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Equine Anthelmintic Resistance

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Equine Anthelmintic Resistance

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Abstract

The rise of parasitic resistance to anthelmintic medication in horses is one of the most concerning topics to today's horse owners, veterinarians, and those surrounding the industry. Much like the trend of resistance to antibiotics in human medicine, anthelmintic resistance poses a severe threat to overall horse population health as well as individual animal health. Many studies, both in the United States and countries across the world, have noted anthelmintic resistance in *Parascaris equorum*, cyathostomins, and other prominent equine parasites. These studies were conducted by determining the Fecal Egg Count (FEC, or the amount of parasite eggs shed) in the manure of horses before and after treatment with anthelmintic. Of the three major anthelmintic classes (benzimidazoles, tetrahydropyrimidines, and macrocyclic lactones), resistance to benzimidazoles is the most commonly observed for reasons that remain unknown. However, studies suggest that resistance to tetrahydropyrimidines and macrocyclic lactones is in the early stages of development. As there are no new active compounds coming onto the market in the near future, most researchers conclude that we must look for better ways to manage the anthelmintic medication distribution based on necessity and not necessarily on prevention. Furthermore, some studies have evaluated herbal parasiticide sources such as garlic, but these studies have been predominantly *in vitro* and have not been evaluated in live animals. Should natural therapies become a viable parasiticide, it appears to be the most readily available and logical anthelmintic. It is my conclusion that, in order to slow the progression of anthelmintic resistance in equine parasites, enforceable restrictions must be placed on existing deworming agents and research of natural therapies must be further explored.

I. Background Information

The effects of parasitic infestation on equine herd and individual health can be chronic and even fatal. Often, the results of equine parasite infestation are not observed until internal damage is done. A horse which appears visibly healthy can be host to over 500,000 parasites in the gastrointestinal tract (Seyoum et al., 2017). The observable symptoms of parasite infestation in horses may include a poor or rough hair coat, coughing, weight loss, colic, pruritus, and decreased performance.

There are two classifications of parasites in horses: internal and external parasites. External parasites are organisms that inflict negative effects on their equine host and spend the majority of their lifecycle on the outside of the horse, primarily associated with the hair coat. A lice infestation would be an example of an external parasite affecting the horse (Figure 1A; Thal, 2016). An internal parasite is an organism that produces negative effects in horses and spends the majority of the lifecycle within the horse's body cavity. Most internal parasites complete their lifecycle in the gastrointestinal tract of the horse because it provides the ideal environment for the parasite's reproduction and survival (Seyoum et al., 2017). Internal parasites are also known to migrate through the cardiovascular and respiratory systems. If left untreated, internal parasites reproduce quickly and can rapidly lead to health issues in the horse. For example, *Parascaris equorum* (*P. equorum*, also known as roundworms or ascarids) are prevalent in young horses and the eggs are ingested with contaminated feed, water, or grass. Once consumed, the eggs hatch into larvae which burrow into the small intestine and migrate to the veins, liver, heart, and lungs. The larvae irritate the lungs, causing the horse to cough. The coughed-up larvae are re-swallowed and return to the small intestine to mature and reproduce (Wood, 2015). In just three months, infestation can cause scarring of the liver and lungs and intestinal blockage and rupture, which can quickly lead to death (Figure 1B; Apfel, 2013).

Due to the negative impact that parasites have on horses, veterinarians have been working to chemically kill worms and their larvae in horses since the early 1900s. Previous parasite control strategies included giving the horse mercury (which was toxic to both the worm and the horse) or using natural plants such as licorice, flaxseed, and tobacco leaves (Potter, 2012). Many natural deworming strategies were tried with limited success from the 1600s until the mid-1900s. Equine specialists at the time acknowledged that tobacco was readily ingested by the horses, but while nicotine (tobacco's active ingredient) was proven to possess anthelmintic properties, it was extremely toxic in the dosage needed to be effective against parasites (Briggs et al., 2004). Most current anthelmintics (also known as "dewormers") are chemical parasiticides given orally. Every equine anthelmintic on the market has one of three active ingredients: benzimidazole, tetrahydropyrimidine, or macrocyclic lactone (Matthews, 2014).

Many horse owners administer anthelmintic medications to horses because they are readily available and relatively inexpensive (Figure 1C; Janicki, 2016). This has led to the decline of major parasite-related health concerns but has resulted in the overuse of these products. Anthelmintics exert selective pressure on the parasites in the horse (Tydén et al., 2014). Ivermectin, a macrocyclic lactone, first came onto the market in the 1980s and was to be administered orally to horses bimonthly (Tydén et al., 2014). Resistance was noted when horses who had been dewormed with the Ivermectin paste continued to show signs of parasite infestation long after treatment. The development of parasitic resistance to Ivermectin was hypothesized to be a result of the overuse of Ivermectin which had led to the selection of resistance-associated alleles present within the parasite population (Tydén et al., 2014).

Only the parasites with the resistance-associated genes survive anthelmintic treatment and reproduce. Eventually the entire population of parasites within a horse or within a herd are resistant to one or several classes of parasiticide (Tydén et al., 2014). Internal parasite resistance is a concern to horse owners and veterinarians because it means that, in the near future, all current brands of equine anthelmintics will be ineffective against dangerous equine parasites. There are a variety of research-based suggestions for anthelmintic management aimed at prolonging or avoiding equine parasitic resistance. The most pressing issues surrounding this subject are controlling the overuse of anthelmintics, preventing the need for these drugs, and developing new larvicides and parasiticides to prevent and treat infestations in the future. Therefore, current anthelmintics must be more strategically used and new larvicides and parasiticides must be developed.

II. Local Relevance

Many Nebraska equine veterinarians are advocating for more frequent fecal egg count (FEC) tests to better determine how to use anthelmintics. Records of equine patient FEC tests performed from 2014 to present at Hillcrest Animal Clinic (HCAC) in Lincoln, Nebraska were evaluated. Strongyle eggs (Figure 1D; Anoka Equine, 2012) were the most commonly seen egg in fecal samples, with roundworm eggs observed in less than 10% of tests. Other species of internal parasites were rarely observed. About a third of the FEC tests performed at HCAC were negative for fecal parasite eggs.

FEC tests at HCAC are performed by weighing out 1.0 gram of the fecal sample and thoroughly mixing with a pre-mixed sucrose solution (Sugar-Med™, specific gravity 1.27). The fecal-sugar solution is then strained through a tea strainer and 13 mL of the collected liquid is poured into a 15 mL conical tube. The tube is centrifuged at room temperature for five minutes at

1200 rpm. After centrifugation, additional sugar solution is added to the tube to form a meniscus. A coverslip is then placed on top of the tube and the tube is allowed to sit at room temperature for 10 minutes. The coverslip is removed and placed onto a microscope slide and the number and type of parasite eggs are determined (Sewolt, 2018).

HCAC veterinarians consider horses which shed fewer than 250 eggs per gram (EPG) of feces to be “Low Shedders.” This value correlates with what many research teams and universities consider to be standard for a low-shedding horse (Lloyd, 2009). HCAC recommends that low shedders be dewormed with Ivomec (a macrocyclic lactone) or Strongid (pyrantel pamoate) once in the spring and then again using a combination of Ivomec and Praziquantel (such as Zimectrin Gold) on December 1. After a horse is confirmed to be a low shedder following a second FEC test, it is recommended to deworm the horse using a combination of Ivomec and Praziquantel annually on December 1.

A “Moderate Shedder” is a horse who sheds 250-500 EPG of feces. HCAC veterinarians recommend that these horses be given either Ivomec or Strongid, altering every three months in addition to the Ivomec/Praziquantel combination once annually.

“High Shedders” are horses who shed more than 500 EPG of feces. It is recommended to deworm these horses every two months with any anthelmintic paste, alternating active ingredients according to the season to effectively control each parasite during its active time of the year. Another option for these horses is to start on a daily feed-through dewormer (such as SafeGuard Equibits Pellets or Strongid C2X Pellets) in addition to an anthelmintic paste once in the spring and fall.

According to HCAC’s written records, only four of 193 horses have tested into the “High Shedder” category in the last four years (Figure 2). This supports the efficacy of the prescribed deworming schedule. However, these chronic “High Shedders” may represent equine parasites developing anthelmintic resistance. The 193 horses vary in ages from yearlings to aged horses and have been on regular, rotational deworming schedules since their first FEC test. The presence of parasites in such high quantities in the fecal material of the four horses suggests that they may have developed resistance to the parasiticides used. Horses that are chronically infested with internal parasites will have compromised immune systems as well as damage to the gastrointestinal tract. The FEC-based, individualized deworming method is part of newer protocols concerning equine anthelmintics prescribed in recent years. Up until three to five years ago, the majority of local equine veterinarians were recommending a bimonthly deworming schedule, which has been determined to be excessive for the majority of horses. While this schedule still included the rotation of active ingredients bimonthly, it proved to be unnecessary for “Low Shedders” which represent nearly half the local population.

HCAC veterinarians and other local equine veterinarians work to stay current on emerging anthelmintic-related research. While resistance isn’t currently a serious problem locally, the veterinarians are starting to see parasites in individual animals which cannot be eradicated by the current dewormers on the market.

III. Discussion

Matthews, 2014 proposed that the indiscriminant application of equine parasiticides was the main reason for the development of anthelmintic resistance. A comparison of the resistance of *P. equorum* and *Strongyloides vulgaris* (*S. vulgaris*) to the three major drug classes showed that both parasite species were minimally affected by benzimidazole and tetrahydropyrimidine, but were better controlled by a macrocyclic lactone-containing dewormer. These results demonstrated the urgency and severity of equine anthelmintic resistance that horse health professionals will be dealing with if not addressed.

Matthews, 2014 indicated that the most conclusive method to determine parasite resistance was to perform a FEC pre- and post-anthelmintic treatment. Pre-treatment, a fresh fecal sample is collected from an individual horse and the number of EPG of feces is determined. The horse is then treated with the selected anthelmintic and a second FEC is performed 14-17 days after anthelmintic treatment (Matthews, 2014). A comparison of the FEC pre- and post-treatment allows the level of resistance to be calculated. If the deworming agent decreased the number of fecal eggs shed by 90-95%, it was determined that no resistance had developed to the parasites in that individual animal. However, if the FEC in the second sample was greater than 5-10% of the original FEC, “resistance” to the anthelmintic had developed (Matthews, 2014). Matthews, 2014 concluded that the methods by which nematodes are managed must be changed in order to ensure global equine health in the future. Anthelmintics, especially macrocyclic lactones, must be used sparingly and horse owners must be given access to knowledge to make informed decisions regarding equine dewormers. A global body making informed decisions regarding anthelmintics and their uses needs to be established to determine and enforce guidelines on the proper use of anthelmintic medications.

Tydén et al., 2014 studied resistance markers in the equine parasite species *P. equorum*. A previous study had identified the presence of a TAC mutation in codon 167 of cyathostomin parasites from horses never previously dewormed (Blackhall et al., 2011). In addition, Diawara et al., 2009 identified mutations in the whipworm DNA at codon 200 in Haitian horse populations long before benzimidazole treatment was available in Haiti (Diawara et al., 2009). Acknowledging that benzimidazoles are the most commonly used substance to treat ascarid infestations, Tydén et al., 2014 designed an experimental protocol to locate the allele in *P. equorum* that coded for benzimidazole resistance. A herd of horses maintained at the University of Kentucky and not subjected to anthelmintic medications since 1979 were evaluated. Fecal eggs were collected and allowed to develop into third stage (L3) larvae. DNA was then extracted and amplified using the Polymerase Chain Reaction (PCR). Amplified DNA was sequenced and compared using Illumina HiSeq compatible sequencing libraries (Tydén et al., 2014).

Results of the study failed to identify any Single Nucleotide Polymorphisms (SNPs) associated with benzimidazole resistance in codons 167, 198, or 200 of the beta-tubulin isotype 1 or isotype 2 genes (Tydén et al., 2014). They hypothesized that resistance was most likely a recessive trait, based on the presence of SNPs in codon 200 in other nematodes (Diawara et al., 2009; Tydén et al., 2014).

Tydén et al., 2014 concluded that SNPs in the beta-tubulin gene, known to confer resistance in other nematode species, were not present in *P. equorum*, stressing the need for further research to identify resistance markers in *P. equorum*.

Fischer et al., 2015 evaluated the efficacy of Ivermectin and Pyrantel in horses using the FEC reduction test. FEC tests were conducted on 428 horses from 24 horse facilities in Germany, each with unique backgrounds concerning previous anthelmintic usage. Twelve species of cyathostomins were detected 14 days after anthelmintic treatment suggesting parasite resistance consistent with previous studies (Table 1; Fischer et al., 2015). Results indicate that four of the 24 farms had horses with cyathostomins resistance to Ivermectin 14 days after treatment. This was a total of five out of 428 horses evaluated with positive EPG counts post-treatment (Fischer et al., 2015). In only 78.3% of the farms, the egg reappearance period (ERP) was calculated to be longer than 42 days, which is standard for Ivermectin (Fischer et al., 2015).

The first finding of benzimidazole resistance in Germany was noted in 1983 (Bauer et al., 1983). Additionally, tetrahydropyrimidine resistance was reported in 2009, which led to the increased use of macrocyclic lactones (Traversa et al., 2009). Fischer et al., 2015 maintains that the shortening of the ERP is a strong indicator of loss of anthelmintic efficacy, or the early stages of resistance.

Three methods of analysis, each involving FEC reduction tests, were used to evaluate resistance associated with Pyrantel (a tetrahydropyrimidine) usage. The first analysis, the World Association for the Advancement of Veterinary Parasitology (WAAVP) method, indicates that resistance occurs when the FEC reduction is less than 90% (Coles et al., 1992). Using the WAAVP method of analysis, four farms showed resistance to Pyrantel. The second method for analysis, the Bootstrap Method, involved the re-testing of each farm and entering the data into an equation outlined by Coles et al., 1992. The Bootstrap Method equation (derived from the Bootstreat® computer program) detected resistance on seven of the farms evaluated. Finally, the Markov Chain Monte Carlo (MCMC) method further delineated the classification of resistance by determining that one farm was "susceptible" to resistance, four were "suspect resistant", and the remaining 16 farms were classified as "resistant" (Fischer et al., 2015). Significantly, only two parasite species consistently remained, and therefore showed resistance: *Cyathostomin goldi* and *Cyathostomin insigne* (Fischer et al., 2015).

Based on the data, Fischer et al., 2015 concluded that Ivermectin's efficacy can still be considered excellent in the region, but the results indicate Pyrantel resistance is present in horses on at least four farms with resistance in the early stages in horses on at least three other farms (Fischer et al., 2015).

Lassen and Peltola, 2014 conducted a FEC study of 41 young (less than three years old) trotting horses in four Estonian stables. Based on the FEC reappearance period and the number of EPG 14 days post-treatment with pyrantel, up to 27% of horses showed parasitic resistance. Equine anthelmintics are widely used in Estonia, which may have contributed to the resistance seen in the horses. These results agree with both those of Fischer et al., 2015 and the results of a study conducted in Finland, which detected an increase in EPG post-treatment with pyrantel in two young horses (Näreaho et al., 2011). Peltola and Lassen, 2014, speculated that the negative FEC reappearance reported by Näreaho et al., 2011was likely representing resistance to pyrantel in those two individuals.

With the need for reform in the equine anthelmintic industry, research has turned its focus to natural parasiticides such as plants. Most of these studies are in their early stages and have been conducted only *in vitro*.

Rakhshandehroo et al., 2017 tested the anthelmintic properties of five plants: *Artemisia dracunculus* (tarragon), *Eucalyptus camadulensis* (red river gum tree), *Mentha pulegium* (squaw mint/pudding grass), *Zataria multiflora* (Avishan-E-Shirazi), and *Allium sativum* (garlic). Using *P. equorum* worms and larvae gathered from foals who had died from the parasite infestation, the plant extracts were tested in four different concentrations: 50, 75, 100, and 125 mg/mL (Rakhshandehroo et al., 2017). Larval viability was defined by the observation of whip-like movement following treatment while immobilized larvae were considered to be affected or destroyed by the plant extract (Rakhshandehroo et al, 2017).

Results showed that *A. dracunculus* and *M. pulegium* extracts were highly lethal against larvae while *E. camadulensis* and *A. sativum* were considered ineffective with little effect on larval viability (Rakhshandehroo et al., 2017). *Z. multiflora* was considered effective at doses of 100 and 125 mg/mL but had no effect on larvae at lower concentrations (Figure 3; Rakhshandehroo et al., 2017).

Other studies claim that *Z. multiflora* is capable of stimulating the innate and acquired immune responses in animals, which could be an underdeveloped route of research in the near future (Erzurumlu et al., 1997; Shokri et al., 2006; Khosravi et al., 2007). In addition, garlic has been studied *in vivo* and determined to be incapable of reducing fecal egg counts, which further confirms the results of the *in vitro* study (Worku et al., 2009). Rakhshandehroo et al., 2017 concluded that plant extracts may be effective alternatives to the current parasiticides on the market.

Juyal and Rahman, 2006 supported the use of natural plant-based routes for parasite eradication and suggested that these plants be used as immunomodulators, which they defined as "a substance, biological or synthetic, which can stimulate, suppress, or modulate any of the components of the immune system." Their theory was that the use of certain plants would be helpful in increasing the efficacy of vaccines or parasiticides. A study by Padmavathi et al., 1988 showed that levamisole improved birds' resistance to caecal coccidiosis by stimulating the birds' own immune system. Plants such as Akanadi (*Cissampelos pareira)*, Kachnar (*Bauhinia variegata)*, Rajhans (*Blechnum orientale)*, and Jatashnkar (*Dryopteris ccochleata)* have been long understood in Indian medicine to hold anthelmintic properties (Table 2; Juyal and Rahman, 2006). The target of these plant-based immunomodulators is the generation of free radicals, nitric oxide, and cytokines which activate natural killer cells.

IV. Conclusion

In conclusion, equine anthelmintic resistance is emerging as an important equine health issue. Research has shown equine internal parasites are developing resistance to the drug classes currently available. Perhaps even more troubling is the fact that there is very limited research that considers what to do about this problem. Many authors state that the best we can hope for is to better educate those who are administering anthelmintic medications to horses and better manage equine practices, but even those measures will only buy time. Nearly every major research team and organization concludes that we will have a significant parasite resistance issue in the near future.

Establishing a governing body to create anthelmintic standards and guidelines is essential. Many authors state that they have trouble defining “resistance” because there is no set standard for what that means in relationship to equine parasiticides. Some veterinarians are better at staying updated about new information regarding this subject than others, but not all who administer these drugs are aware of the potential global risks. I believe researchers, veterinarians, owners, and parasitologists should unite under one organization to develop standards and usage guidelines for the currently available anthelmintics on the market and to be on the forefront for the development of safe, effective alternatives for the future.

The equine industry may benefit by funding research on plant-based therapies. The development and use of an herbal parasiticide in the case of “low-shedding” horses would limit the use of the current major larvicides on the market. Plant-based larvicide studies have primarily been conducted *in vitro*, so funding research of these potential parasiticide plants *in vivo* is necessary.

Based on current research, the major points needing to be addressed to decrease anthelmintic resistance include:

1. The creation of a governing body for the development of standards and guidelines on the proper use of livestock anthelmintics.
2. Elevate the status of equine parasiticides to “prescription-only” drugs to limit overuse. A FEC test would be required to determine the need for anthelmintics.
3. Encourage veterinarian education of clients on the subject of parasite prevention by creating incentive (through the proposed governing body) for evidence of proper and effective parasite control.
4. Have the new organization lobby for additional research funding for the development of new anthelmintics.

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VI. Figure Legends

Figure 1: External and internal parasitic infestation can have adverse effects on equine health. A) Horse suffering from a lice infestation. Notice the "rough hair coat" associated with the external parasitic infestation (Thal, 2016). B) Fatal intestinal rupture due to ascarid infection in the equine small intestine (Apfel, 2013). C) The most popular current deworming method for horses is an oral paste which can be administered by the horse's owner (Janicki, 2016). D) Equine strongyle egg detected as part of a fecal egg count (Anoka Equine, 2012).

Figure 2: Results of 193 FEC tests conducted at Lincoln's Hillcrest Animal Clinic between 2014 and 2018. FEC tests categorized 88 horses as "Low Shedders (fewer than 250 eggs per gram), 10 horses as "Moderate Shedders" (250-500 EPG), four horses as "High Shedders" (more than 500 EPG) and the remaining 91 horses as negative with no parasitic eggs observed (Sewolt, 2018).

Figure 3: Plant-based parasiticide effectiveness against *Parascaris equorum in vitro* (Rakhshandehroo et al., 2017).

VII. Figures

Figure 1A (Thal, 2016)

Figure 1B (Apfel, 2013)

Figure 1C (Janicki, 2016)

Figure 1D (Anoka Equine, 2012)

Figure 2 (Sewolt, 2018)

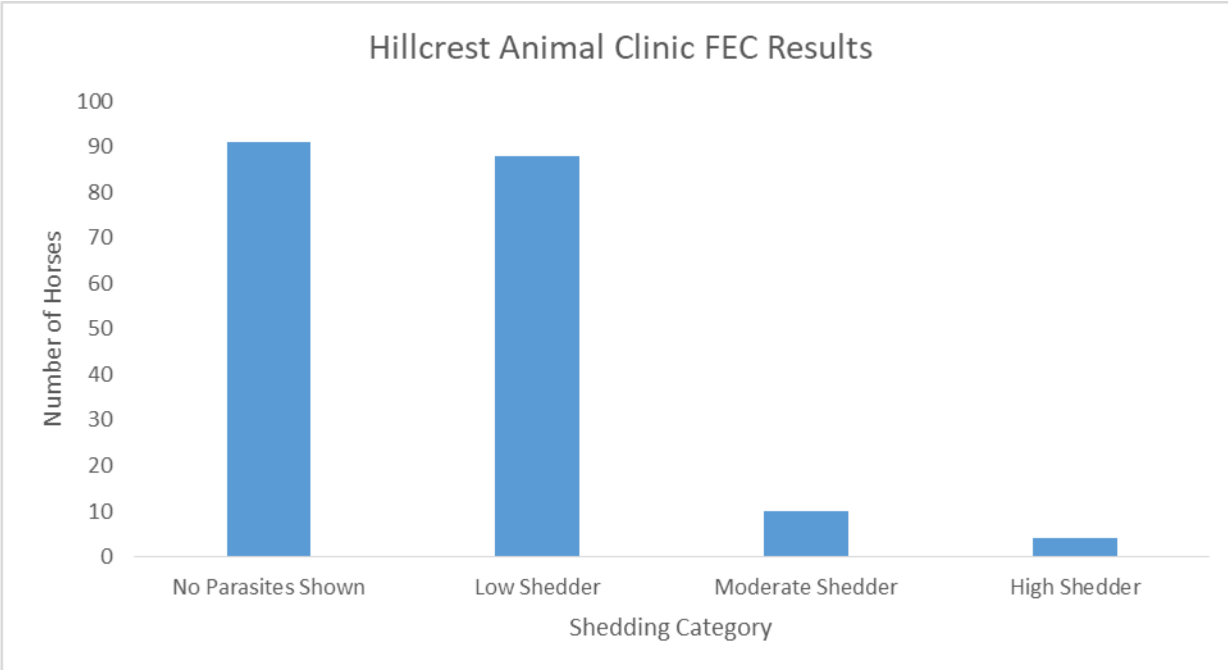

Figure 3 (Rakhshandehroo et al., 2017)

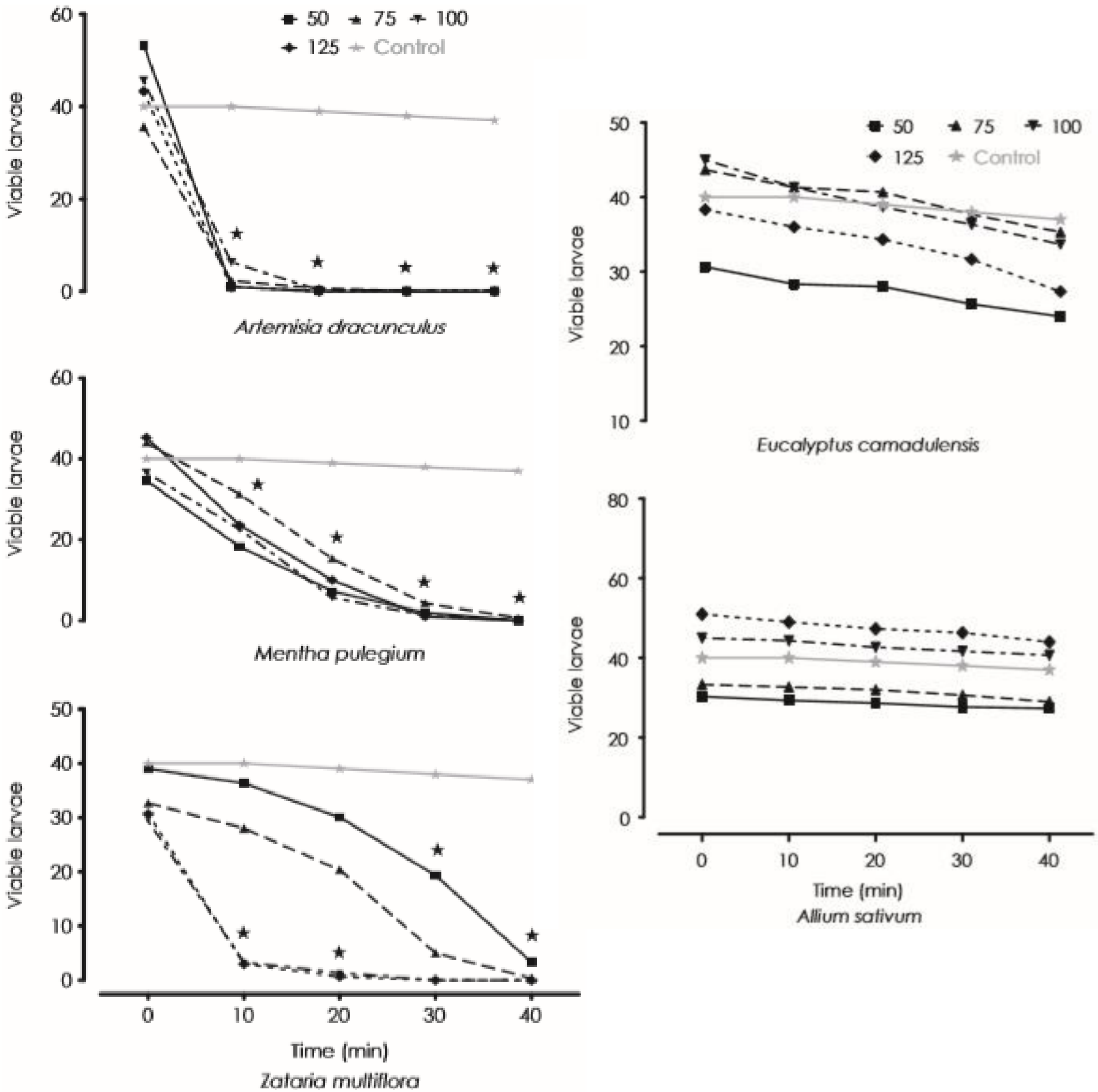

Table 1: Indication of Early Parasite Resistance to Ivermectin (Fischer et al., 2015). FECR = Fecal Egg Count Reduction, LCL= Lower Confidence Intervals, UPL = Upper Confidence Intervals. The definition of resistance is “FECR <90%, LCL <80%” which is present in a minimum of four farms, highlighted above.

Sample	Farm	FECR%	LCL (95%)	UPL (95%)
1	1	100	99.9	100
2	2	65.3	0	89.6
3	3	91.1	63.4	97.8
4	5	88.1	29.7	98
5	6	88.1	15.5	98.3
6	7	97.4	90.8	99.3
7	8	98.6	95.5	99.6
8	9	99.1	96.6	99.7
9	11	94.9	65.5	99.2
10	12	98	79.3	99.8
11	13	100	99.9	100
12	14	90.2	41.3	98.8
13	15	92.2	73.3	97.7
14	16	78.8	0	95.9
15	17	91.7	56.9	98.4
16	18	98.3	60.3	98.9
17	19	94.9	72.8	99
18	20	98.4	96	99.4
19	22	97.7	85.3	99.6
20	23	93.9	74.5	98.5
21	24	97.8	85.2	99.7

Table 2: Plant Species and their Anthelmintic Activity (Juyal and Rahman, 2006)

Plant species	Activity spectrum
Areca catechu	Gastrointestinal nematodes of poultry, pig and ruminants. Tapeworms of dog and poultry.
Boswellia dalzeli *Eurythrina senegalensis* *Khaya senegalensis* *Lawsonia intermis*	Fasciolosis in ruminants
Oil of chenopodium list in British Veterinary Codex	Used against *Ascaris* in horses & pigs, *Toxocara* in dogs and strongyle in horses.
Artemisia maritima	*Toxocara vitulorum* in buffalo, *Toxascaris* in dogs and *Ascaris suum* in Pigs
Caesalpinia crista	*Toxocara vitulorum* in calves
Melia azedarach	*Haemonchus, Chabertia, Trichuris, Trichostrongylus* and other gastrointestinal nematodes in sheep. *Ascaridia galli* in poultry.
Mallotus philippensis	Intestinal cestodes in goats(Akhtar and Ahmad, 1992)
Chrysanthemum spp.	Several nematode species in different mammals
Matteuccia orientalis	*Fasciola* in cattle (Shiramizu *et al*, 1993)
Carica papaya	Ascariosis in pigs (Satrija *et al*, 1994)
Heracleum *spp.*	Gastrointestinal nematodes in sheep
Hedysarvum coronarium	*Trichostrongylus* spp. in sheep (Niezen *et al*.1995)
Aloe barteri	*Nippostrongylus* spp. in rats
Diospyros mollis	*Nector americanus, Nematodirus dubius and Hymenolepis nana* in golden hamsters and mice.
Jantana (Commercial ayurvedicby Mycon Pharma ,Pune India)	*Strongylus, Trichostrogylus, haemonchus* and *Nematodirus* in cattle (Sharma , 1993)
*Tribulus terrestris (*Perrenial plant*)*	*Ascaridia galli* in poultry
Holerrhena antidysentrica, *Barberia* spp and *Alium* spp	Combination of drug gave antococcidial activity against all the endogenous stages of *Eimeria tenella*
Azadirachta indica	Anticoccidial efficacy in terms of oocyst count and lower mortality (Tipu *et al* 2002).
Barberis aristata	Property of healing unhealthy ulcers, sores and even protozoan infections like malaria, fever in man. Also have effect on development of coccidia and healing effects on lesions of poultry (Roy *et al* 1990).
Zycox {(IHP-250C) (Ayurvedic preparation from Indian herbs, India)}	Quite effective against different levels of *E. tenella* infection and caused least interference in the development of immunity (Tuli, 2002).
Xanthium strumarium	*Trypanosoma evansi*@ 100 and 300 $mg.kg^{-1}$. Survival time of *T. evansi* significantly increased (Talakal *et al*, 1995a)
Parthenium hysterophorus	Significantly reduced the parasitaemia and increase the Survival time of *T. evansi* significantly increased (Talakal *et al*, 1995b)
Artemisinin (qinghaosu) derivatives viz. artesunate, arteether and artemether	Effective against multiple drug resistance cases of human falciparum malaria and *Babesia* Infection (Bunnag *et al*, 1992, Pittler et al, 1999 and Kumar *et al*, 2003)
Artemisia annua	Provided significant protection against lesions due to *E. tenella* (Allen *et al* 1997).