. (1999). Control strategies for human intestinal nematode infections. *Adv. Parasitol*.**42:**277–341.

[3.] Anderson, T.J.C., Blouin, M.S. and Beech, R.N. **(**1998). Population Biology of Parasitic Nematodes: Applications of Genetic Markers. *Adv. Parasitol*. **41**:220-281.

[4.] Boersema, J.H. (1983). Possibilities and limitations in the detection of anthelmintic resistance. In: Facts and Reflections IV: Resistance of Parasites to Anthelmintics. A workshop held at the Central Veterinary Laboratory: 207–218.

[5.] Boudsocq, A., Chartier, C., Cabaret, J. (1999). Breeding Management and Development of Benzimidazole Resistance on Goat Nematode Species Diversity. WAAVP 17th International Conference, Copenhagen, Abstract Book, A107.

[6.] Bundy, D.A. and De Silva, N.R. (1998). Can we deworm this wormy world? *Br. Med. Bull*. **54:** 421-432.
[7.] Catherine, E. J., Amanda, L. H. and Mary W. D. (2007). Drug resistance mechanisms in helminths: is it survival of the fittest? *Trends Parasitol.* **25(7):**328-335.
[8.] Chitsulo L., Engels D., Montresor A., Savioli L (2000). The global status of schistosomiasis and its control. *Acta Trop*. **77:**41–51.
[9.] Coles, G.C., Bauer, C., Borgsteede, F.H.M., Geerts, S., Klei, T.R., Taylor, M.A., Waller, P.J., (1992). World Association for the Advancement of Veterinary Parasitology (WAAVP) methods for the detection of anthelmintic resistance in nematodes of veterinary importance. *Vet. Parasitol*. **44:** 35–44.
[10.] Coles, G.C., Borgsteede, F.H.M. and Geerts, S. **(**1994). Anthelmintic resistance in nematodes of farm animals. Proceedings of a seminar organized for the European Commission, held in Brussels from 8–9 November 1993.
[11.] Coles, G.C., Tritschler II, J.P., Laste, N.J., Schmidt, A.L. (1988). Larval development test for detection of anthelmintic resistant nematodes. *Res. Vet. Sci*. **45:** 50–53.
[12.] De Clercq, D., Sacko, M., Behnke, J., Gilbert, F., Dorny, P. and Vercruysse, J. (1997). Failure of mebendazole in treatment of human hookworm infections in the southern region of Mali. *Am. J. Trop. Med. Hyg.* **57:** 25-30.
[13.] Dorny, P., Claerebout, E., Vercruysse, J., Sani, R., Jalila, A. **(**1994). Anthelmintic resistance in goats in peninsular Malaysia. *Vet. Parasitol*. **55:**327-342.
[14.] Elard, L., Cabaret, J. and Humbert, J.F**. (**1999). PCR diagnosis of benzimidazole-susceptibility or -resistance in natural populations of the small ruminant parasite, *Teladorsagia circumcincta. Vet. Parasitol*. **80:**231-238.
[15.] Geerts, S. and Gryseels, B. (2000). Drug Resistance in Human Helminths: Current Situation and Lessons from Livestock. *Clin. Microbiol*.**13 (2):** 207-222.
[16.] Giordano, D.J., Tritschler, J.P., Coles, G.C., 1988. Selection of ivermectin-resistant *Trichostrongylus colubriformis* in lambs. *Vet. Parasitol*. **30:** 139–148.
[17.] Grant, W., Bisset, S., Prichard, R.K., Hotez, P., Bthony, J., Gillard, J., Sternberg, p., Gasser, R., mardis, E.R., Wilson, R.K., and Mitreva, M. (2010). Anthelmintic resistance in parasitic nematodes.
[18.] Hotson, I.K., Campbell, N.J. and Smeal, M.G. (1970). Anthelmintic resistance in *Trichostrongylus colubriformis*. *Aust. Vet. J*. **46:** 356–360.
[19.] http://www.wpro.who.int/NR/rdonlyres. Schistosomiasise, WHO.
[20.] Ismail, M., Metwally, A., Farghaly, A., Bruce, J., Tao, L.F., Bennett, J.L. (1998). Characterization of isolates of *Schistosoma rnansoni* from Egyptian villagers that tolerate high doses of praziquantel. *Am. J. Trop. Med. Hyg*. **55:** 214-218.
[21.] John, I.B., Luiz, C.D., Yung-San, L., and Gerald, C. (1987). Drug resistance in Schistosomes. *Intern. Symp. On Schistosomiasis*. **82(4):** 143-150.
[22.] Kelly, J.D., Sangster, N.C., Porter, C.J., Martin, I.C.A., Gunawan, M. (1982). Use of guinea-pigs to assay anthelmintic resistance in ovine isolates of *Trichostrongylus colubriformis*. *Res. Vet. Sci*. **30:** 131–137.
[23.] Kwa, M.S.G., Veenstra, J.G. and Roos, M.H. **(**1994). Benzimidazole resistance in *Haemonchus contortus* is correlated with a conserved mutation at amino acid 200 in ß-tubulin isotype 1. *Mol. Biochem. Parasitol.* **63**:299-303
[24.] Lacey, E., Snowden, K.L. (1988). A routine diagnostic assay for the detection of benzimidazole resistance in parasitic nematodes using tritriated benzimidazole carbamates. *Vet. Parasitol*. **27:** 309–324.

[25.] Martin, I.C.A. (1982). Guidelines related to the registration of anthelmintics with efficacy against resistant nematodes of sheep. *Vet. Proc*. **18:** 53–58.

[26.] Martin, P.J., Anderson, N., Jarrett, R.G. (1989). Detecting benzimidazole resistance with faecal egg count reduction tests and in vitro assays. *Aust. Vet. J*. **66:** 236–240.

[27.] Monteiro, A.M., Wanyangu, S.W., Kariuki, D.P., Bain, R., Jackson, F. and McKellar, Q.A. **(**1998). Pharmaceutical quality of anthelmintics sold in Kenya. *Vet. Rec*. **142:**396-398.

[28.] Presidente, P.J.A. (1985). Methods for the detection of resistance to anthelmintics. **In:** Resistance in Nematodes to Anthelmintic Drugs (Anderson, N., Waller, and P.J. Eds.). Division of Animal Health, CSIRO, Australia. 13–27.

[29.] Prichard, R.K. (2008). Mechanisms of anthelmintic resistance: Implications for the future of parasite control.

[30.] Reinemeyer C.R., Rohrbach B.W., Grant V.M., Radde G.L (1992). A survey of ovine parasite control practices in Tennessee. *Vet. Parasitol.* **42:**111–122

[31.] Reynoldson, J.A., Behnke, J.M., Pallant, L.J. (1997). Failure of pyrantel in treatment of human hookworm infections *(Ancylostoma duodenale)* in the Kimberley region of North West Australia. *Acta Trop.* **68:** 301-312.

[32.] Roos. M.H., Kwa, M.S.G. and Grant, W.N. **(**1995). New genetic and practical implications of selection for anthelmintic resistance in parasitic nematodes**.** *Parasitol. Today*. **11:**148- 150.

[33.] Sangster, N.C., Prichard, R.K. and Lacey, E. (1985). Tubulin and benzimidazole resistance in *Trichostrongylus colubriformis* (nematoda). *J. Parasitol.* **71:** 645–651.

[34.] Scott, E.W., Mitchell, E.S., Armour, J., Bairden, K., Soutar, A., Bogan, J.A. (1989).Level of benzimidazole resistance in a strain of *Ostertagicircumcincta* studied over several infections in lambs. *Vet. Parasitol*. **30:** 305–314.

[35.] Smith,G., Grenfell, B.T., Isham,V. and Cornell, S. **(**1999). Anthelmintic resistance revisited: under-dosing, chemoprophylactic strategies, and mating probabilities. *Int. J. Parasitol*. **29:** 77-91.

[36.] Stringfellow, F. (1986). Cultivation of *Haemonchus contortus* (Nematoda: Trichostrongylidae) from infective larvae to the adult male and egg laying female. *J. Parasitol*. **72:** 339–345.

[37.] Stringfellow, F. (1988). An in vitro test for drug resistance in *Haemonchus contortus*. *P. helm. Soc. Wash.* **50:** 19–23.

[38.] Sutherland, I.A., Lee, D.L., Lewis, D. (1988). Detection of benzimidazole resistance in trichostrongylid nematodes. *Parasitol Today.* **4(1):**22-4.

[39.] Taylor, M.A. (1990). A larval development test for the detection of anthelmintic resistance in nematodes of sheep. *Res. Vet. Sci.* **49:** 198–202.

[40.] Van Wyk, J.A. (2001). Refugia – overlooked as perhaps the most potent factor concerning the development of anthelmintic resistance. *J. Vet. Res*. **68**: 55-67.

[41.] Warren, K. S. (1993). For the new millennium: control of helminth diseases throughout the world. *Med. J. Aust.* **159:**461–463

[42.] Warren, K. S. An integrated system for the control of the major helminth parasites (1990). *Acta Leiden*. **59:** 433–442.

[43.] WHO (2008). Monitoring Anthelmintic Efficacy for Soil-Transmitted Helminths (STH), WHO.

[44.] WHO, 2002. Prevention and control of schistosomiasis and soil-transmitted helmentiasis.WHO, Geneva.