Anthelmintic Resistance-Clinician's Present Concern

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Introduction

1. Nematodes parasites are of major economic importance in domesticated animals throughout the world.
2. Anthelmintics have been developed and employed to control these parasites and to reduce disease and production losses due to infection.
3. Beneficial uses of these anthelmintics have resulted in their wide and intensive use.
4. Intensive use select individual nematodes that is genetically and physiologically resistant to the anthelmintics.
5. Further treatments will progressively select for anthelmintic resistance in the worm population.
6. In severe cases, disease may not be checked by anthelmintic (Pichard, R., 1994).

Defining resistance:

"Resistance is present when there is a greater frequency of individuals within a population able to tolerate doses of a compound than in a normal population of the same species and is heritable".(Prichard et al.,1980) **OR**

"Situation where a population of nematodes originally sensitive to anthelmintic, inherit the ability to survive treatment after repeated exposure to the drug".(Taylor& Hunt,1989)

Other Definitions:

Cross Resistance: Ability of parasite strains to survive the therapeutic doses of chemically unrelated drugs or drugs with different modes of action". (Singh et al., 2002).

Side Resistance: Phenomenon where the resistance to a compound is the result of selection by another compound with a similar mode of action.

1. Side resistance among benzimidazole(BZ) compounds. (*Barger*, 1975; *Hall,et.al.*,1978).
2. Levamisole resistant strains show side resistance to morantel.(*Le Jambre and Martin*, 1979).
3. Ivermectin resistant strain to moxidectin (*Conder et al.,* 1993 and Shoop, 1993)

Multiple Resistances: Parasites are resistant to two or more anthelmintics because of either selection by each group independently or by side resistance.

1. Field strains of *H.contortus* resistant to BZ., levamisole(LEV) and naphthalphos.(Green et al.,1981)
2. Multiple resistances in livestock in South Africa.(*Van Wyk,et.al.,*1997),Multiple resistances in India.(*Uppal,et.al.*,1992; Swamkar,et.al.,1999).

Reversion: Refers to return of individuals to susceptiblity from an originally resistant strain with decrease in the frequency of resistant individuals following removal of selecting agent.

1. Reversion to susceptibility of BZ resistant nematodes. (*Simpkin and Coles*, 1978; *Kelly and Hall*, 1979).
2. Reversion of BZ after selection with LEV. (*Donald,et.al.,*1980; *Waller,et.al.*,1989).

Prevalence of Anthelmintic Resistance

World Scenario:

1. Anthelmintic resistance throughout the world among the three broad spectrums (Prichard, 1990; Jackson,1993; Waller,1994).
2. Anthelmintic resistance to narrow spectrum anthelmintics(*Rolfe,et.al.*,1990).
3. Anthelmintic resistance in nine nematodes. (*Kettle,et al.*,1982; *Riffkin,et.al.*,1984; *Sivaraj et.al.*,1993).
4. Anthelmintic resistance in caprine nema-todes from France.(Kerboey and Hubert, 1985).
5. Anthelmintic resistance from U.K. (*Scott et al.*, 1989).

Indian Scenario:

1. First report of *Haemonchus contortus* resistant to phenothiazine and thiabendazole in sheep. (Varshney and Singh, 1976).
2. BZ resistant *H.contortus* from sheep. (Yadav, 1990).

3. Multiple resistance against BZ, LEV, Morantel(MT) and Thiophenate in *H.contortus* from goats. (*Uppal et al.*,1992)

Western Region (Rajasthan):

1. Resistance in *H. contortus* from sheep for BZ. (*Singh et al.*,1995).
2. Rafoxanide resistance in *H.contortus* in sheep. (*Singh et al.*,1996)

North Temperate Himalayan Region: BZ resistance in *H.contortus* from sheep(*Singh et al.,* 1992).

Country Wide Survey: First conducted by Gill in 1991-1992 revealed existence of BZ and LEV resistance in all the five sheep farms- Sandyallha and Kalapakkam in T.N.,Palmner in A.P., Dantiwada in Gujarat, Bikaner in Rajasthan. In cattle only single report of MT resistance strain of *H.placei* from Haryana.(Yadav and Verma, 1997).

Reports of Anthelmintic Resistance from India

Host	Anthelmintic	Region	Reference
Sheep	Phenothiazine, BZ,LEV,	UP, Haryana, H.P., RAJ.	*Singh, et.al.* (1995) Goat
	BZ, LEV, MT	Haryana,U.P	*Yadav,et.al,* (1992)
Cattle	MT	Haryana	Yadav and Verma (1997)

Properties of Anthelmintics

Ideally, it should:

1. Be effective against all parasitic stages.
2. Should be non-toxic to the host.
3. Should be rapidly cleared and excreted by the host.
4. Should be easily administered
5. Should have a reasonable cost.

Therapeutic Usage

1. If not effective against all stages, it should be effective against the pathogenic stage.
2. Cessation of clinical signs should occur with the removal of the parasite.

Prophylactic Usage

1. Cost should be justifiable.
2. Should not interfere with development of acquired immunity.
3. Avoid anthelmintic resistance (use of 1 drug).

Mechanisms and Molecular Genetics of Anthelmintic Resistance:

Levamisole/Morantel: Resistant mutants*(e.g. Caenorhabdits elegans)* lack acetyl-choline receptors and do not respond to cholinergic agonists like acetylcholine,nicotine,carbamyl chloride and levamisole leading to flaccid paralysis. (*Lewis,et. al.*,1980). Binding of cholinergic agonists varies between levamisole susceptible and resistant parasites(*Lewis,et.al.*,1987).

Altered Neuromuscular coordination process leading to differential effects on hatchability of eggs among resistant strains of *H.contortus and Trichostrongylus colubriformis*(*Sangster,et.al* .,1988).

Ivermectin Resistance: Alteration in the

Anthelmintics (Major Groups)

Anthelmintic Group	Examples	Mode of Action
Macrocyclic Lactones (Macrolids)	Ivermectin Eprinomectin Doramectin Moxidectin Milbemycin oxime Selamectin	Bind to glutamated chlorine channels causing paralysis (Arena et al., 1992)
Benzimidazoles	Thiabendazole Mebendazole Fenbendazole Oxfendazole Oxibendazole Albendazole	Interfere with energy metabolism by inhibition of polymerization microtubules (Lacey&Gill et al.,1994)
Pro-benzimidazoles	Febantel	Same as benzimidazoles
Imidazothiazoles	Tetramisole Levamisole	Cholinergic agonists (Roberson, 1988)
Tetrahydropyrimidines	Morantel Pyrantel	Cholinergic agonists (Roberson, 1988)
Organophosphates	Dichlorvos Haloxon Trichlorfon	Inhibitors of Cholinesterase (Roberson, 1988)
Piperazines	Piperazine salts	Anticholinergic action - block neuromuscular transmission (Roberson 1988)

glutamate/ivermectin chloride channel receptors thus preventing opening of glutamate dependent chloride channel of neuromuscular membranes of nematodes and arthropods. (Johnson,1993).

Benzimidazole Resistance: Decrease in high affinity binding receptors.(Lubega and Prichard, 1990).

In fungi, BZ resistance is associated with an alteration in beta tubulin genes and their products.(*Fujimura et al*.,1992;*Jung et al*.,1992). Change of phenylalanine to tyrosine at position 167 of beta tubulin (*Orbach,et.al*.,1986). Deletion of ben-I gene coding for beta tubulin and also for phenylalanine (*Driscoll,et.al*.,1989). Alteration in the beta tubulin isoforms and not the alpha-tubulin isoforms (Lubega and Prichard,1991).

Detection of Resistance

1. In Vivo Techniques
2. In Vitro Techniques

***In Vivo* Techniques**

1. **Fecal egg count reduction test (FECRT)** - Done by comparing worm egg counts from animals before and after treatment. (*Coles et al .,1992*).
2. **Critical anthelmintic test-** Based on the collection of feces from animals for atleast four days after treatment from which the no. of expelled worms are estimated.(Hall & Foster,1981)

***In Vitro* Techniques**

1. **Egg hatch assay(EHA)-**Based on ovicidal property of BZ which prevents egg embryonationand hatching. (LeJambre,1976; Colesand Simpkin,1979).
2. **Larval paralysis test-** Done by comparing the proportion of paralysed L3 over a range of drug concentrations. (Martin & Le Jambre,1979 for morantel resistance).

Larval development assay- Used to detect resistance against all drugs irrespective of their mode of action.(Lacey,1990).

Tubulin binding assay- Based on differential binding of BZ to tubulin prepared from susceptible and resistant nematodes.(Lacey and Prichard, 1986).

Other Tests

Biochemical assay: To compare nonspecific esterase & acetylcholine esterase of susceptible & resistant strains.(Sutherland and Lee,1989)

Micromotility test: To differentiate resistant & susceptible parasite where resistant are more motile (*Folz,et.al*.,1987).

DNA probes: To detect the presence of resistance/ susceptibility genes in individual nematodes (PCR primers to analyze isotype-I & isotype-II beta tubulin genes) [Prichard,et.al.,1993].

Strategies to minimize Anthelmintic Resistance:

1. **Administration of correct dose of anthelmintics**-Underdosing permit survival of resistant heterozygous individuals and increases their chances of producing highly resistant offsprings. (Waller et al.,1983)
2. **Reduction in frequency of treatment-** selection occurs at faster rate with increasing frequency of treatment due to high selection pressure. (Barton,1983;Martin et al.,1984)
3. **Slow rotation between anthelmintic types-** frequent change may select for multiple resistant species of worms.(Le Jambre et al .,1978)
4. **Avoid introduction of resistant worm into farm-** Prior to mixing the animals treat with IVM or macrocyclic lactones and held for atleast 30 hrs. away from pasture (*Singh,et.al*.,2002)
5. **Regular monitoring with invivo or invitro tests.**
6. **Avoid wrong choice of anthelmintics-**

* Use narrow spectrum anthelmintics when

Techniques for detection of anthelmintic resistance in parasitic nematodes

Test	Spectrum	Application	References
A. In vivo			
FECRT	All Anthelmintics	Widespread	Coles et al.,(1992)
Critical Anthelmintic Test Controlled	All Anthelmintics	Limited	Presidente, (1985)
B. In vitro			
Egg hatch	BZ	Widespread	Le Jambre, (1976)
Egg embryonation	BZ	Limited	Coles, (1977)
Larval paralysis	LEV/MT	Limited	Martin, (1979)
Larval development	BZ,LEV,IVM	Comercialized	Lacey, (1990)
Tubulin binding	BZ	Research	Sutherland, (1988)

resistance in a particular agroclimate locality is known.

* When anthelmintic resistance is confirmed discontinue that anthelmintic group.

7. Controlling the density of livestock (stocking rate): Overstocking forces the animals to graze closer to faecal material and closer to the ground, and may result in the consumption of a higher number of infective larvae.

1 Strategic deworming when conditions are most favourable for larval development on the pasture.

1 Separating age groups in the more intensive production systems.

8. Use of non- chemical measures:

* Use of genetically engineered worm vacccines. (Thompson,1999).
* Biological control of free-living stages on pasture by use of predacious fungi.(Eg. *Arthrobotrys oligospora etc.*) (Waller and Faedo,1993 ;Sanyal 2000 ; Khan et al.,2000).
* Improvement of the natural immunity by breeding for disease resistance (Woolaston,1996).

9. Maximising persistency of anthelmintics:

1. Incorporating the drug into sustained release device.(*Le jambre,et.al.*,1981).
2. Slowing down of flow of digesta through the host by restricting feed intake [fasting]. (*Singh,et.al.*,1999)
3. Maintaining the animals on dry fodder prior to dosing (*Singh,et.al.*,1999).

10. Use of computer models: Computer modelling to simulate variety of climatic and managemental conditions are of great benefit.(Gettinby,1989;Barnes and Dobson,1990).

* Also predicts the evolution of anthelmintic resistance for any new control scheme (Waller, 1994).

To Sum Up:

* **Use of anthelmintic combination-** Formulations containing two anthelmintics with different modes of action. Eg BZ+LEV(Anderson et al.,1988).
* Do have a practical worm control policy and stick to it.
* Do not treat unnecessarily.
* Do not underdose by guessing animal weights or using faulty equipment.
* Do not use the same wormer family year after year.
* Do not buy in resistant worms.
* Do not use the same pastures for sheep and goats.

Conclusions:

To prevent anthelmintic resistance,most valuable weapons are:

1. To conserve susceptibility in helminthic population.
2. To develop effective methods for detection of anthelmintic resistance.

2 In India true prevalence may be higher than reported because of treatment failures at farmers flocks are seldom reported.

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Veterinary Events

Global Meet on Veterinary Public Health, 2008

Global Meet on Veterinary Public Health and Symposium on New Horizons in Food Security with Special Reference to Veterinary Public health and Hygiene - Evolving Strategies with Global Perspectives will be held from 19th - 21st November 2008 at Hotel Taj Residency, Lucknow, India. Details regarding registration and programme are available at the site http://www.publichealthglobalmeet.org/

XIX National Congress of Veterinary Parasitology, Ludhiana

The 19th National Congress of Veterinary Parasitology is to be held from 18-20 November, 2008 at Ludhiana. The theme is on "National Impact of Parasitic Diseases on Livestock Health and Production and with focal theme "Changing Trends in Parasitology from Eggs to Genomics". The organizers call for the abstracts of papers and the details of submission of abstracts and registration are available at the web site www.IAAVP.nxom.org or email to ncvp19@gmail.comand juyalpd@rediffmail.com