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Für meine Eltern, meinen Mann Mike und meine Tochter Felina

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Abbreviations

ATP	=	Adenosintriphosphate
CNS	=	Central Nervous System
cDNA	=	Complementary DNA
DNA	=	Deoxyribonucleic acid
EHT	=	Egg hatch test
FECRT	=	Faecal egg count reduction test
GABA	=	Gamma-(γ)-amino butyric acid
GluCl	=	Glutamate-gated chloride channel
GI	=	Gastrointestinal
GTP	=	Guanosin triphosphate
HypCH4-V	=	Name of the restriction endonuclease (enzyme for RFLP)
MALDT	=	Microagar larval development test
Mdr	=	Multidrug resistant
PCR	=	Polymerase chain reaction
RFLP	=	Restriction fragment length polymorphism
Spp	=	Subspecies
WAAVP	=	World Association for the Advancement of Veterinary Parasitology

1 Introduction

Diseases have a great damaging effect on livestock production. Although parasitic infections, in particular with nematode infections, may not be the most important of diseases in ruminants with regard to animal mortalities, they have a high economic impact because they cause retarded growth, weight loss, disorder in fertility and loss in milk production [Loyacano, 2002]. In order to control these economic losses in intensive beef calf rearing systems – as they are common, for example, in New Zealand and South America – farmers resort to frequent anthelmintic treatments. For example, in 1994, cattle farmers in New Zealand have spent about 28 million US $ for anthelmintic products [Bisset, 1994]. In 1999, worldwide about 3,5 billion US $ were spent for antiparasitic agents (about 1,1 billion US $ for cattle), thereof 53 % for products consisting of the three main anthelmintic classes (35% macrocyclic lactones, 10,5 % benzimidazoles, 7,5% imidazothiazoles) [Coles, 2001].

The exclusive and frequent use of anthelmintic drugs to control nematode infections in cattle has drawbacks on the larger perspective, namely the development of anthelmintic resistance. To avoid the development of anthelmintic resistance, no worms should survive anthelmintic treatment. While the efficacy of highly effective anthelmintics reach more than 98 % [Wood et al., 1995], no anthelmintic product can always guarantee a 100 % cure rate in treated animals. Due to the immense selection pressure on nematode population, it is inevitable that anthelmintic resistance has and will develop, but to what extent and when? These are the crucial but also difficult to answer questions.

This thesis will give a snapshot of the global situation regarding anthelmintic resistance in cattle as of 2006. The global occurrence of drug resistance in nematodes of cattle and the resistant nematode species will be reviewed. Furthermore, the thesis addresses the questions, how resistance develops, how anthelmintic resistance can be detected and how development of anthelmintic resistance can be delayed.

2 Overview: Current anthelmintics for cattle

On the basis of the mode of action, anthelmintic compounds can be subdivided into five classes. The list is modified from Bjorn (1992):

- Class I ß-tubulin-interfering compounds (Benzimidazoles / Probenzimidazoles)
- Class II Neuromuscular acting compounds(Imidazothiazoles / Tetrahydropyrimidines)
- Class III Glutamate-gated chloride channel acting compounds (Macrocyclic lactones)
- Class IV Salicylanilides and substituted nitrophenols
- Class V Acetylcholine esterase inhibitors

The three major families of broad-spectrum anthelmintics – the benzimidazoles, imidazothiazoles / tetrahydropyrimidines and macrocyclic lactones – will be highlighted in the following chapters, because these are the main used anthelmintic classes today. Firstly, some comments should be made about the class IV and V anthelmintics.
Some of the best known compounds of class IV anthelmintics are closantel and rafoxanide. They are especially used in *Haemonchus* spp. and *Fasciola* spp. infections in sheep and cattle. Niclosamid is an other example of an halogenated salicylanilide, which is used as anticestodal substance [Robertson, 1995; Swan, 1999].
Well known members of the class V anthelmintic compounds are dichlorvos, trichlorophon, coumaphos and haloxon [Bjorn, 1992]. They are all organophosphorus compounds having a narrow spectrum against nematodes and are mainly used against different ectoparasites. The mode of action is based on an more or less irrevesible inhibition of actylcholine esterase. Organophosphate antiparasitics have a greater affinity and binding to enzymes of the parasites than to enzymes of the host animals. There are also differences in affinity and binding between different nematode species [Ungemach, 1994; Adam and Christ, 1987]. Organophosphate anthelmintincs were introduced to market in the 1950s. Today only dichlorvos is still used as anthelmintic for horses, dogs and cats, but it is not approved in Germany [Manger, 1991; Ungemach, 1994].

2.1 Available anthelmintics and generic medicinal products

The purpose of the following chapter is to illustrate the wide range of anthelmintic products, but also to show the small repertory of compounds. Currently, there are primarily three anthelmintic classes in use worldwide for the control of nematodes in cattle, namely the

benzimidazoles, imidazothiazoles / tetrahydropyrimidines and macrocyclic lactones. A list of available anthelmintics and generic products is given in table 2.1.1.
Firstly, some comments are made about the chemical structures and qualities of compounds of this three main anthelmintic classes.
All benzimidazoles have the same central chemical structure (1,2-diaminobenzene). The different molecules of the benzimidazoles result from a substitution on carbon 5 of the benzene ring (see table 2.1.1). All are insoluble or only slightly soluble in water. Albendazole and oxfendazole are soluble in alcohols, fenbendazole in dimethylsulfoxide [McKellar and Scott, 1990].

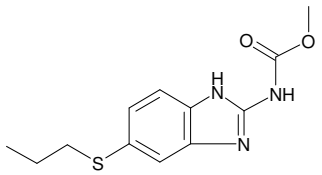
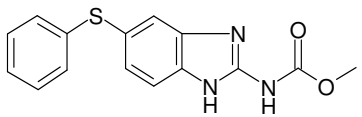
Levamisole is the *l*-isomer of *dl*-tetramisole (mixture of *l*-tetramisole = levamisole and *d*-tetramisole). The chemical name is (-)-2,3,5,6-tetrahydro-6-phenylimidazo [2,1-b] thiazole. Levamisole is available either as hydrochloride (bolus, drench) or as phosphate (injectable) salt. Levamisole hydrochloride is a white powder which is highly soluble in water [Courtney and Roberson, 1995].

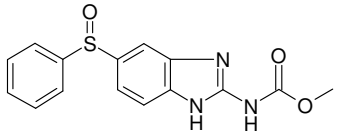
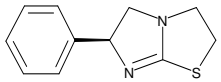
Morantel is a cyclical amidine belonging to the tetrahydropyrimidines and is a methyl-substituted analogue of pyrantel. The tartrate salt of morantel is soluble in water [Plumb, 1991].

The avermectins / milbemycins – belonging to the macrocyclic lactones – are chemically related products which are originated from actinomycetes, from the genus *Streptomyces*. There are different strains of *Streptomyces*; some of them produce milbemycin-type compounds, while others produce avermectin-type compounds. Apart from the fact, that both – avermectins and milbemycins – are produced by *Streptomyces* spp., they correspond in their pharmacophore (macrocycle, benzofuran and spiroketal) [Stapley and Woodruff, 1982]. The pharmacophore is that part of a molecule which is responsible for the pharmacological effects [Gund, 1977]. Ivermectin, eprinomectin and moxidectin are semisynthetic derivatives of different *Streptomyces* spp. [Campbell, 1993; Shoop et al., 1996, Takiguchi et al., 1980]. Abamectin and doramectin are natural products of *Streptomyces avermitilis* [Shoop et al., 1995].

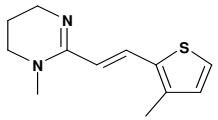
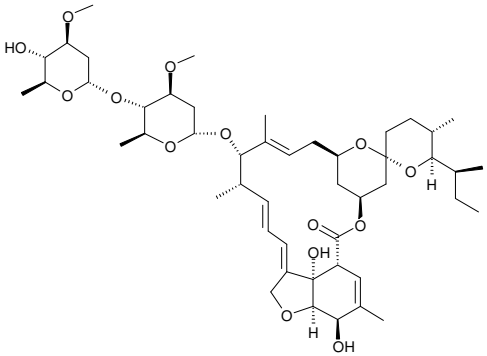
Table 2.1.1 Overview of some available anthelmintics and generic medicinal products

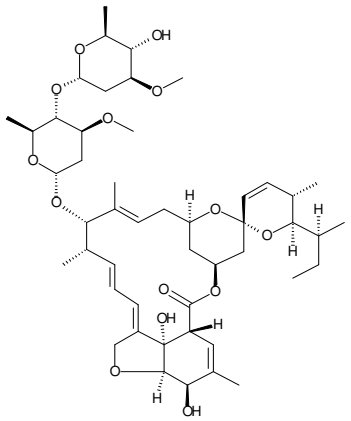
(Licend in Germany* and/or Europe°)

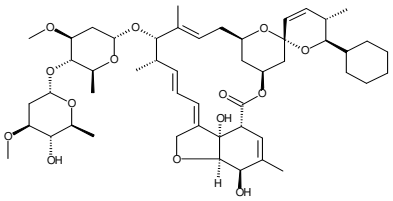
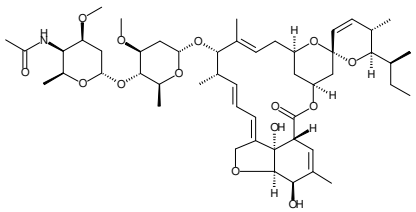
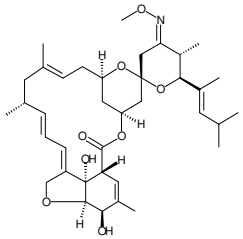
Anthelmintic class	Active ingredient / Chemical structure	Trade name	Registrant
Class I Benzimidazoles	**Albendazole** 	Albenil®	Virbac Ltd °
		Albex®	Chanelle Animal Health Ltd °
		Albendazol®	aniMedica GmbH*
		Endospec®	Bimeda°
		Valbazen®	Pfizer Ltd °/*
		Vermitan®	CEVA Tiergesundheit GmbH *
	Fenbendazole 	Coglazol®	CEVA Tiergesundheit GmbH*
		Fenbendatat®	aniMedica GmbH *
		Fendazole®	Bimeda °
		Fenzol®	Norbrook Laboratories Ltd °
		Orystor®	Bioptivet Tierarzneimittel GmbH & Co.*

Anthelmintic class	**Active ingredient / Chemical structure**	**Trade name**	**Registrant**
		Panacur®	Intervet Ltd. ○ / *
		Zerofen®	Chanelle Animal Health Ltd ○
	Oxfendazole 	Autoworm Finisher®	Schering-Plough Animal Health ○
		Bovex®	Chanelle Animal Health Ltd ○ / *
		Oxfenil®	Virbac Ltd *
		Parafend®	Norbrook Laboratories (GB) Ltd ○
		Systamex®	Schering-Plough Animal Health / ESSEX Pharma GmbH*
<u>Class II</u> Imidazo-thiazoles	**Levamisole** 	Armadose Breakwormer®	Bayer ○
		Aethrol L®	Pharmacia GmbH*
		Belamisol®	Bela-Pharm GmbH & Co.KG*
		Chanaverm®	Chanelle Animal Health Ltd ○
		Citarin-L®	Bayer *
		Combinex® (levamisole + triclabendazole)	Novartis Animal Health Ltd ○

Anthelmintic class	Active ingredient / Chemical structure	Trade name	Registrant
		Concurat-L	Bayer *
		Decazole Forte®	Bimeda ○
		Levacide®	Norbrook Laboratories (GB) Ltd ○
		Levacur®	Intervet (UK) Ltd ○
		Levafas Diamond®	Norbrook Laboratories Ltd ○
		Levamisol R®	Klat-Chemie Vertrieb GmbH*
		Levamisol 10®	CP-Pharma GmbH*
		Nematovet-10®	aniMedica GmbH *
		Nilverm Gold®	Schering-Plough Animal Health ○
		Niratil®	Virbac Ltd *
		Rafazole®	Chanelle Animal Health Ltd ○
		Ripercol®	Janssen Animal Health ○ / *
		Sure LD®	Young´s Animal Health ○

Anthelmintic class	Active ingredient / Chemical structure	Trade name	Registrant
		Vermisole®	Bimeda ○
		Vetamisol®	aniMedica GmbH *
Class II Tetrahydro-pyrimidines	**Morantel** 	Paratect Flex®	Pfizer Ltd ○ / *
Class III Macrocyclic lactones	**Ivermectin** 	Animec®	Chanelle Animal Health Ltd ○
		Bimectin®	Bimeda ○
		Chanectin®	Chanelle Animal Health Ltd.*
		Diapec R®	Bimeda *
		Ecomectin®	ECO Animal Health Ltd *
		Ivomec®	Merial Animal Health Ltd ○ / *
		Noromectin®	Norbrook Laboratories Ltd ○
		Panomec®	Merial Animal Health Ltd ○
		Paramectin®	Norbrook Laboratories Ltd *

Anthelmintic class	**Active ingredient / Chemical structure**	**Trade name**	**Registrant**
		Qualimec®	ECO Animal Health Ltd*
		Sumex®	Chanelle Animal Health Ltd ○ / *
		Virbamec®	Virbac Ltd ○ / *
		Wedemec R®	Bimeda*
	Abamectin 	Enzec®	Merial Animal Health Ltd ○

Anthelmintic class	Active ingredient / Chemical structure	Trade name	Registrant
	Doramectin 	Dectomax®	Pfizer Ltd ○
	Eprinomectin 	Eprinex®	Merial Animal Health Ltd ○ / *
	Moxidectin 	Cydectin®	Fort Dodge Animal Health ○ / *

(Source: Data about trade name and registrant are taken from NOAH (Compendium of data sheets for veterinary products, 2001-2002) and Bundesamt für Verbraucherschutz und Lebensmittelsicherheit (BVL), Berlin, 2007)

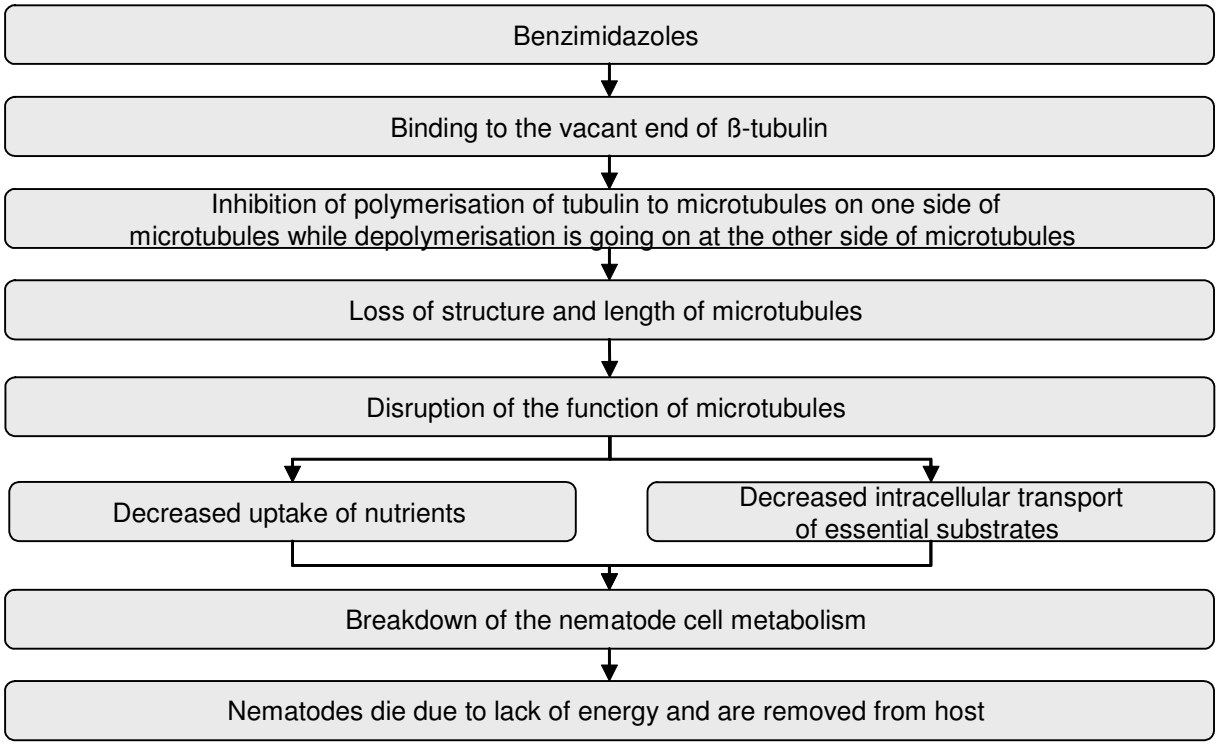
2.2 Mode of action of anthelmintics

To understand the processes which are involved in resistance mechanisms, it is essential to know the targets of the different anthelmintic drugs. In the following chapter, the mode of action of the three main anthelmintic classes is reviewed.

2.2.1 Benzimidazoles

The mode of action of benzimidazoles is based on their binding to ß-tubulin of parasite cells (Figure 2.1).

Figure 2.1 Mode of action of benzimidazoles



Tubulin is the protein which makes up microtubules. These filamentous proteins are assembled from dimers of α- and β-tubulin. Microtubules are intracellular structures that are responsible for various kinds of movements in all eukaryotic cells. Microtubules are involved in cell division, organization of intracellular structure and intracellular transport [Lacey, 1990; Samson-Himmelstjerna, 2006].

There is a dynamic balance between dimer and polymer molecules of tubulin, between polymerisation and depolymerisation. Binding of benzimidazoles to the vacant end of ß-tubulin ("capping") prevents polymerisation of tubulin to microtubules. At the same time depolymerisation is going on at the other side of microtubules. This mechanism leads to the loss of structure and length of microtubules. Therefore, microtubules are unable to function, which causes decreased uptake of nutrients and decreased intracellular transport of essential substrates [Lacey, 1988; Lacey, 1990]. Especially a reduction of glucose uptake leads to a decreased consumption and a reduced synthesis of endogen glycogen (a highly branched glucose polymer). In addition, adenosintriphosphate (ATP)-synthesis is highly reduced because the resource for this nucleotide is glucose [Rahman and Bryant, 1977]. ATP is an energy rich compound which is used for different energy consuming processes of the cell metabolism. After exhaustion of all endogenous glycogen reserves, parasites die due to lack of energy. Nematodes are then removed from the host 2-3 days after treatment of cattle with benzimidazoles [Ungemach, 1994].

The different tasks of microtubules are not only essential for nematode cells but also for all other eukaryotic cells, including mammals. However, benzimidazoles selectively act on parasitic ß-tubulin. This selective toxicity of benzimidazoles can be explained by a much lower affinity of these drugs for mammalian tubulin compared to the affinity for tubulin from helminth [Lacey and Gill, 1994]. Benzimidazoles bind at a special site ("pocket") of ß-tubulin when this is opened either by formation of the dimer, or the GTP (Guanosintriphosphate) binding. This "pocket" is closed by a hydrogen bond between residues of amino acids 200 and 165. In contrast to mammals, the position 200 in nematodes is filled by a phenylalanine, which is unable to form a hydrogen bond. Thus, the "pocket" is open for benzimidazole entry [Robinson et al., 2004].

Benzimidazoles seem to have not only nematocidal but also ovicidal activity. This inhibition is also based on the special functions of microtubules during developmental period [Lacey et al., 1987].

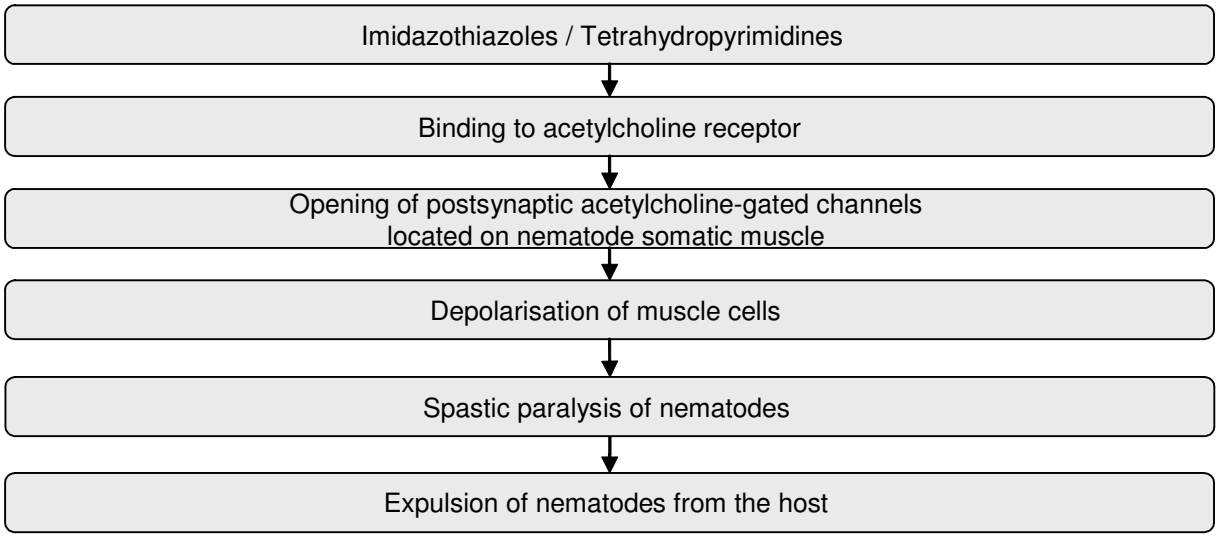
Recent findings suggest that the main transport mechanism of the benzimidazole anthelmintics into nematodes is a passive drug transfer through the external cuticule [Mottier et al., 2006].

2.2.2 Imidazothiazoles (Levamisole)

The mode of action of levamisole (Figure 2.2) is based on its direct cholinergic effects on the acetylcholine receptor [Martin, 1997]. The acetylcholine receptor is a transmembrane protein which consists of five subunits, two α, one ß, one γ and one δ. These five subunits form a channel which is gated by acetylcholine. Levamisole acts as an agonist binding to the α-subunit leading to a constant depolarisation of the cells, such as muscle cells, causing spastic paralysis of nematodes [Coles et al., 1975].

The effect of levamisole depends on the level of the drug concentration at the parasite and it is independent from exposition time [Guerrero, 1980; Atchison et al., 1992;].

Figure 2.2 Mode of action of anthelmintics acting as agonists of acetylcholine receptors



2.2.3 Tetrahydropyrimidines

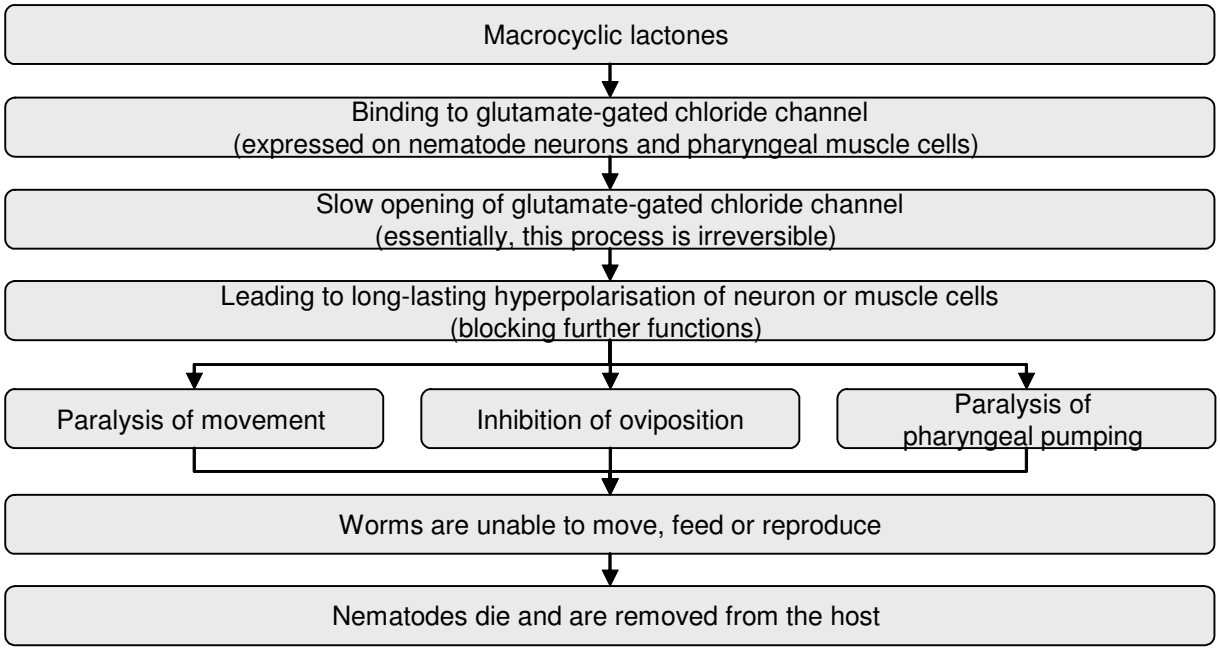
Pyrantel and morantel, belonging to the tetrahydropyrimidines, act in a similar way to levamisole. The mode of action is based on a direct agonistic effect on muscarinic and nicotinic acetylcholine receptors. In addition, in higher concentrations the acetylcholinesterase is blocked by tetrahydropyrimidines. This neuromuscular blockade leads to a spastic paralysis of parasites [Bamgbose et al., 1973; Ungemach, 1994].

2.2.4 Macrocyclic lactones

The macrocyclic lactones include antimicrobial, antifungal, insecticidal and antiparasitic macrolides. Avermectins and milbemycins – belonging to the macrocyclic lactones – do not show antimicrobial or antifungal effects, but act against various endo- and ectoparasites [McKellar and Benchaoui, 1996; Wolstenholme and Rogers, 2005]. Avermectins and milbemycins are natural products of microorganisms or modified compounds thereof. Avermectin producing organisms were originally isolated from soil at the Kitasato Institute in Japan and were identified as actinomycetes, *Streptomyces avermitilis*. Substrate of one of the isolated cultures was active against a gastrointestinal nematode, *Nematospiroides dubius* [Stapley and Woodruff, 1982; Campbell et al., 1983; Sutherland, 1990]. This nematocidal activity was utilized and ivermectin, as the first product of avermectins, was introduced into the market in 1981.

The mode of action of avermectins is based on an increased permeability of neural cell membranes of nematodes and of neural and muscular cell membranes of arthropods for chloride ions [Turner and Schaeffer, 1989]. Such drugs, which act on both, nematodes and arthropods, are called endectocides. The target of avermectins and milbemycins are glutamate-gated chloride channels. In Figure 2.3 the mode of action of macrocyclic lactones is shortly summarized.

Figure 2.3 Mode of action of the macrocyclic lactones



(Source: [Wolstenholme and Rogers, 2005])

Initially, ivermectin was thought to inhibit γ-aminobutyric acid (GABA)-gated chloride channels. However, high concentrations of macrocyclic lactones are required for the binding to GABA receptor channels. These concentrations are out of clinical relevance. Presently, it is accepted that glutamate-gated chloride channels are the essential target in the mode of action of avermectins and milbemycins. In experiments with *Caenorhabditis elegans*, a glutamate-gated chloride channel, which was sensitive to ivermectin at lower concentrations, was discovered and described by Arena et al. (1992).

Glutamate-gated chloride channels are characteristic for invertebrates. Binding of macrocyclic lactones – and surely of glutamate, which is the naturally endogenous transmitter acting at these receptors – to these receptors lead to an opening of the channel, which results in an influx of chloride ions into the cell. This influx causes a hyperpolarisation of neuron or muscle cells [Cully et al., 1996; Jagannathan et al., 1999]. In contrast to glutamate, the blockade caused by ivermectin is usually irreversible.

Three main effects can be observed if macrocyclic lactones are given to nematodes: paralysis of movement, paralysis of pharyngeal pump and inhibition of oviposition due to effect on uterus muscles. Therefore, nematodes are unable to move, to feed or to reproduce. Finally, they are quickly removed from the host animal [Wolstenholm and Rogers, 2005].

The effect on reproduction has also been described by Campbell et al. (1983). They have demonstrated that avermectins could also induce a long-lasting reduction in larval production. The suppressive activity against eggs and larval stages of nematodes has been confirmed by Wang et al. (1989).

Avermectins show a low affinity for mammalian ligand-gated chloride channels in rat brains 100-fold less than in *C.elegans* [Burkhart, 2000]. Toxic side effects in the host animal are based on GABA receptors which exist not only in nematodes but also in the brain and spinal cord of mammals. However, since macrocyclic lactones don't cross the blood brain barrier, side effects occur only due to overdosing or a defect blood brain barrier. In an experiment with Murray Grey cattle idiosyncratic reactions could be observed at a dose of 200 µg/kg [Seaman et al., 1987]. Similar signs – including ataxia, paralysis of the tongue and blindness – could be monitored in some Collie dogs after an injection of 200 µg / kg ivermectin [Pulliam et al., 1985].

The blood brain barrier is a natural barrier for ivermectin [Campbell and Benz, 1984]. Intoxication with ivermectin is caused by high concentrations of ivermectin in the central nervous system (CNS). In ivermectin-sensitive animals – especially Collie dogs and related breeds – extremely high concentrations of ivermectin were measured [Mealey et al., 2001]. The reason for this abnormal accumulation of ivermectin in the CNS is due to a genetic defect, a deletion in the mdr-1 gene. The mdr-1 gene encodes for a large transmembrane protein, the P-glycoprotein which is integrated in the blood-brain barrier and functions there as a pump for drug transport. P-glycoprotein transports different drugs – ivermectin is one of its substrates – from the brain back into the blood. In ivermectin-sensitive Collies P-glycoprotein is lacking [Mealey et al., 2001; Roulet et al., 2003].

2.3 Spectrum of anthelmintics in cattle

In the following chapter, the spectrum of the different anthelmintic classes is summarized in Table 2.3.1. It is important to know the range of activity of the different anthelmintics to decide which one is the best therapeutic compound for the particular situation.

Only one of these classes shows activity against endo- and ectoparasites. Macrocyclic lactones are active against a variety of nematodes and arthropods.

Additionally, not every compound is active against both, adult and larval stages of nematodes. Levamisole, for example, is only active against adult stages of *Ostertagia* spp. but not against

inhibited larvae of this nematode species, whereas ivermectin is highly effective against both, inhibited larvae and adults.

Furthermore, the kind of formulation should be considered. There are many formulations in use to treat parasites in cattle, but not every compound is available in each formulation. Anthelmintic drugs can be administered in form of an injection (subcutaneous application), it can be given as a pour-on formulation (dermal application), it can be administered as sustained release bolus (intraruminal application) or it can be given as an oral drench. However, in some cases the application form is not practicable. In a large cattle herd, for example, the intraruminal application form is not feasible. In this case the subcutaneous or the dermal application is preferable. Today, the dermal (pour-on) application is the most used form of anthelmintic delivery.

Finally, it is important to know the state of resistance of nematodes in an animal herd or area, in order to use the proper therapeutic agent.

Table 2.3.1 Spectrum of anthelmintics for cattle

Drug class	Anthelmintic compound	Dosage in mg/kg body weight	Spectrum	
Benzimidazoles	Albendazole	7,5 oral	Gastrointestinal (**GI**) nematodes	*O.ostertagia* (incl.inhibited larvae)*, Cooperia* spp.*, Haemonchus* spp.*, Trichostrongylus* spp.*, Nematodirus* spp.*, Oesophagostomum* spp., *Bunostomum* spp.*, Strongyloides* spp.
			GI cestodes	*Moniezia* spp.*, Taenia saginata*
			Liver flukes	*Fasciola hepatica*, *Fascioloides magna*
			Lungworms	*Dictyocaulus viviparus*
	Fenbendazole	7,5 oral	GI nematodes	*O.ostertagi* (incl.inhibited larvae)*, Cooperia* spp.*, Haemonchus contortus*, *Trichostrongylus* spp., *Nematodirus* spp., *Oesophagostomum* spp., *Bunostomum* spp., *Strongyloides* spp., *Trichuris* spp., *Toxocara vitulorum*
			GI cestodes	*Moniezia* spp.

Drug class	Anthelmintic compound	Dosage in mg/kg body weight	Spectrum	
			Liver flukes	*F.hepatica*
			Lungworms	*D.viviparus*
	Oxfendazole	4,5 oral	GI nematodes	*O.ostertagi* (incl.inhibited larvae), *Cooperia* spp., *Haemonchus* spp., *Trichostrongylus* spp., *Nematodirus* spp., *Oesophagostomum* spp., *Bunostomum* spp., *Strongyloides* spp., *Trichuris* spp.
			GI cestodes	*Moniezia* spp.
			Lungworms	*D.viviparus*
Imidazothiazoles	Levamisole	7,5 oral	GI nematodes	*Cooperia* spp., *O.ostertagi* (insufficient against inhibited larvae), *Haemonchus* spp., *Trichostrongylus* spp., *Bunostomum* spp., *Oesophagostomum* spp., *Nematodirus* spp., *Trichuris* spp., *Toxocara vitulorum*, *Strongyloides papillosus*
			Lungworms	*D.viviparus*

Drug class	Anthelmintic compound	Dosage in mg/kg body weight	Spectrum	
Tetrahydro-pyrimidines	Morantel	10,0 oral	GI nematodes	*Haemonchus* spp., *O.ostertagi, Cooperia* spp., *Trichostrongylus* spp., *Nematodirus* spp., *Oesophagostomum* spp., *Bunostomum* spp., *Strongyloides* spp., *Trichuris* spp.; insufficient against histiotroph inhibited larvae
			Lungworms	*D.viviparus*
Macrocyclic lactones	Abamectin	0,2 subcutaneous	GI nematodes	*O.ostertagi* (incl.inhibited larvae)*, Cooperia* spp., *Haemonchus* spp., *Trichostrongylus* spp., *Oesophagostomum* spp., *Nematodirus* spp., *Bunostomum* spp., *Trichuris* spp.
			Lungworms	*D.viviparus*
			Lice	*Linognathus vituli, Haematopinus eurysternus, Damalinia bovis*
			Mite	*Psoroptes ovis, Sarcoptes bovis*
	Doramectin	0,2 subcutaneous	GI nematodes	*O.ostertagi* (incl.inhibited larvae)*, Cooperia* spp., *Haemonchus* spp., *Trichostrongylus* spp., *Nematodirus* spp., *Bunostomum* spp.,

Drug class	Anthelmintic compound	Dosage in mg/kg body weight	Spectrum	
		or 0,5 pour on		*Strongyloides* spp., *Oesophagostomum* spp., *Trichuris* spp.
			Lungworms	*D.viviparus*
			Ticks	*Boophilus microplus*
			Lice	*Linognathus vituli, Haematopinus eurysternus, Damalinia bovis*
			Mite	*Sarcoptes bovis, Psoroptes bovis, Chorioptes bovis*
			Flies	*Haematobia irritans*
			Warbles	*Hypoderma bovis, Hypoderma lineatum*
	Eprinomectin	0,5 pour on	GI nematodes	*O.ostertagi* (incl.inhibited larvae), *Cooperia* spp., *Haemonchus* spp., *Trichostrongylus* spp., *Bunostomum* spp., *Nematodirus* spp., *Oesophagostomum* spp., *Trichuris* spp.
			Lungworms	*D.viviparus*

Drug class	Anthelmintic compound	Dosage in mg/kg body weight	Spectrum	
			Lice	*Linognathus vituli, Haematopinus eurysternus, Damalinia bovis*
			Mite	*Psoroptes bovis, Chorioptes bovis, Sarcoptes bovis*
			Warbles	*Hypoderma* spp.
	Ivermectin	0,2 subcutaneous 0,5 pour on	GI nematodes	*O.ostertagi* (incl. inhibited larvae), *Cooperia* spp., *Haemonchus* spp., *Trichostrongylus* spp., *Nematodirus* spp., *Bunostomum* spp., *Oesophagostomum* spp., *Strongyloides* spp., *Trichuris* spp., *Toxocara vitulorum*
			Lungworms	*D.viviparus*
			Ticks	*Boophilus microplus, Ornithodoros savignyi*
			Lice	*Linognathus vituli, Haematopinus eurysternus, Damalinia bovis*
			Mite	*Psoroptes bovis, Sarcoptes bovis, Chorioptes bovis*
			Warbles	*Hypoderma bovis, Hypoderma lineatum*

Drug class	Anthelmintic compound	Dosage in mg/kg body weight	Spectrum	
	Moxidectin	0,2 subcutaneous or 0,5 pour on	GI nematodes	*O.ostertagi* (incl. inhibited larvae), *Cooperia* spp., *Haemonchus* spp., *Trichostrongylus* spp., *Nematodirus* spp., *Oesophagostomum* spp., *Bunostomum* spp.
			Lungworms	*D.viviparus*
			Ticks	*Boophilus microplus*
			Lice	*Linognathus vituli, Haematopinus eurysternus, Bovicola bovis, Damalinia bovis*
			Mite	*Psoroptes bovis, Sarcoptes bovis, Chorioptes bovis*
			Warbles	*Hypoderma bovis, Hypoderma lineatum*

(Source: NOAH, Compendium of data sheets for veterinary products, 2001-2002)

3 Resistance against anthelmintics

In the following chapter, the term ´resistance` will be defined, types of anthelmintic resistance and mechanisms of resistance will be described, the development of resistance and methods for the detection of anthelmintic resistance will be summarized. The global occurrence of drug resistant nematodes in cattle, the resistant nematode species in cattle and the speed of resistance development in cattle nematodes will be presented. Finally, management of anthelmintic resistance will be described.

3.1 Definition

"**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]. Resistance is the genetically transmitted loss of sensitivity in (parasite) populations previously sensitive to the same drug.

Resistance to an anthelmintic drug can be observed by an increased number of nematode eggs, higher survival rates of adults in the host and consequently greater numbers of immature stages on the pasture after treatment.

Multidrug resistant parasites show resistance to several classes of anthelmintics. An example is the multidrug resistance in a *H.contortus* isolate which showed resistance to benzimidazoles and macrocyclic lactone anthelmintics [Anziani et al., 2004].

Side resistance includes all anthelmintics which are chemically related (members of the same class of anthelmintics) or which do act on the same drug mechanism. An example is the simultaneous resistance of an isolate of *O.ostertagi* resistant to levamisole (imidazothiazoles) and morantel (tetrahydropyrimidines) [Borgsteede, 1991].

Finally, it should be added, that there is a difference between resistance and tolerance. 'Tolerance' is characterized as the intermediate stadium between susceptibility and complete drug failure [Hastings and Watkins, 2006]. Coles (2006) argued that this description is not completely correct. In his opinion 'tolerance' can be total or stage specific. In an example he argued that ivermectin is not effective in killing inhibited cyathostomes in horse but in killing the luminal stages.

3.2 Mechanisms of anthelmintic resistance

Mechanisms of resistance can broadly be divided into two main processes: firstly, into a change in the target molecule and secondly, into a mechanism which inactivates or removes the drug from the environment of the target molecule. In the following chapter, the mechanisms of anthelmintic resistance of the main nematocidal compounds are described.

3.2.1 Benzimidazoles

As described in chapter 2.2.1, the mode of action of benzimidazole is based on the binding to the ß-tubulin subunit of microtubules. This binding results in an interruption of the polymerisation of microtubules [Lacey, 1988; Lacey, 1990].

Benzimidazole resistance is associated with mutations in ß-tubulin genes. Until today, three different point mutations were detected in nematodes, leading to benzimidazole resistance. One of the mutations is the change in codon 200 of ß-tubulin [Kwa et al., 1994]. The codon 200 polymorphism has been detected in benzimidazole resistant *C.oncophora* of cattle [Njue and Prichard, 2003; Winterrowd et al., 2003]. This mutation concerns only a single nucleotide switch, from TTC (phenylalanine) to TAC (tyrosine) [Winterrowd et al., 2003]. The knowledge of this nucleotide change is an important diagnostic aid for the detection of nematode resistance to benzimidazoles (see chapter 3.4).

A second point mutation has been detected in benzimidazole-resistant populations of *H.contortus.* It was described as a phenylalanine-tyrosine polymorphism at codon 167 of the ß-tubulin [Prichard, 2001]. This mutation at codon 167 was also detected in benzimidazole-resistant *T.circumcinta*, a gastrointestinal nematode of sheep and goats [Silvestre and Cabaret, 2002], and in highly benzimidazole-resistant cyathostomes from horses [Drogemüller et al., 2004a].

Furthermore, a mutation at codon 198 of ß-tubulin (glutamate to alanine) is supposed to be involved in benzimidazole resistance in *H.contortus* [Mäser et al., 2006]. This mutation was found in multidrug resistant *H.contortus* from South Africa and Australia. The same polymorphism, at codon 198, was previously described in benomyl-resistant isolates of phytopathogenic fungi such as *Monilinia fructicola* [Ma et al., 2003].

Finally, it is suggested that the cell membrane efflux pump, P-glycoprotein, could also be involved in benzimidazole resistance. Experiments for the localisation of these P-glycoproteins were carried out with *C.elegans* and *H.contortus* [Kerboeuf et al., 2003a, b].

3.2.2 Imidazothiazoles (Levamisole) and tetrahydropyrimidines

Levamisole, and also tetrahydropyrimidines, act as agonists at nicotinic acetylcholine receptors. It is thought that resistance to these drugs is either due to a change in binding characteristics or a reduction in number of acetylcholine receptors [Hoekstra et al., 1997]. In *C.elegans* – the most important nematode for developmental biology – one GABA and two acetylcholine receptors were found at neuromuscular junctions. One of these two acetylcholine receptors was activated by levamisole, whereas mutants which did not express the α- and non-α-subunit of the acetylcholine receptor lost their susceptibility for levamisole [Richmond and Jorgensen, 1999]. Different subtypes of acetylcholine receptors were also found in other experiments, like N-type (nicotine sensitive) and L-type (levamisole sensitive) receptors. It is suggested that levamisole resistance is related with a loss of L-type acetylcholine receptor [Martin et al., 2003].

A heterogeneous receptor population with different subtypes, named G25, G35, G40 and G45, was found in levamisole-sensitive isolates of *Oesophagostomum dentatum*. In levamisole-resistant isolates the G35 subtype was missing. It is suggested that this change in the quality of levamisole receptor population (subtypes) leads to levamisole resistance [Robertson et al., 1999].

It can be concluded that levamisole resistance is supposed to be associated with a lowering of its affinity to the acetylcholine receptor or with the loss of levamisole binding sites at the receptor.

Additionally, it could be demonstrated that nematodes which show resistance to levamisole were also resistant to pyrantel and morantel sharing the same mode of action (side resistance) [Sangster et al., 1998].

3.2.3 Avermectins and milbemycins

There are different theories about the mechanism of avermectin / milbemycin resistance.

The genetic variability of two genes, GluCl-alpha3 and GluCl-beta (encoding for subunits of glutamate-gated chloride channel) was analysed in an ivermectin-susceptible and an ivermectin-resistant isolate of *C. oncophora.* Statistical analysis supports an association between glutamate-gated chloride channels (GluCl) and ivermectin resistance [Njue and Prichard, 2004]. Similar analyses and results were previously found by Blackhall et al. (1998a). They found a correlation between changes in allele frequencies of the alpha-subunit gene of a glutamate-gated chloride channel and resistance to ivermectin and moxidectin.

The involvement of P-glycoproteins in resistance to macrocyclic lactones is a further observation which was first described by Blackhall et al. (1998b). As it is mentioned in chapter 2.2.4, P-glycoproteins are transmembrane proteins which act as a cell-membrane efflux pump. In mammalian cells and some protozoan parasites, like trypanosomes, it is supposed that this pump is involved in an active drug export [Sangster et al., 1999; Kerboeuf et al., 2003a, b; Drogemüller et al., 2004b].

There are further observations in macrocyclic lactone resistance of *H.contortus*. In search for the exact sites of avermectin and milbemycin resistance, the amphidial neurons have been found. These neurons are located in the cephalic end of the nematode in a pair of channels, the amphids. Amphidial neurons can be found on either side of the pharynx. Via pores they are connected to the external environment. Electron micrograph analysis and comparison of the amphidial neurons of ivermectin-susceptible and ivermectin-resistant *H.contortus* showed a generalized degeneration of the neurons in resistant nematodes [Guerrero and Freeman, 2004].

More experiments are necessary to clarify, if amphidial neurons could be one of the factors taking part in resistance or susceptibility to macrocyclic lactones.

In conclusion, alteration in Glutamate-gated chloride channels, P-glycoproteins and amphidial neurons may contribute or cause resistance to macrocyclic lactones, but there is no clear evidence until today.

3.3 Development of anthelmintic resistance

The development of anthelmintic resistance is a complex theme and many factors are involved in the process of resistance selection. However, it is important to know these factors to prevent resistance if possible or at least to delay the development of resistance. The factors could be differentiated into 3 groups:

- drug related factors (pharmacokinetics, formulation and mode of application of anthelmintics)
- management related factors (incorrect dosing of anthelmintics, frequency of anthelmintic treatment, use of the same anthelmintic class for several years, pasture management of livestock)
- parasite related factors (number of nematodes in refugia, frequency of genes for resistance in an unselected parasite population, genetic factors as mode of inheritance, fitness and fecundity of resistant nematodes, generation time)

The pharmacokinetics of a drug can exert an essential influence on the development of resistance. Differences in the anthelmintic pharmacokinetics between the various host species could be responsible for the variable expression of resistance in the different host animals [Coles, 2002a; Kaplan, 2004].

Subcurative drug doses are an important risk factor in the development of resistance and it was the most likely reason in the evolution of macrocyclic lactone resistance in nematodes in goats [Escudero et al., 1999]. It can be expected that an anthelmintic substance with a long half-live will select more for resistance, because of subcurative drug levels at the “tail” of its elimination phase. If there is a rapid fall in drug concentration due to a quick metabolism – as it was shown for thiabendazole in cattle – sublethal concentrations in the host won’t occur [Coles and Stafford, 1999].

Not only the concentration of an anthelmintic is an evident factor, but also the time of contact between parasite and drug. The contact time has to be long enough to get the full effect of an anthelmintic compound. Especially for benzimidazoles, it is important to have an extended period of time due to a sufficient interference of benzimidazoles with the microtubule synthesis [Lanusse and Prichard, 1993]. Therefore, it is important that the drug reaches the area in the animal – for example the abomasum, duodenum or blood – where parasites are located. In that context, it is supposed that the effect of benzimidazoles is reduced if the oesophageal groove reflex of ruminants is acting at drenching, so that an essential part of the drug will pass the forestomachs straight forward into the abomasum [Prichard and Hennesy, 1981].

Management factors do also contribute to the development of anthelmintic resistance. The frequent use of anthelmintics is one of the most surveyed reasons for the appearance of resistance, because the selection pressure for anthelmintic resistance survivors increases [Coles, 2002b; Waller, 1993; Bjorn, 1992; Anziani et al., 2004; Mejia et al., 2003; Fiel et al., 2001]. It is supposed that this is one of the main reasons why anthelmintic resistance in bovine nematodes is not as common yet [Waller, 1993; Coles, 2002a]. Due to the frequent use of anthelmintics, selection pressure is raised and susceptible nematodes die, while nematodes with genes for resistance are filtered out. Thus, resistant nematodes have a greater chance than susceptible nematodes of contributing to the next generation [Martin et al., 1984].

A further reason for development of resistance is the intensive use of the same drug for several consecutive years which is often combined with a frequent use [Anziani et al., 2004;

Bjorn, 1992]. Mathematical models had shown that anthelmintic resistance can be delayed if an annual rotation scheme (change anthelmintic class every year) is used [Barnes et al., 1995].

Pasture management is a further resistance influencing factor. In the past, the ´dose and move` scheme – where animals are treated and moved to rested pastures – was recommended as an effective method of nematode control [Michel, 1969]. However, it has been widely accepted that this method is almost creating the development of resistance [Coles, 2002b; Bjorn, 1992]. Treatment surviving ´resistant` nematodes will produce eggs that contaminate the ´nematode-free` pasture, and newly infected host animals will ingest a majority of these ´resistant` nematodes.

Apart from drug and management related factors, it is supposed that the biology of nematode populations plays a major role in development of resistance.

It is presumed that genes for resistance are already present in very low numbers in a nematode population before anthelmintics are used [Georghiou and Taylor, 1977]. The frequency of genes for resistance in an unselected nematode species is not known for any nematode population, nevertheless, it contributes to the development of resistance.

The inheritance of genes is also an essential component in the development of resistance. If resistance is dominant, heterozygotes as well as homozygotes will contribute to the next generation and this will ensure that resistance is spread to a greater extent, when resistance is recessive. It is presumed that the inheritance of benzimidazole resistance as well as levamisole resistance is recessive [Sangster et al., 1998]. In avermectins, inheritance of resistance seems to be dominant [Le Jambre et al., 2000]. However, these results are only evaluated for *H.contortus*.

Fecundity and the number of eggs which are produced by nematodes vary between the different species. For instance, only a few hundred eggs per day are laid by *O.ostertagi* whereas in *H.contortus* some hundred thousand eggs are produced per day. The greater the number of eggs of a parasite the bigger is the chance to have resistance genes in a nematode population. Therefore, nematodes have a greater chance to respond to anthelmintic selection pressure [Bjorn, 1992].

The generation interval of a nematode population may also contribute to the development of resistance. For example, adult nematodes of *H.contortus* in sheep can be found for several months in their host animals, whereas adults of *O.ostertagi* do only survive for a relatively

short period. This is a great advantage for *H.contortus* because there is enough time to produce the next generation [Michel, 1969; Coles, 2002a].

However, it is supposed that the number of nematodes in refugia (different nematodes stages which are not exposed to anthelmintic) is the most important issue in the development of resistance [van Wyk, 2001; Coles, 2002a]. There are three possible locations for nematodes in refugia: first, larvae on pasture, second, adult nematodes in untreated animals and third, inhibited stages in the host animal which survived treatment [Coles, 2005]. The idea of nematodes in refugia was first described by Martin (1981) and than again picked up by van Wyk (2001). He argued that those nematodes which survive an anthelmintic treatment form the next generation of the nematode population. This surviving ´resistant` population will produce eggs that contaminate the pasture. However, when there are enough nematodes on the pasture which are still susceptible for the used drug (nematodes in refugia), the treatment surviving ´resistant` nematodes will be diluted from these susceptible nematodes. The re-mixture of resistant nematodes with susceptible nematodes will help to delay the development of resistance [Martin, 1981; van Wyk, 2001]. Therefore it is important to save as much nematodes as possible in refugia. That means: keep pasture contaminated with nematode larvae, avoid the killing of all developmental stages (inhibited larvae) and do not treat all animals in a given flock.

3.4 Methods for detection of anthelmintic resistance

Resistance cannot be measured on the basis of an apparent clinical failure to anthelmintic treatment [Kelly and Hall, 1979]. Other reasons could make clinical signs similar to those normally associated with nematode diseases. Therefore, detection methods are an important means to prove if resistance to an anthelmintic compound is true.

The generally adopted threshold of anthelmintic resistance of the World Association for the Advancement of Veterinary Parasitology (WAAVP) is a treatment failure to reduce faecal nematode egg counts (FEC) by at least 95 % [Coles et al., 1992; McKenna, 1994]. The different detection methods to diagnose anthelmintic resistance include an in vivo test, the faecal egg count reduction test (FECRT) and various in vitro tests like the egg hatch test (EHT), the microagar larval development test (MALDT) and molecular based tests. Carven et al. (1999) found a poor correlation between FECRT, EHT and MALDT, so it is recommended to use more than one test for the confirmation of anthelmintic resistance [Craven et al., 1999].

For cattle, currently the only methods for detection of anthelmintic resistance are the faecal egg count reduction test (FECRT) and necropsy [Coles, 2004]. The latter method is not practicable because it is expensive and labor-intensive.

3.4.1 The faecal egg count reduction test (in vivo test)

The World Association for the Advancement of Veterinary Parasitology (WAAVP) gives an exact operation procedure how this test should be performed [Coles et al., 1992, 2006].

The FECRT is the most widely used method to detect the presence of anthelmintic resistance. It is applicable for the detection of resistance in all anthelmintic classes. The FECRT measures the number of eggs per gram faeces before treatment and at a defined time after treatment [Coles et al., 1992]. The period between first and second collection of faecal samples varies between the different types of anthelmintics (see table 3.4.1.).

Table 3.4.1 Collection time of faecal samples for FECRT

Anthelmintic group	**Time before treatment (day 0) and 2nd egg count**
Benzimidazoles	8 – 10 days
Levamisole / Tetrahydropyrimidines	3 – 7 days
Macrocyclic lactones	14 – 17 days

(Source: [Coles et al., 2006])

The reason for this procedure is the suppression of egg production due to a temporarily sterilization of – eventually resistant – female nematodes with benzimidazoles and macrocyclic lactones. These female nematodes will not be removed if they show resistance to one of the named anthelmintics. Therefore, it is important to wait for 10-14 days (compromise period of the different anthelmintics) after treatment before taking the second faecal sample [Taylor et al., 2002; Coles, 2005].

Faecal samples are collected from animal groups of at least 15 cattle with a minimum individual count of 100 eggs per gram. For the interpretation of data, Coles et al. (1992) recommended to calculate the arithmetic mean, percentage reduction of egg counts and 95% confidence interval.

The percentage reduction in egg counts is:

$$\mathbf{FECR\ (\%) = 100 \cdot (1 - X_T / X_C)}$$

FECR: faecal egg count (in eggs per gram) reduction in percent

X_T: arithmectic mean of eggs per gram faeces (treated group)

X_C: arithmetic mean of eggs per gram faeces (control group)

“Resistance is present, if the percentage reduction in egg count is less than 95% and the confidence interval is less than 90%” [Coles et al., 1992].

FECPAK (FECPAK International Ltd.) is a commercial kit, which is available for counting nematode eggs in faecal samples. This kit is designed for field use by farmers, veterinarians and scientists. The FECPAK unit contains all the equipment which is needed for calculating quickly and accurately the number of eggs in the faecal sample. This method could be used with cattle faecal samples and is sensitive to 10 eggs per gram. Detailed information is given by Coles (2003).

One of the disadvantages of the FECRT is the high expenditure of time. Another one is its low sensitivity. The test is only significant if more than 25% of the parasitic nematodes are resistant [Martin et al., 1989]. Nevertheless, it is the best test for the screening of anthelmintic resistance in the field and again – in contrast to the other tests – it can be used for all anthelmintic classes [Johansen, 1989; Coles et al., 1992].

3.4.2 The egg hatch test (in vitro test)

There is also a guideline from the WAAVP for the egg hatch test (EHT). The EHT was first described by Le Jambre (1976). He tested strains of *H.contortus* and *O. circumcinta* on resistance to thiabendazole. He could show that eggs of thiabendazole resistant strains hatched in higher concentrations of thiabendazole than non-resistant strains [Le Jambre, 1976].

The EHT is an in vitro method which can only be used with anthelmintics of the benzimidazole class [Coles et al., 2006]. Benzimidazoles show ovicidal activity and therefore prevent embryonation and hatching of nematode eggs [Lacey et al., 1987]. This property of benzimidazoles is used in the EHT. Therefore undeveloped eggs are incubated in serial

concentrations of the anthelmintic, mostly thiabendazole because of its high solubility in water [Coles et al., 1992].

For an interpretation of results, Coles et al. (2006) recommend to use the discriminating dose. This is the dose – 0,1 µg/ml thiabendazole – at which 99 % of susceptible eggs will not hatch and eggs which will hatch are resistant. A discriminating dose for eggs from nematodes of cattle has not been ascertained till now [Whitlock et al., 1980; Taylor et al., 2002; Coles et al., 2006].

One of the problems with the EHT is the requirement of undeveloped eggs [Coles, 2005]. The essential aim is to prevent the development of nematode eggs during transit to the laboratory. Several methods for the storage of eggs have been described. One of these methods is the cooling of eggs at 4°C. This allows storing eggs up to 3 days from the date of collecting [Smith-Buijs and Borgsteede, 1986]. Another method is the keeping of eggs under anaerobic conditions [Hunt and Taylor, 1989].

3.4.3 The microagar larval development test (in vitro test)

Currently, the microagar larval development test (MALDT) is only applicable for the testing of benzimidazole or levamisole resistant nematodes [Grimshaw et al., 1994; Amarante et al., 1997; Coles et al., 2006]. There is also a commercial test available, DrenchRite® (Horizon Technology, Australia).

The test is based on the development of eggs to L3 larvae. For that, eggs must be incubated for 7 days in a special medium with different concentrations of anthelmintic drugs. In contrast to the EHT, the age of the eggs (developed or undeveloped) is not important for the test. Anthelmintic resistance is probable, if 50% of the eggs, respectively the larvae, have developed to the next stage. The advantage of the MALDT is, that nematodes develop to L3 (Larvae 3) stage. In this stage of larval development the different species can be identified [Hubert and Kerboeuf, 1992].

The test cannot be advised for the control of resistance of cattle nematodes because of the inadequate evaluation of this test for bovine nematodes [Coles et al., 2006].

3.4.4 The molecular based test

The molecular test is based on a simple PCR (polymerase chain reaction). It can only be used for benzimidazoles because only for this substance class the molecular resistance mechanism is fairly understood [Coles et al., 2006]. Kwa et al. (1994) were pioneers in the research for a

molecular test detecting resistance in parasitic nematodes. They designed a special primer located at amino acids 194-200 on the coding strand. The resistance to benzimidazoles is mainly based on a mutation at codon 200 (phenylalanine to tyrosine) of the ß-tubulin gene, so if there is a change in this codon, it can be identified by a PCR test [Kwa et al., 1994]. After amplification of DNA with the PCR, susceptible and resistant nematode species can be distinguished with the restriction fragment length polymorphism (RFLP) [Coles et al., 2006]. However, there are other mutations than that at codon 200, like polymorphisms at codon 167 and codon 198 (see chapter 3.2.). The codon change at position 198 creates a RFLP enzyme site for HypCH4-V (restriction enzyme). This would allow a rapid identification of a mutation at position 198 [Mäser et al., 2006]. However, if there are additional, unknown mutations present, current tests will not detect resistance [Coles et al., 2006].

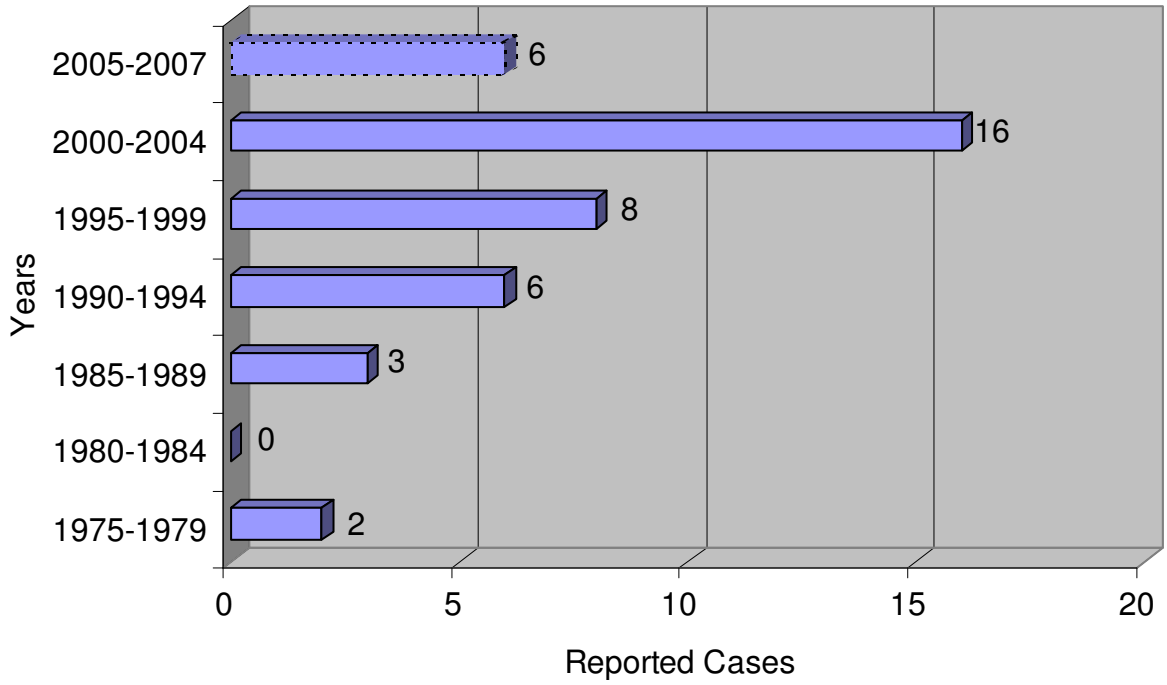
For PCR the DNA will be extracted from exsheathed larvae (special stage in the development of larvae). For the extraction, a protein digestion with the Kawasaki method should be done. Further guidelines for the exact process of this PCR test are given by WAAVP [Coles et al. 1992, 2006].

This molecular test was originally developed for *T.circumcincta*, *T.colubriformis* and *H.contortus*. There is a similar test available for one cattle nematode species, *C.oncophora* [Njue and Prichard, 2003].

3.5 Global occurrence of drug resistant nematodes in cattle

In comparison to the situation in sheep, anthelmintic resistance in cattle nematodes is not established to that extent. The increase of case reports of anthelmintic resistance in cattle nematodes between 1975 and 2007 is presented in Figure 3.1.

Figure 3.1 Number of case reports of anthelmintic resistance in cattle nematodes since 1975



One of the earliest cases known from anthelmintic resistance came from a levamisole-resistant strain of *T.colubriformis* in Brazil [Santiago et al., 1977]. Since 2000, there are increasing records on resistant nematodes in cattle from South America. The first report of anthelmintic resistance in cattle in Argentina came from Corrientes Province. Since then, cases of avermectin and benzimidazole resistance were frequently documented from different regions of Argentina [Anziani and Fiel, 2004]. Firstly, a strain of *H.contortus* was found showing multiple resistance to avermectin and benzimidazole [Anziani et al., 2004]. *Cooperia* species were the predominant parasite genus in all regions of Argentina and were mostly resistant to avermectins. The latest report came from the Pampa region (Argentina), where investigations of 25 cattle herds were conducted to asses the prevalence of resistance [Suarez and Cristel, 2007]. *Cooperia* spp. and *Ostertagia* spp. were the predominant nematode species which could be found in these cattle herds. Anthelmintic resistance could be detected in 16 of the observed farms, in 15 of these herds ivermectin resistance was present and in 8 of them benzimidazole resistance could be detected. Multidrug resistance was found in seven of the surveyed herds. Levamisole resistance was not detected.

The worldwide occurrence of drug resistant nematodes in cattle is listed in Table 3.5.1. The existing case reports were tabulated by continents and only countries where reports exist were listed.

Table 3.5.1 Worldwide occurrence of anthelmintic resistance in cattle

Country	**Number of case reports**	**Nematode species**	**Anthelmintics (B=Benzimidazoles, L= Levamisole, T= Tetrahydropyrimidines ML= Macrocyclic Lactones)**	
South America				
Argentina	**9**	*Cooperia* spp., *Ostertagia* spp.	B / ML	[Suarez and Cristel, 2007]
		Cooperia spp.	ML	[Descarga, 2005]
		H.placei, C.oncophora, C.pectinata	B / ML	[Anziani et al., 2004]
		C.oncophora, C.punctata, O.ostertagi, H.placei	B / ML	[Mejia et al., 2003]
		C.pectinata	ML	[Anziani et al., 2000]
		C.oncophora	ML	[Fiel et al., 2001]
		C.pectinata, Trichostrongylus spp.	ML	[Fiel et al., 2001]
		C.oncophora, T.colubriformis, T.longispicularis	ML	[Fiel et al., 2000]
		C.pectinata	ML	[Anziani et al., 2000]
Brazil	**5**	*D.viviparus*	ML	[Molento et al., 2006]

Country	Number of case reports	Nematode species	Anthelmintics (B=Benzimidazoles, L= Levamisole, T= Tetrahydropyrimidines ML= Macrocyclic Lactones)	
		H.placei	ML	[Rangle et al., 2005]
		H.placei, C.punctata	ML	[Paiva et al., 2001]
		H.placei	B	[Pinheiro and Echevarria, 1990]
		T.colubriformis	L	[Santiago, 1977]
Venezuela	**1**	Gastrointestinal nematodes (Species not determined)	L / ML	[Sandoval et al., 2001]
North America				
USA	**1**	*H.contortus, C.punctata*	B / ML	[Gasbarre and Smith, 2004]
Australia / New Zealand				
Australia	**3**	*T.axei*	B	[Eagleson et al., 1992]
		T.axei	B	[Eagleson and Bowie, 1986]
		O.ostertagi	B / L	[Anderson, 1977]
New Zealand	**14**	*Cooperia* spp., *Ostertagia* spp.	B / ML	[Waghorn et al., 2006]
		C.oncophora	ML	[Mason and McKay, 2006]
		C.oncophora, T.longispicularis	ML	[Loveridge et al., 2003]
		C.oncophora	B	[Winterrowd et al., 2003]
		Cooperia spp.	ML	[Familton et al., 2001]

Country	Number of case reports	Nematode species	Anthelmintics (B=Benzimidazoles, L= Levamisole, T= Tetrahydropyrimidines ML= Macrocyclic Lactones)	
		C.oncophora, O.ostertagi	B	[Hosking et al., 1996]
		Cooperia spp., *Ostertagia* spp., *Trichostrongylus* spp.	B	[McKenna, 1996]
		Cooperia spp.	ML	[Vermunt et al., 1996]
		Cooperia spp.	B / ML	[Vermunt et al., 1995]
		Cooperia spp.	B / ML	[Watson et al., 1995]
		Cooperia spp.	ML	[West et al., 1994]
		Ostertagia spp.	B	[Hosking et al., 1991]
		Cooperia spp., *Ostertagia* spp., *Trichostrongylus* spp.	B	[McKenna, 1991]
		C.oncophora	B	[Jackson et al., 1987]
Europe				
GB	**2**	*Cooperia* spp.	ML	[Coles et al., 2001]
		C.oncophora	ML	[Coles and Stafford, 1999]
Poland	**1**	Gastrointestinal nematodes (Species not determined)	B	[Balicka-Ramisz and Ramisz, 1999]
The Netherlands	**2**	*O.ostertagi*	L / T	[Borgsteede, 1991]
		O.ostertagi	L	[Geerts et al., 1987]

Country	Number of case reports	Nematode species	Anthelmintics (B=Benzimidazoles, L= Levamisole, T= Tetrahydropyrimidines ML= Macrocyclic Lactones)	
Asia				
India	**1**	*H.placei*	T	[Yadav and Verma, 1997]
Bangladesh	**1**	*Haemonchus* spp., *Trichostrongylus* spp.	B	[Hoque et al., 2003]
Africa				
Nigeria	**1**	Gastrointestinal nematodes (Species not determined)	B / L / T	[Fashanu and Fagbemi, 2003]

In New Zealand the situation is similar to that in Argentina. Increasing reports of anthelmintic resistance are made from different regions from all over the country. The first reported case of resistance against an anthelmintic substance came from Waikato (North-West of New Zealand), where an oxfendazole-resistant strain of *C. oncophora* was found [Jackson et al., 1987]. In all documentations about anthelmintic resistance in cattle in New Zealand *Cooperia* was the main genus.

Furthermore, there are also multidrug resistant *Cooperia* species from cattle which show resistance to both ivermectin and oxfendazole [Vermunt et al., 1995]. But also multigeneric (resistance in more than one species) resistance to avermectins has been observed from *C. oncophora* and *T.longispicularis* in cattle in New Zealand [Loveridge et al., 2003]. In the latest report, Waghorn et al. (2006) observed 62 beef cattle herds in the North Island of New Zealand. Anthelmintic resistance to ivermectin could be detected in 92 % of the farms, to albendazole in 76 %, to both in 74 % and to levamisole in 6 % of the observed farms. *Cooperia* spp. and *Ostertagia* spp. were the main parasite species which were involved in these cases [Waghorn et al., 2006].

The first report of anthelmintic resistant nematodes in cattle in Australia is by Anderson (1977). He observed levamisole, thiabendazole and fenbendazole resistance in *O.ostertagi* in

cattle. Furthermore, there were two cases of benzimidazole resistance found from *T.axei* in cattle in Australia [Eagleson and Bowie, 1986; Eagleson et al., 1992].

But not only South America, New Zealand and Australia are affected by resistant nematodes in cattle. Also in Europe there are several reports of anthelmintic resistant nematodes in cattle. The first case of levamisole resistance in *O.ostertagi* had been detected in Belgium, in a cattle farm in Merchtem, Flanders [Geerts, 1987]. Borgsteede (1991) from The Netherlands observed a morantel tartrate resistant strain of *O.ostertagi* which additionally showed side resistance to levamisole. The first reported failure of macrocyclic lactones in Europe came from South-West Britain. In this record an ivermectin-resistant strain of *C. oncophora* was found in calves on a farm in Somerset [Coles and Stafford, 1999]. A second case of macrocyclic lactone resistance was detected in a herd of first year calves in Gloucestershire [Coles et al., 2001]. Another case of resistant nematodes in cattle in Europe was found in northwest part of Poland, where benzimidazole-resistance of gastrointestinal nematodes in cattle could be demonstrated [Balicka-Ramisz and Ramisz, 1999].

In other parts of the world, there are cases of benzimidazole-resistance reported from Bangladesh [Hoque et al., 2003], morantel tartrate resistance from India [Yadav and Verma, 1997] and a case of multidrug resistance in Nigeria [Fashanu and Fagbemi, 2003].

There is also a report from USA about multidrug resistance in cattle nematodes [Gasbarre and Smith, 2004]. Gasbarre and Smith (2004) have identified multidrug resistant nematodes in cattle from Wisconsin, USA. *H.contortus* and *C.punctata* were the major species in the 4000 animal large cattle herd. But also *H.placei*, *C. oncophora* and *C. spatulata* were present in the calves. *H.contortus* showed multiple resistance to various macrocyclic lactones and to albendazole, whereas the remaining nematodes showed resistance only to macrocyclic lactones.

3.6 Resistant nematode species in cattle

In the existing records, only five main parasitic nematode species were found to be resistant to one or all of the available anthelmintics. In table 3.6.1 the frequency of occurrence of the different nematode species is documented and the species are itemized into the subspecies which were found in the present case reports.

Table 3.6.1 Frequency of cattle nematode species with anthelmintic resistance

Nematode species	Number of case reports including this species
***Cooperia* spp.** *C.oncophora* *C.punctata* *C.pectinata* *C.curticei* *C.spatulata*	**26**
O.ostertagi	**10**
***Trichostrongylus* spp.** *T.axei* *T.longispicularis* *T.colubriformis*	**9**
***Haemonchus* spp.** *H.contortus* *H.placei*	**8**
D.viviparus	**1**

Globally, *O.ostertagi* is regarded as the most important trichostrongylid of cattle. This species is involved in enormous losses in cattle all over the world [Miller, 1994]. First year calves with ostertagiosis show severe diarrhoea, oedema and weight loss. Ostertagiosis frequently leads to emaciation and death.

In New Zealand and Argentina, *Cooperia* is the predominant genus which shows resistance to benzimidazoles and macrocyclic lactones. But also in Europe resistant *Cooperia* species were found [Coles and Stafford, 1999; Coles et al., 2001]. Altogether, *Cooperia* was the genus mostly noted in the existing reports. Especially macrocyclic lactone resistance is widespread in *Cooperia* species, and has been found in Argentina, Brazil, New Zealand, the UK and the USA. In cattle, the dose-limiting species for ivermectin are *Cooperia* spp. and *Nematodirus* spp [Shoop et al., 1995; McKellar and Benchaoui, 1996]. It was found by Benz et al. (1989) that *C.oncophora* as well as *T.colubriformis* is less susceptible to endectocidal anthelmintics than other nematode species . Presumably, a species which is less susceptible to a given drug would develop resistance quicker than a species which is highly susceptible. This could be an

explanation for the frequent occurrence of *Cooperia* spp. An overview of the case reports is given in table 3.4.1.

To a lesser extent, *Trichostrongylus* and *Haemonchus* species were found to be resistant in cattle. The first case of *T.colubriformis* in cattle came from Brazil [Santiago et al., 1977]. Two cases of *T.axei* could be detected in Australia [Eagleson and Bowie, 1986; Eagleson et al., 1992]. And two more cases of *T.colubriformis* and *T.longispicularis* could be reported from Argentina [Fiel et al., 2000; Fiel et al., 2001].

Also in Argentina, a multidrug resistant strain of *Haemonchus* could be detected [Anziani et al., 2004]. In another case, *H.placei* resistant to benzimidazoles was found [Mejia et al., 2003]. Resistance of *H.placei* has also been detected in Brazil [Rangle and Leite, 2005; Paiva et al., 2001; Pinheiro and Echevarria, 1990].

Another remarkable detection was the single report of a resistant *D.viviparus* population. This case was not a field situation but occurred under experimental conditions. Efficacy of the macrocyclic lactones ivermectin, doramectin and abamectin should be detected in naturally infected calves. It was determined by faecal larval counts that none of the substances were able to eliminate *D.viviparus* for up to 28 days after treatment. Molento et al. (2006) suggest that resistance to macrocyclic lactones has developed in this *D.viviparus* population. This development under experimental conditions could – as the author argued – be due to a combination of factors including suppressive treatment, high infection rates on pasture, continuous usage of dose-and-move strategy, presence of resistant genes in this *Dictyocaulus* population and ideal climatic conditions [Molento et al., 2006]. Furthermore, *D.viviparus* is the most susceptible species to macrocyclic lactones [Stromberg et al., 1999; Burden and Ellis, 1997].

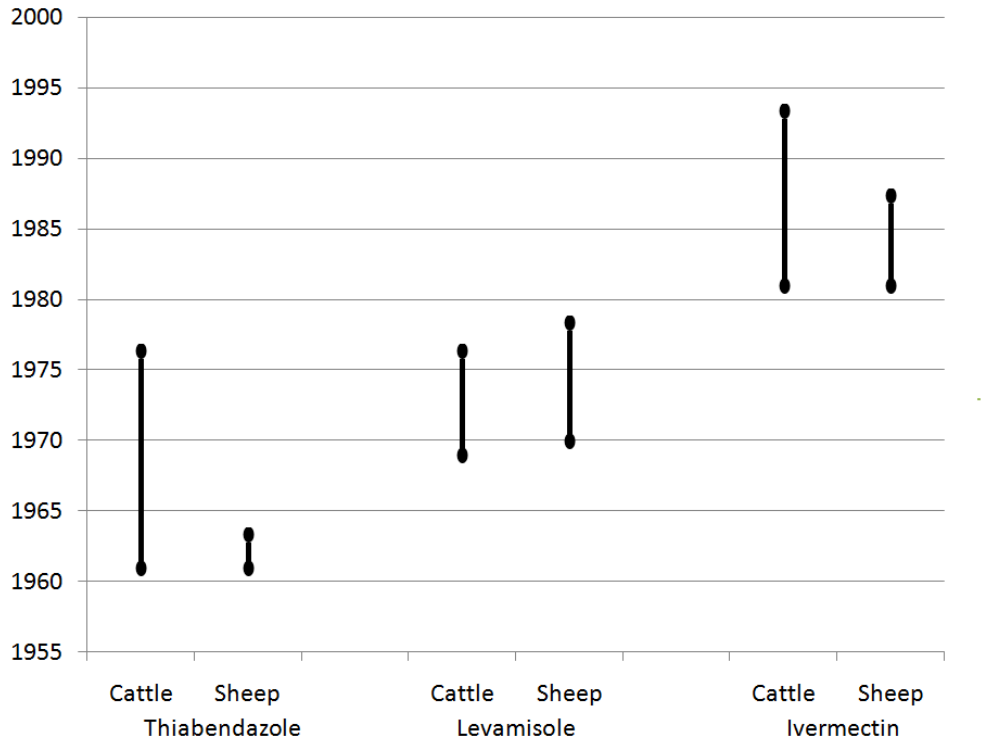
3.7 Speed of resistance development in cattle nematodes

The introduction of anthelmintics to the market happened in periodic intervals. Benzimidazoles were introduced in the early 1960s, levamisole and the tetrahydropyrimidines in the 1970s and macrocyclic lactones in the early 1980s. In the beginning of anthelmintic usage, each class showed excellent efficacy against a wide range of nematodes. But within a few years, resistance to every one of them was reported [Kaplan, 2004].

In contrast to the development of resistance in bacteria, the evolution of nematode resistance occurred rather slowly. The time between introduction and the occurrence of resistance against any anthelmintic class was approximately 10 years [Waller, 1994, 2003, 2006]. But

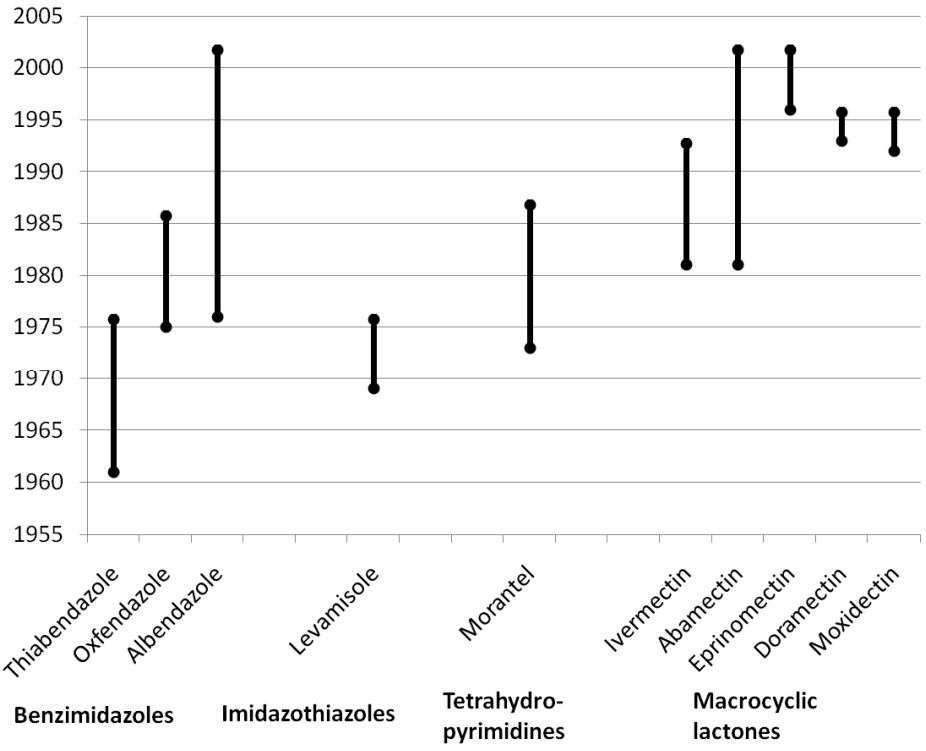
the development of resistance could vary between the different host species. The development of resistance (period between introduction of an anthelmintic product and the first report of anthelmintic resistance) in cattle nematodes to different anthelmintic classes is documented in figure 3.7.1 and 3.7.2. For a better assessment of this time periods, the resistance development of cattle nematodes is compared (Fig.3.7.1) with the resistance development of sheep nematodes (only for those anthelmintics which are used in cattle and sheep).

Figure 3.2 Comparison of periods between initial drug approval and first published report of anthelmintic resistance for cattle and sheep



* The exact approval date will vary between countries

Figure 3.3 Year of commercial release of anthelmintic drugs* and the first published case report of resistance in cattle nematodes



* The exact approval date will vary between countries

The first thing which could be observed from the two figures is that no new anthelmintic class has been introduced since 1981 [Kaplan, 2004; Besier, 2007]. Secondly, for drugs of macrocyclic lactones it could be detected: the later an anthelmintic of this class was released, the shorter was the period between release and first report of resistance. This could be due to the effect of side resistance. It could be presumed that if resistance had happened to one member of an anthelmintic class that resistance is conferred to the other members of this anthelmintic group [Wolstenholm et al., 2004].

In the comparison between cattle and sheep it could be seen that development of resistance in sheep nematodes was in parts rather fast. For thiabendazole, the period was only 3 years (Figure 3.7.1).

Compared to the situation of sheep, case reports of resistant cattle nematodes were few and development of resistance in this host was or rather is a slow process. The occurrence of resistance against benzimidazoles for example needed between 11 to 27 years. The first report of benzimidazole resistance came from Australia, where both, thiabendazole and also

levamisole failed to eliminate adult as well as early fourth stage larvae of *O.ostertagi* [Anderson, 1977].

For mebendazole and oxibendazole no records were found about resistance development in nematodes of cattle. The reason for this observation could be the fact that there are no products with these ingredients available for cattle or no investigations were undertaken. The same phenomenon was found for pyrantel where no drugs are available for cattle.

Morantel resistance in cattle nematodes were firstly observed after 15 years. The species, which showed resistance to morantel tartrate, was *O.ostertagi*, which was isolated and evaluated for anthelmintic resistance by Borgsteede (1988).

Levamisole was one of the quicker resistance developing substances. The first report came from Brazil where *T.colubriformis* showed resistance to levamisole [Santiago et al., 1977]. In this case, resistance was firstly observed 8 years after the introduction of levamisole.

Development of resistance in cattle nematodes occurred also against macrocyclic lactones. The earliest record came from *Cooperia* species in New Zealand, which showed resistance to ivermectin. Eprinomectin, introduced to market 1996, is one of the younger avermectins. Resistance in this anthelmintic substance came within 7 years. Loveridge et al. (2003) observed the resistance against eprinomectin and also against abamectin in *C. oncophora* and *T.longispicularis* in New Zealand. The shortest time for resistance development in cattle nematodes was found for doramectin. The first case of resistance against doramectin was observed 3 years after its introduction. In this case, inefficacy of doramectin as well as moxidectin was found. Vermunt et al. (1996) conducted investigations in two different farms: in the first farm resistance by *Cooperia* spp.had still been demonstrated to ivermectin, moxidectin and oxfendazole in the previous year, and in the second farm ineffectiveness of ivermectin against *Cooperia* spp. had also still been demonstrated. On the second farm moxidectin as well as doramectin were never used before and on this farm Moxidectin was not effective against *Cooperia* spp. Doramectin was not effective in either of the two farms against *Cooperia* spp. This result must be due to – in the opinion of Vermunt et al. (1996) – side-resistance to moxidectin and doramectin of the ivermectin-resistant *Cooperia* spp. Due to similar chemical structure and the relationship between avermectins and milbemycins a common mode of action can be assumed.

Furthermore, multidrug resistance in cattle nematodes could be observed from different regions of the world. In table 3.7.3 the reports of multidrug resistance in cattle nematodes are summarized.

Table 3.7.1 Development of multidrug resistance (mdr) in cattle nematodes

Anthelmintic classes	Documented reports of multidrug resistance in cattle nematodes
- Benzimidazoles / Levamisole	1977 [Anderson, 1977]
- Benzimidazoles / Macrocyclic lactones	1995 [Vermunt et al., 1995], [Watson et al., 1995]
- Benzimidazoles / Macrocyclic lactones	2003 [Mejia et al., 2003]
- Benzimidazoles / Macrocyclic lactones	2004 [Anziani et al., 2004], [Gasbarre and Smith, 2004]
- Benzimidazoles / Macrocyclic lactones	2006 [Waghorn et al., 2006] , [Suarez and Cristel, 2006]

The first observation came from Anderson (1977). He demonstrated levamisole and thiabendazole resistance of *O.ostertagi* in cattle in Australia. More than one case could be reported from New Zealand. Vermunt et al. (1995) observed resistance to ivermectin and oxfendazole in an isolate of *Cooperia*. In another case in New Zealand, bull beef weaners were infected with *Cooperia* species resistant against benzimidazoles and macrocyclic lactones [Watson et al., 1995]. Additionally, there are case reports of multidrug resistant *Cooperia*, *Ostertagia* and *Haemonchus* species from Argentina with resistance to avermectins and benzimidazoles [Mejia et al., 2003; Anziani et al., 2004; Suarez and Cristel, 2007]. Finally, one record on multidrug resistance came from Wisconsin, USA, where nematodes of cattle showed resistance to macrocyclic lactones and benzimidazoles [Gasbarre and Smith, 2004].

3.8 Management of anthelmintic resistance

Management of resistance in nematodes is not easy to deal with and many papers and articles have been written on this topic. Unfortunately, there is not a general solution which can be adopted for every farm system. Solutions for the management of resistance have to be defined for every single farm.

The following points are universal recommendations and should be recognised to prevent or at least delay the development of resistance in cattle herds [Bjorn, 1992; Barnes et al., 1995; Dobson et al., 2001; van Wyk 2001 ; Coles, 2002a, 2004, 2005; Wolstenholm et al., 2004]:

- Presence of nematode eggs/larvae should be examined before drug treatment and success of the treatment should be checked
- Resistance/susceptible status of nematodes should be monitored regularly
- Treating of only first-year calves, not second year or adult cattle
- Treating first-year calves only when there is a serious problem with nematodes in older animals
- Underdosing should be avoided; short acting drugs should be chosen to prevent exposure of nematodes from sub-therapeutic drug concentrations (extended half-life of a drug)
- Giving first-year calves the chance of a sufficient contact to nematodes to enable the development of immunity
- Changing pasture of first-year calves every year
- Changing the type of anthelmintic every year
- Finally, consider – in either case – to keep nematodes in ´refugia`(conserve anthelmintic susceptibility)

In the nearer future maybe newer management strategies or alternative control methods could be integrated in ´traditional` control programs. These strategies could include the inset of bioactive forages, parasite resistant animal breeds, biological control with nematophagous fungi or perhaps vaccines [Tzamaloukas et al., 2005, 2006; Sarkūnas et al., 2000; Hertzberg et al., 2002; Stear et al., 2007; Claerebout et al.,2003].

There is only little experience with bioactive forages, and the few trials with these additives were performed with sheep. In these trials grazing lambs were fed with different bioactive forages after infection with larvae of *T.circumcincta*. There was a significant reduction in adult nematode burdens after 35 days (lambs were slaughtered) when they had eaten *Cichorium intybus*. So, there is some evidence that the used forages, respectively their secondary metabolites, could be candidates for the support of pasture management [Tzamaloukas et al., 2005, 2006].

The great potential of biological control using the nematophagous fungus *Duddingtonia flagrans* has been demonstrated in various trials with cattle, sheep, horses and pigs [Hertzberg

et al., 2002]. This fungus produces thick-walled spores which survive passage through the gut of ruminants. In an experiment in goats, the influence of these spores on both L3 larvae from *T.circumcincta* and L1 larvae from *Muellerius capillaris* has been demonstrated. It was shown that *D.flagrans* was highly effective in reducing the larval development of *T.circumcincta* on the pasture [Paraud and Chartier, 2003]. However, the daily application of spores is inhibiting the introduction of this biological control approach.

Furthermore an interesting control practice would be a vaccine for nematode infections. Until now the only available vaccine to control nematode infections is the radiation-attenuated larval vaccine against *D.viviparus*, Dictol® [Eckert and Deplazes, 1996; Schnieder, 2004]. It is no longer licensed in Germany and Switzerland. However, it would be desirable to have such a vaccine also for other nematode species. There are numerous research projects pursued on this theme, mostly on vaccines against *O.ostertagi* [Claerebout et al., 2003]. This is not surprising, because it is supposed that *O.ostertagi* is the most important nematode parasitizing the abomasum of cattle. There is little success in the vaccination of cattle with an *Ostertagia* polyprotein allergen [Vercauteren et al., 2004]. However, there is currently no commercial vaccine against gastrointestinal nematodes available and further research will be needed on this theme.

4 Discussion

The present work gives an overview about the current situation of the worldwide occurrence of anthelmintic resistance in cattle nematodes. It shows that until today reports of anthelmintic resistance in cattle nematodes were mainly documented from New Zealand and South America. In contrast, there are only few cases known from other countries (see table 3.5.1). Therefore, resistance in cattle nematodes is today perceived as a relatively minor problem. However, it would be naïve to assume that resistance in cattle nematodes will remain at this low level. More likely, it will further develop to a serious problem, as increasing numbers of reports from several regions of the world indicate. It is more likely that it will become as important as it is today in other livestock species. The sheep industry for example is already heavily affected by resistance of parasitic nematodes all over the world [Waller et al., 1996; Waller, 1997; van Wyk et al., 1997; Coles, 1998; Jackson and Coop, 2000; Kaminsky, 2003; Besier and Love, 2003; Besier, 2007].

In the literature on drug resistant nematodes in cattle, two major questions emerge:

1. Which factors in the different case reports were responsible for the development of resistance in cattle nematodes?
2. What are the reasons for the relatively small number of reports on anthelmintic resistance in cattle nematodes?

1. As described more detailed below, the factors which led to resistance in cattle nematodes – in the reviewed papers – can be divided into two main groups:

- Factors according to management systems (cattle husbandry)
- Factors according to treatment

Firstly, different management systems of cattle housing and their influence on resistance development should be regarded closer. Here we want to highlight three basic forms of cattle husbandry: firstly, the system where cattle are kept solely indoors (for example dairy cows or bulls for fattening), secondly, the system where cattle are temporarily kept indoors and are turned out to pastures in the late spring/summer months and are put back into barns in autumn, and thirdly, the system where cattle are kept the whole year round on the pasture. These three types of cattle management systems differ regarding the risk of nematode infections, and therefore, differ in the use of anthelmintics. Not in every system anthelmintic

treatments are required. If the risk of infection is low, anthelmintic treatments should normally be low too.

The system of cattle husbandry with the lowest risk of infection is that where cattle are kept the whole year round in barns. The risk of infection with nematode larvae is reduced. Cattle may harbour an infection and thus, may keep some adult nematodes. In such cases these adults will produce eggs, which will be shed into the environment. However, it is unlikely that these eggs respectively the developing larvae will be ingested again, because cattle take their food from mangers and dung will be removed daily. So, normally all eggs are removed from the barn and thus, the life cycle of nematodes is disrupted. One example for this cattle husbandry system is bull beef-rearing in barns in Germany. None of the presented case reports of resistant nematodes included this type of cattle husbandry.

The cattle husbandry systems which includes both, barn and pasture management will be that with medium risk of infection with nematode larvae. The risk to be infected with nematode larvae is mainly given in that time of grazing on pasture. Representative regions for such a system are for example the alpine and subalpine regions. In these areas, young cattle stay from the early summer to early autumn on alpine pastures and they are commonly treated two times a year, once before they are turned to pasture and after return from there. Two treatments a year seem sufficient to get a good nematode control but not too much to favour the development of anthelmintic resistance [Balmer et al., 1998].

The third form of cattle housing (whole year grazing) will be that with the greatest risk of infection, because in this system the life cycle of the parasites is closed. Adult nematodes in the host animals shed eggs which continuously contaminate the pasture. These eggs will develop to larvae which will be ingested by grazing cattle. In addition, if there is a high stocking density on the pasture, the risk to ingest infective larvae is higher than on pastures with low stocking density. One example is the whole year grazing system in the plain regions of central Argentina, where cattle production is mainly based on year round grazing [Anziani et al., 2004]. In this case, the same pastures are used for grazing of young bulls for several years without interruption. Additionally, frequent treatments were used to control nematode burdens and to promote good growth of the bull calves. Both factors, year round grazing on the same pasture and frequent treatments increase the selection pressure on nematodes.

The different cattle husbandry systems vary between countries. In a report of IFCN (International Farm Comparison Network) different farm systems with cattle beef production in 11 countries were presented [Deblitz et al., 2002]. They point out for example differences

in fattening of bulls between Germany/France (European Countries), South America and USA. In Germany and France bull fattening is mainly performed in barns and is based on intensive feeding with grass, silage and grains, whereas bull fattening in Argentina is based on year round grazing. In USA bull fattening is a mixture between pasture management and cattle housing, in the end of fattening the bulls are kept in so-called feedlots.

In the present case reports, the form of cattle husbandry seems to have a considerable part in the development of anthelmintic resistance. The main reports of anthelmintic resistance came from Argentina and New Zealand where intensive beef calf rearing with year round grazing is the primary management system of cattle [Suarez, 2002; Anziani et al., 2004]. As it was mentioned before, the year round pasture management is the most risky form of cattle husbandry for the infection with nematode larvae. Therefore, anthelmintics are widely used in these countries in attempts to control nematode infections. Especially, macrocyclic lactones are used, primarily for tick control without considering the consequences on the development of resistance in nematodes [Coles, 2002a].

In addition to the above, differentiation between barn and pasture management systems, Coles (2002a) differentiates between beef suckler herds, in which mother animals are held together with their calves on pastures, and beef calf rearing, where young calves are bought from different places and turned out together on one pasture. In the first form, where adult and young cattle are kept together, the main part of the grass will be taken by mother animals, which generally have already developed a good immunity against nematodes. Calves will ingest low amounts of grass and therefore they will be infected with a lower number of nematodes than the adult cattle. Calves in this animal husbandry system get the chance to develop a good immunity. Coles (2002a) argued that in this management system anthelmintic treatments are seldom required and therefore it would be unlikely that anthelmintic resistance will develop.

Coles (2002a) described a second management system: beef calf rearing which is a very common farming system in Argentina and New Zealand. In this type of cattle housing, often animals are bought from different farms and put together on large pastures [Deblitz et al., 2002; Mejia et al., 2003; Anziani et al., 2004; Suarez and Cristel, 2007]. The purchase of animals from different farms comprises the risk of introduction of new animals with nematodes of a different drug susceptibility status, i.e. drug-resistant nematodes. Putting these animals together without initial quarantine increases the risk of introduction of resistant nematodes into the herd.

A common problem with the grazing systems is that the same pasture is utilized for consecutive years. This has been observed in Argentina, where calves were put for three or four consecutive years on the same pasture [Anziani et al., 2004]. The same phenomenon could be observed in the south west of England. In a questionnaire about nematode control practices, 65 per cent of 97 farmers reported that they turned out first year calves on the same pasture every year [Stafford and Coles, 1999]. If in these systems calves are treated regularly with anthelmintics, practically all eggs which are on the pasture derived from those nematodes which survived anthelmintic due to reduced drug susceptibility.

Apart from suboptimal pasture management systems, incorrect usage of anthelmintics is the second main reason for the development of resistance.

The main reason for the development of resistance in the present case reports seems to come from “too frequent treatments”. Many of the farmers treated their calves too often per year. Mejia et al. (2003) reported from a farm in Argentina with multidrug resistant nematodes where cattle were treated four times per year. Similar conditions were observed by Suarez and Cristel (2007), who tested twenty-five cattle herds for anthelmintic resistance in the Pampeana region, Argentina. They discovered that in herds with anthelmintic resistance, cattle were treated 4,0 ± 1,3 times per year and in herds without anthelmintic resistance they were treated 1,9 ± 1,0 times per year. But also in New Zealand the correlation between treatment frequency and the occurrence of anthelmintic resistance could be seen. In the study of Jackson et al. (2006) 62 beef cattle-rearing farms were observed in the Northern Island of New Zealand. From a questionnaire on management of nematode parasites they discovered that on one of four farms calves were treated 8 to 12 times per year. The prevalence of resistance was high for ivermectin (82 per cent) as well as for albendazole (60 per cent). In these cases anthelmintic resistance is most likely a result of a high selection pressure on nematodes due to frequent treatments. Nematodes which survive treatment will produce the next generation. In systems with low selection pressure, surviving resistant nematodes, respectively their larvae, will be diluted by already existing susceptible nematode larvae on the pasture. But if treatments are repeated too often, the susceptible nematodes will be diluted out and only resistant nematodes will establish the infectious population.

In addition, the exclusive use of the same anthelmintic class for several years led in many of the case reports to anthelmintic resistance. In a mathematical model, Barnes et al. (1995) could show that annual rotation between two different anthelmintic classes was better than 5-year- or 10-year-rotation between the two drugs. In a model with annual rotation,

susceptibility of nematodes to both drugs could be maintained for a little longer than in a model with 5-year- or 10-year-rotation.

In conclusion, all kinds of combinations between cattle management systems and treatment frequencies are possible. If inappropriate combinations of a pasture management and of treatment frequency are chosen the risk of resistance development is increased.

2. What are the reasons for the relatively small number of anthelmintic resistance reports in cattle nematodes? Does this snapshot – as we can see it today – reflect reality correctly?

We are probably looking only on a part of the whole problem. Anthelmintic resistance is not a local but a world wide problem. As it can be seen from above arguments, anthelmintic resistance is correlated with inappropriate nematode control practices. Therefore, it can be hypothesized that there are more case reports from countries other than South America and New Zealand.

There are several possible explanations for this lack of reports: perhaps, management systems and anthelmintic treatment in countries with currently low prevalence of anthelmintic resistance or no case reports are probably others and/or are better than that in South America and New Zealand. But more likely, no investigations have been carried out in those contries to monitor the real situation on nematode control methods and the distribution of anthelmintic resistance [Coles, 1988, 2002a], or investigations have been performed but without public reporting.

It might be possible, that the lack of reports is based on lacking of knowledge/notice on the importance of anthelmintic resistance by veterinarians`as well as by farmers`. As Sargison et al. (2007) argued, the true prevalence of multi drug resistance in sheep in the UK is not known, because only a little percentage of farmers does routinely check anthelmintic efficacy. This seems also be true for cattle farmers. Stafford and Coles (1999) had maid a survey in south west of England. But due to the low participation in this trial, a prediction about the real resistance status in cattle nematodes in south west England cannot be made.

Preliminary results from Kleinschmidt et al. (2007) about the occurrence of ivermectin resistance in cattle nematodes in Northern Germany suggests that resistance in cattle nematodes could be more common in those countries from which only few or no case reports exists on anthelmintic resistance in cattle nematodes. Resistance in these dairy farms

developed because only ivermectin was used over years. Nematode control was performed following the advice of the farm veterinarian.

Both articles, the one from England and the one from Germany are perhaps only the tip of the iceberg in these countries and certainly there are more cases of anthelmintic resistance in other regions and countries from which only few or no case reports exists.

In conclusion: there are at least four recommendations following the above mentioned observations:

- farmers as well as veterinarians should be pointed to the worldwide problem of increasing anthelmintic resistance respectively multidrug resistance; this may prompt a rethinking concerning a reduction of treatment frequency
- more surveys on anthelmintic resistance are needed, especially in countries from which there are today no or few case reports only on anthelmintic resistance in cattle; one could initially start with those farms with a high risk of nematode infection
- the relative slow development of anthelmintic resistance in cattle nematodes should be taken as chance to keep some of the still effective drugs working
- new anthelmintic compounds should be developed and made available before resistance will occurre to all currently available anthelmintics

In conclusion, the increasing number in recent years of reports on anthelmintic resistance in cattle nematodes indicates that this potential threat to cattle production may currently be underestimated. Cattle farmers may still have the chance to learn form the devastating situation in sheep farming where multidrug resistance is a global phenomenon, and delay the development of resistance by using a holistic approach and apply appropriate anthelmintic treatment and farm management.

5 Summary

The purpose of the present work was to highlight the increasing number of reports on resistant nematodes in cattle. In addition, the currently available anthelmintics (in Germany and Europe) including the spectrum of the different drugs, the mode of action and mechanisms of drug resistance, the detection methods for drug resistant nematodes, the development and management of resistance are reviewed.

Today, there are three main anthelmintic classes for veterinary use: the benzimidazoles, the imidazothiazoles (levamisole)/tetrahydropyrimidines, and the macrocyclic lactones. Anthelmintic resistance in cattle nematodes has been detected against all compounds of the three classes. Particularly, macrocyclic lactones are involved in many of the present case reports, but also benzimidazoles resistance was detected. There have been a few case reports from cattle nematodes which showed resistance to imidazothiazoles (levamisole) or tetrahydropyrimidines. Also multidrug resistance – especially to macrocyclic lactones and benzimidazoles – was observed.

Anthelmintic resistance has primarily been observed in New Zealand and South America, where intensive beef calf rearing is practiced. In addition, there are single reports on anthelmintic resistance in cattle nematodes from the USA, Europe, Asia and Africa.

Cooperia spp. was the main parasite genus. In the majority of these reports, *Cooperia* spp. showed resistance to macrocyclic lactones. Further species, which were involved in anthelmintic resistance in cattle, were *Ostertagia* spp., *Trichostrongylus* spp. and *Haemonchus* spp. So far, there was only one case report of a resistant *D.viviparus* population in cattle.

The increasing number of case reports on anthelmintic resistance in cattle nematodes is alarming and might in the future threaten beef and milk production in a similar way as it does constrain sheep industry today. Appropriate management and correct use of anthelmintics is crucial to delay the development of anthelmintic resistance in cattle. The most important step to retard the development of anthelmintic resistance is the reduction of drug treatments per animal and year.

6 Zusammenfassung

Das Ziel der vorliegenden Arbeit war es, die zunehmende Anzahl von Berichten resistenter Nematoden beim Rind zu analysieren. Darüber hinaus wird ein Überblick über die derzeit in Deutschland und Europa verfügbaren Anthelmintika gegeben. Außerdem werden das Wirkungsspektrum der verschiedenen Medikamente, die Wirkungsweise und die Resistenzmechanismen betrachtet. Schließlich wird auf die Nachweismethoden für resistente Nematoden, die Entstehung und das Management von Resistenzen eingegangen.

Es gibt hauptsächlich drei für den veterinärmedizinischen Gebrauch relevante Klassen von Anthelmintika: die Benzimidazole, die Imidazothiazole (Levamisol) / Tetrahydropyrimidine und die Makrozyklischen Laktone.

Resistenzen gegen Anthelmintika bei Rindernematoden wurden bei allen Verbindungen der drei Klassen festgestellt. Besonders Resistenzen gegenüber den Makrozyklischen Laktonen wurden in vielen der vorliegenden Berichte beschrieben, aber ebenso konnten Benzimidazol-Resistenzen festgestellt werden. Es wurden nur wenige Fallberichte gefunden, in denen Resistenzen gegenüber Imidazothiazolen oder Tetrahydropyrimidinen auftraten. Auch Mehrfachresistenzen, besonders gegen Makrozyklische Laktone und Benzimidazole, wurden beobachtet.

Anthelmintika-Resistenzen wurden vorwiegend in Neuseeland und Südamerika beobachtet, wo intensive Rinderzucht betrieben wird. Außerdem gibt es einzelne Berichte von Anthelmintika-Resistenz bei Rindernematoden aus den USA, Europa, Asien und Afrika.

Cooperia spp. war die häufigste Parasitenspezies. In der Mehrheit der Berichte zeigten *Cooperia* spp. Resistenzen gegen Makrozyklische Laktone. Weitere Spezies, die an Anthelmintika-Resistenz im Rind beteiligt waren, waren *Ostertagia* spp., *Trichostrongylus* spp. und *Haemonchus* spp. Bislang gibt es erst einen Fall einer resistenten *D.viviparus* Population im Rind.

Die zunehmende Zahl der Fälle von Anthelmintika-Resistenzen bei Nematoden des Rindes ist alarmierend und könnte in der Zukunft die Fleisch und Milchproduktion gefährden, ähnlich wie sie bereits die Schafindustrie beeinträchtigt. Sachgerechtes Management und der korrekte Einsatz von Anthelmintika sind ausschlaggebend, um die Entwicklung von Anthelmintika-Resistenz im Rind zu verzögern. Die wichtigste Massnahme, um die Entwicklung von Anthelmintika-Resistenz zu verlangsamen, ist die Reduktion der Arneimittelgaben pro Tier und Jahr.

7 References

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