ETHNOBIOLOGY LETTERS Perspectives

Plant-Based Solutions to Global Livestock Anthelmintic Resistance

Katherine E. French[1*]

[1]Department of Plant Sciences, University of Oxford, Oxford, UK
*katherine.french@plants.ox.ac.uk

Abstract Anthelmintic resistance in livestock is increasing globally. Livestock intestinal parasites now develop resistance to synthetic anthelmintics within 2–10 years, collectively costing billions of dollars annually in lost revenue around the world. Over-reliance on commercial drugs and dips and changes in livestock management practices are key drivers of this trend. To date, current research has focused on identifying new anthelmintics from bacterial and fungal sources or even synthesizing new drugs that target parasite metabolism or reproduction. Plant-derived anthelmintics are a promising alternative, yet to date major research funders and scientists have overlooked this option. Until the mid-20th century, rural communities relied on plant-based methods of controlling livestock parasites. These methods include feeding livestock specific medicinal plants and trees, grazing livestock on herbal leys, and changing where livestock grazed based on ecological factors (e.g., flooding) that increased parasite burdens. Many historic texts and ethnological accounts record the ethnobotanical knowledge of rural communities and the plants they used to control livestock intestinal parasites. Some traditions persist today yet the farmers, graziers, and shepherds who hold this knowledge are rapidly disappearing and with them perhaps a potential long-term solution to anthelmintic resistance. This short perspective piece will cover recent research using ethnobotanical data as a means to identifying potential new anthelmintics; the morphological, physiological, and metabolic effect of plant secondary metabolites on parasites; and an overview of "best practices" which can reduce bias in assessments of plant bioactivity and increase reproducibility of test results. This will hopefully bring recent advances in ethnobiology, chemistry, and ecology to new audiences, and, potentially, spark new interest in using medicinal plants to improve livestock health.

Received May 19, 2017
Accepted February 19, 2018

OPEN ACCESS
DOI 10.14237/ebl.9.2.2018.980

Keywords Drug-resistance, Livestock, Local ecological knowledge, Conservation, Ecology

Supplementary Files available at ojs.ethnobiology.org/index.php/ebl/article/view/980

Introduction

Anthelmintic resistance in livestock is increasing globally. In the USA, South America, and South Africa, current pharmaceutical anthelmintics (e.g., benzimidazoles, avermectins) are now completely ineffective in many regions (Kaplan and Vidyashankar 2012; Shalaby 2013; Vatta and Lindberg 2006). In the UK and northern Europe resistance is also on the rise, but complete resistance has yet to occur (Taylor et al. 2009; Traversa and von Samson-Himmelstjerna 2016). Climate change and global trade have also increased helmintic infections in livestock by increasing the abundance of specific zoonotic parasites (or their hosts), and by introducing new parasites into new regions (Fayer 2000; Fox et al. 2011; Karesh et al. 2012; Patz et al. 2000; Semenza and Menne 2009). Anthelmintic resistant parasites affecting livestock can also spread to humans (e.g., through hybridization of parasites affecting livestock and those affecting humans, as in the case of schistosomiasis and fascioliasis), leading to billions of dollars in economic loss and thousands of human lives every year (King et al. 2015; Waller 2006). This is particularly severe in developing countries due to over-use/misuse of anthelmintics, poor sanitary conditions, and shared land and water use among livestock and humans (King et al. 2015). The use of synthetic anthelmintics also has wider ecological and economic effects. Synthetic anthelmintics reduce soil invertebrate diversity (Spratt 1997; Strong 1993). For example, lab and field studies suggest anthelmintic residues in livestock dung reduce beetle populations by decreasing egg production and increasing larval mortality (Cook et al. 2017; Numa et al. 2012; Ridsdill-Smith 1993). International and national initiatives support the development of new preventative or therapeutic alternatives[1], but current research continues to focus on developing chemical solutions that deactivate specific genes or proteins in parasites that disrupt ability to feed, nicotinic acetylcholine receptors (nAChR), and fertility (Hotez et al. 2010; Kaminsky et al. 2008; Sabatelli 2010).

Exploiting the diversity and bioactivity of plant secondary metabolites may be a viable alternative. Plants naturally produce over 60,000 chemical compounds to deter herbivores, to destroy microbial pathogens, and to communicate with other organisms like pollinators (Wink 2010). Before the creation of synthetic anthelmintics by drug companies in the mid-20th century, humans relied on plants to control livestock intestinal parasites (Corley and Godley 2011). In Europe, medieval herbals and 17th–19th century printed books are filled with descriptions of plants fed to livestock to expel parasites. Today, many small farmers and pastoralists around the world continue to use plants to treat livestock diseases. For example, in South Africa aloes are the premier anthelmintic (Beinart and Brown 2013). In northern Europe, small farmers still value old "traditional" pastures rich in medicinal herbs and legumes for the perceived anthelmintic qualities of specific wild plants (French 2017). Because many of these plants are actively consumed by livestock within traditional agropastoral systems, their toxicity and environmental effects are likely low.

Yet these traditions are rapidly disappearing and with them perhaps a potential long-term solution to anthelmintic resistance. This short perspective piece will cover recent research using ethnobotanical data as a means to identifying potential new anthelmintics; the morphological, physiological, and metabolic effect of plant secondary metabolites on parasites; and an overview of "best practices" which can reduce bias in assessments of plant bioactivity and increase reproducibility of test results. This will hopefully bring recent advances in ethnobiology, chemistry, and ecology to new audiences, and, potentially, spark new interest in using medicinal plants to improve livestock health.

Phyto-Anthelmintics: Old Plants, New Leads

Over the past ten years the number of publications on plants used to treat livestock parasites has doubled (Figure 1). An increasing resistance to traditional synthetic anthelmintics may be responsible. The majority of these publications are from India, although there are a surprising amount of publications from the US and UK. Most research is published in the journals relating to parasitology, pharmacology, veterinary sciences, and plant sciences. A phytochemical database run by the United States Department of Agriculture (USDA) contains 1,029 plants with 58 different chemicals with anthelmintic properties (United States Department of Agriculture 1992-1996) (Supplementary Materials Table 1). The top five plants with the highest number of anthelmintic compounds are *Achillea millefolium*, *Dryopteris filix-mas*, *Peumus boldus*, *Rosmarinus officinalis*, and *Salvia officinalis*. Although the database contains plants from around the world, the majority of these are cultivated or economic plants and many wild plants (even common ones) are excluded. For example, many wild legumes found in British meadows (e.g., *Lathyrus pratensis*, *Vicia cracca*) are absent. In addition, many indigenous plants recently evaluated for anthelmintic qualities are not found in the database. One solution would either be to actively maintain this phytochemical database by allowing users to upload data, or to create a new open-access database specifically for anthelmintic plants.

Perhaps one of the most surprising aspects of anthelmintic plants is that they are often common (Figure 2). For example, Bartha et al. (2015) found that villagers in Romania used *Allium sativum* bulbs, *Cucurbita pepo* seeds, *Daucus carota* ssp. *sativus* roots, and *Quercus petraea* and *Quercus robur* nuts to treat pigs, cattle, and horses. Similarly, in northern Europe many of the plants reported by farmers and pastoralists to have anthelmintic properties are wild plants commonly growing in pastures and meadows (Waller et al. 2001). In addition, a number of studies have shown that the bark, fruits, and nuts of many trees found in traditional rangeland and pasture systems and which are naturally consumed when livestock are ill (zoopharmacognosy) have anthelmintic properties. For example, many of the plants found in arid rangelands of Jordan contain plants such as *Achillea fragrantissima* (Lavender cotton), *Artemisia judaica* (wormwood), and *Thymus bovei*, which have anti-parasitic properties according to Bedouins (Al-Tabini

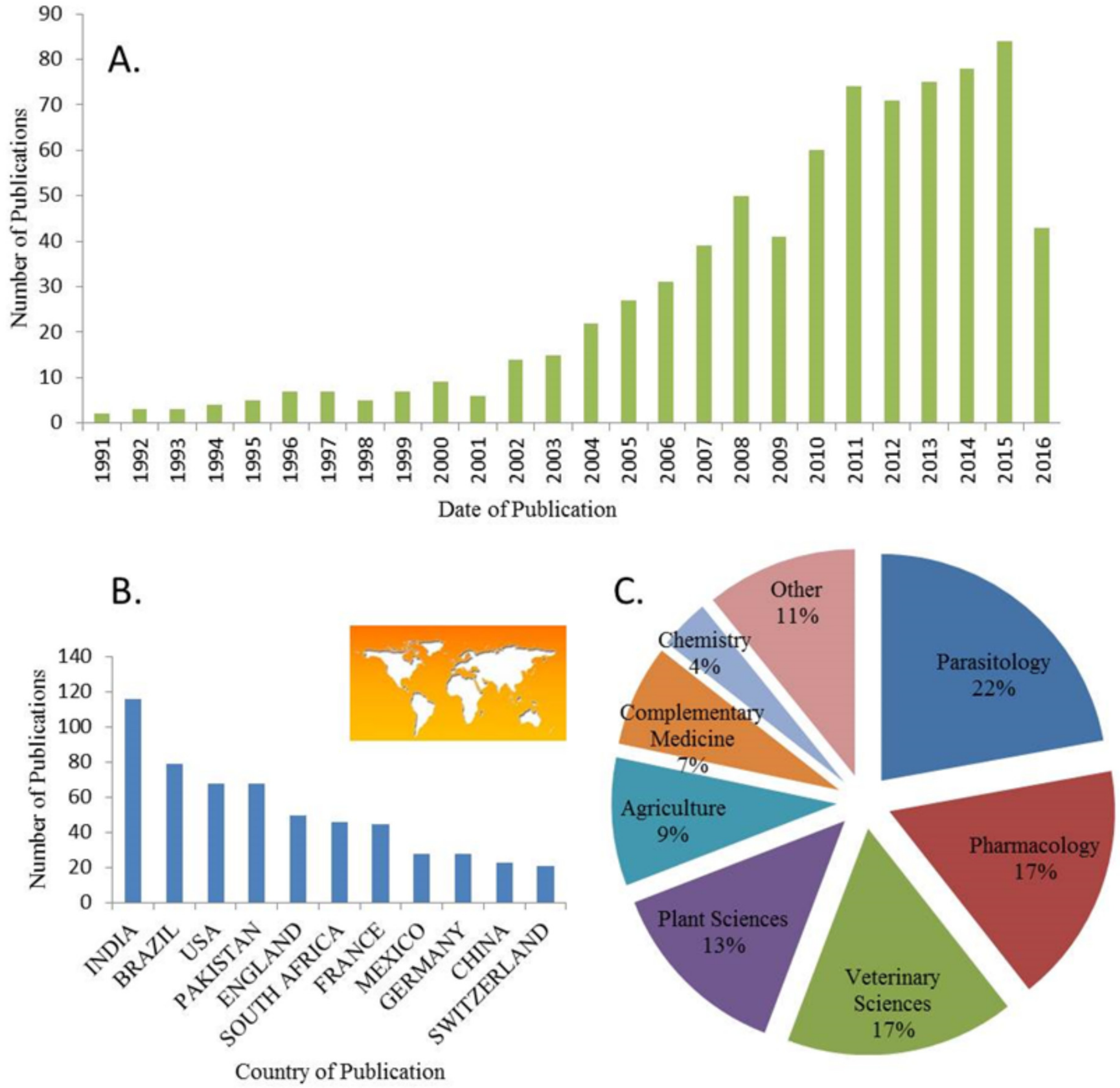

Figure 1 Trends in natural anthelmintic research. A) Publications on plants with anthelmintic properties have tripled in the past ten years. B) The countries producing the majority of these papers are India, Brazil, USA, Pakistan, England, and South Africa, all places where anthelmintic resistance is a prime agricultural and economic issue. C) Most research on anthelmintic plants occurs within the fields of parasitology, pharmacology, veterinary sciences, and plant sciences. All charts were generated using Web of Science data using search terms "anthelmintic" and "plants" (Accessed: June 3, 2016).

et al. 2012; Landau et al. 2014). In British Columbia (Canada), *Juniperus communis* (juniper), *Pinus ponderosa* (Pinaceae) (yellow pine), and *Symphoricarpos albus* var. *laevigatus* (snow berry) branches are used against endoparasites and liver fluke (Lans et al. 2007). The same plants (or another from the same genus) are also used as anthelmintics in geographically dispersed regions. For example, juniper is used to treat liver fluke in Canada, as mentioned above, while the leaves of *Juniperus excelsa* are used by the Wakhi pastoralists of Afghanistan (Soelberg and Jäger 2016). Other anthelmintic plants with widespread, cross-cultural use include: *Urtica dioica* (nettle), *Mentha pulegium* (penny royal), *Digitaria abyssinica* (couch grass), *Salix* spp., and *Carica papaya* (pawpaw) (Nabukenya et al. 2014).

Ethnobotanical research can contribute to the identification of which plants might contain

Figure 2 Plants Rich in Anthelmintic Compounds. Plants store anthelmintic compounds in vacuoles, resin ducts, and trichomes (phytoanticipins) and produce other compounds in response to pathogenic attack (phytoalexins). To date, plants with the greatest number of anthelmintic compounds are common, widespread, and thus, highly studied. From left to right: (A) Salvia officinalis (B) Rosmarinus officinalis (C) Achillea millefolium (D) Dryopteris filix-mas and (E) Peumus boldus. All images are available in the Public Domain (CC0).

anthelmintic properties, as well as how they are prepared. This local knowledge is a form of metadata: time of collection, method of preparation, and dose can direct metabolomic, pharmacological, and epidemiological research (Silva et al. 2014). However, this knowledge is a finite resource. The widespread use of synthetic anthelmintics has spurred the decline of traditional anthelmintics in livestock management around the world in favor of feeds such as maize, soy, and cereals which increase daily live weight gain (Bartha et al. 2015). Farmers who switch from raising local breeds to crossed/exotic livestock breeds and/or increase herd sizes due to governmental incentives also switch from local ethnoveterinary medicines to pharmaceutical alternatives (Nabukenya et al. 2014; Vatta and Lindberg 2006). Decreased medicinal plant availability due to environmental changes or restricted access to natural sources (e.g., by limiting grazing rights) have also contributed to this change (Beinart and Brown 2013; Nabukenya et al. 2014).

How Phytochemicals Affect Parasites

Ethnobotanical data serve as a guide, but not a basis, for plant-based anthelmintic research. Without further chemical analyses and *in vitro* and *in vivo* tests, these data remain "hearsay". Advances in chemical identification using multiple methods from mass spectrometry (liquid-chromatography mass spectrometry, gas-chromatography mass spectrometry) can aid in identifying the secondary metabolites found in medicinal plants. It can also lead to the discovery of new molecules that could serve as drug leads.

Research within the past ten years suggests that plants with anthelmintic properties affect multiple morphological, physiological, and metabolic targets. Plant secondary metabolites with anthelmintic

Table 1 Function of plant-secondary metabolites with anthelmintic properties. "Activity" refers to the antagonistic function of specific metabolites against microbiota and/or helminths. In the table, "PSM" = Plant Secondary Metabolite (or metabolite class).

Activity	PSM	Reference
Inhibit energy metabolism	tannins	(de Macedo et al. 2015)
Cause epidermal lesions	adenine, ascorbic-acid, chymopapain, caricain, genistein, glycyl endopeptidase, lutein, malic-acid, papain	(Duke 1992; Piluzza et al. 2014; Vieira et al. 2001)
Decrease motor activity	tannins, saponins	(Athanasiadou and Kyriazakis 2004; Hoste et al. 2006; Williams et al. 2014)
	terpenoids	(Athanasiadou and Kyriazakis 2004)
	caffeic acid	(Cowan 1999)
Inhibit transformation of eggs to larvae	tannins	(Athanasiadou and Kyriazakis 2004)

properties reduce motility, create epidermal lesions, degrade esophagus and gut tissues, decrease egg production in females, inhibit eggs from transforming into larvae, and cause death within 24–72 hours (see Table 1). Of all the plant secondary metabolites, phenolic compounds show the highest level of bioactivity against parasitic worms. Phenolic compounds inhibit proteins and include flavonoids, coumarins, and condensed tannins (Wink and Schimmer 2010). Condensed tannins have received the most attention, and international research programs such as the EU-wide Legume-Plus initiative have sought to develop new breeds of tannin-enriched lucerne and sainfoin (http://legumeplus.eu/). However, feeding livestock tannin-dense feeds (>7% dry matter) can have detrimental effects including reduced growth rate (Hoste et al. 2006). In addition, lucerne contains phytoestrogens (coumestans) which can reduce livestock fertility (Smith et al. 1979). Lucerne and sainfoin seeds are also expensive and the latter requires fertile, moist calcareous soils to grow. These factors may limit the geographic range and farmer uptake of these plants. Selecting a *range* of plants with different anthelmintic bioactive compounds, instead of one or two, could be a more effective strategy to control livestock parasites. This approach would also satisfy the other dietary needs of livestock (e.g., sugar, protein, fiber, and macro and micro minerals).

The synergy of metabolites found in any given plant, and those found among plants in complex mixtures, may be more effective together than when purified, isolated, and tested on their own *in vitro* and *in vivo*. A recent ethnoveterinary study reported that 70% of all practices in the study area relied on more than one plant (Bartha et al. 2015). For example, the metabolites in Table 1 each target different parts of helminth physiology and/or reproductive cycle. However, to date no study has established whether the efficacy of anthelmintic plants is due to multiple phytochemicals working together. Statistical approaches developed to assess the activity of multi-drug therapies in cancer research could be used to determine synergistic activity. For example, the "MixLow" method combines: (1 multiple nonlinear mixed-effects models, (2 the Lowe Index, and (3 confidence intervals for the Lowe Index to investigate drug interactions (Boik et al. 2008).

The potential synergistic efficacy of plant secondary metabolites differs remarkably from current anthelmintics under development (Table 2). Current synthetic anthelmintics target particular DNA regions, proteins, or biosynthetic pathways (e.g., chokepoints) using synthetic chemicals, proteins from other parasites, or metabolites produced by bacteria and fungi. However, the problem with all of these approaches is the specificity of the anthelmintic under development. By focusing on one target, anthelmintic resistance will continue as parasites evolve and evade current drugs. How rapidly this resistance occurs varies: some studies report resistance in 10 years, while more recent studies have reported resistance to the newest anthelmintics within 2 years (Buckingham et al. 2014). Hotspots of resistance may also emerge in areas where anthelmintics are used heavily (e.g., multiple does per year) and for both humans and livestock (King et al. 2015).

Although many studies report the efficacy of plant-based anthelmintics *in vitro* and *in vivo*, the negative results reported from experimental research should also lend a word of caution. For example, Githiori et al. (2003) tested seven local plants used to treat anthelmintic infections in Kenya and found that only one (*Ananas comosus*) had weak *in vitro* activity. In addition, a recent study found that when goats and kids were fed a commercial herbal feed supplement containing a mix of several herbs traditionally used as vermifuges (*Artemisia absinthium* (wormwood), *Allium sativum* (garlic), *Juglans nigra* (black walnut), *Cucurbita pepo* (field pumpkin), *Artemisia vulgaris* (mugwort), *Foeniculum vulgare* (fennel), *Hyssopus officinalis* (hyssop), and *Thymus vulgaris* (thyme)) at a dose of 19 g for three days, the supplement failed to control intestinal parasites (Burke et al. 2009). This suggests that other factors, including amount of plant consumed (dose and length of administration), the effects of manufacturing and packaging, and even metabolite stability may influence the bioactivity of such supplements.

Developing Best Practices

Many studies on the anthelmintic properties of plants use very different methodological procedures. Different practices—from the initial collection of plant material, to method of metabolite extraction, to assay-design—can introduce bias (of false positive or false negative results). The following provides some suggestions for "best practices" which can help standardize the evaluation of plant bioactivity and increase reproducibility.

1. *Preparation of Plant Material:* Plants should be collected at the same time of day (if collected over an extended period) and dried outside in the shade or indoors in a drying room for 48 hours to one week. If plants are collected for metabolomic analysis, at least 4 replicates of each plant from each site are needed. Alternatively, freezing plants in liquid nitrogen (in Falcon tubes) followed by lycophilization as soon as plants are collected will preserve the metabolite composition (Asami et al. 2003; de Torres et al. 2010). In addition, plants prepared in this way are easier to homogenize into a fine powder which will increase the exposure of plant cell walls to the solvent of choice. Although oven drying is widely practiced, this can lead to loss of aromatic metabolites (e.g., terpenes).

2. *Assay Selection:* Agar and broth dilution assays can be used to establish the Minimal Inhibitory Concentration (MIC) and Lethal Concentration (LC) values of a crude extract on helminths. In agar assays, a petri dish is seeded with nematodes and *E. coli* (their food source) and then exposed to a plant extract. However, many metabolites (e.g., essential oils) do not travel through agar very well which may lead to false negatives. In broth dilution assays, 96-well microtiter plates are filled with a nematode growth medium, nematodes (e.g., 10–50 L-4 stage adults), and the crude extract under assessment (Garvis et al. 2009). The advantages of the 96-well plates are that many compounds can be assayed against nematodes in different life stages (e.g., eggs, larvae, adults) at once and the system can be semi-automated. Screening parasites at different life stages can nuance our understanding of how these plant compounds work; some might be effective at halting egg production, while others interfere with larvae growth and development. A COPAS Biosorter can be used to distribute a specific number of nematodes at a given life-stage into each well in a matter of seconds. Identification of nematode survival can be established by counting under a microscope. This process can be automated using the WormAssay protocol, where a high definition camera is attached to an inverted microscope to detect parasite motility and the captured images are analyzed using specially-designed algorithms (Marcellino et al. 2012; Storey et al. 2014). When feasible, using the latter method will provide more accurate, reproducible results.

3. *Fractionation:* Fractionation takes a specific amount of plant material and extracts metabolites

Table 2 Non-plant based anthelmintics. Current anthelmintics are synthesized from specific lead chemicals, microbial metabolites, or parasitic worms and their activity is based on one specific target.

Type	Source	Active Agent	Target	Reference
Chemical	1-dimethyl-4-phenylpiperazinium (DMPP)	synthetic chemical	nicotinic agonist	(Kaminsky et al. 2008)
	Albendazole	synthetic chemical	eggs	(Taylor et al. 2013)
	Amino-acid derivatives (AAD)	synthetic chemical	nematode-specific clade of acetylcholine receptor subunits affecting movement, growth and viability	(Kaminsky et al. 2008)
	Benzimidazoles	synthetic chemical	α- and β-tubulin monomers	(Demeler et al. 2013)
	Dasatinib	synthetic chemical	protein kinases	(Taylor et al. 2013)
	Diethyllabamazine (DEC)	synthetic chemical	eggs	(Taylor et al. 2013)
	Flavopiridol	synthetic chemical	protein kinases	(Taylor et al. 2013)
	Invermectin	synthetic chemical	eggs	(Taylor et al. 2013)
	Levamisole	synthetic chemical	subtype of nicotinic acetylcholine receptor (nAChR)	(Kaminsky et al. 2008)
	Neomycin	synthetic chemical	protein kinases	(Taylor et al. 2013)
Vaccine	*Dictyocaulus* spp.	x-irradiated L3	L3-stage adults	(Hotez et al. 2010)
	Echinococcus granulosus	recombinant proteins	EG95	(Hotez et al. 2010)
	Fasciola hepatica	cathepson L	egg production and viability	(Sabatelli 2010)
	Fasciola hepatica	anti-H-gal-GP	digestion	(Sabatelli 2010)
	Necator americanus	protein-2	unspecified	(Sabatelli 2010)
	Necator americanus	APR1	inhibit parasite feeding by neutralizing enzyme activity	(Hotez et al. 2010)
	Pichia pastoris	GST1	inhibit parasite feeding by neutralizing enzyme activity	(Hotez et al. 2010)
Microbial	*Arthrobotrys conoides*	secondary metabolites	larvicidal	(Falbo et al. 2015)
	Arthrobotrys musiformis	secondary metabolites	larvicidal	(Acevedo-Ramírez et al. 2015)
	Bacillus circulans	spore crystal suspension	larvicidal	(Sinott et al. 2012)
	Bacillus thuringiensis	Cry5B	p38 mitogen-activated protein kinase; nicotinic acetylcholine receptor (nAChR) agonist	(Cappello et al. 2006; Hu and Aroian 2012; Urban et al. 2013)
	Bacillus thuringiensis	Cry21A	nicotinic acetylcholine receptor (nAChR) agonist	(Hu and Aroian 2012)

(continued on next page)

(continued from previous page)

Table 2 Non-plant based anthelmintics. Current anthelmintics are synthesized from specific lead chemicals, microbial metabolites, or parasitic worms and their activity is based on one specific target.

Type	Source	Active Agent	Target	Reference
Microbial	*Bacillus thuringiensis*	Cry14A	growth and development	(Wei et al. 2003)
	Bacillus thuringiensis var. *kurstaki*	spore crystal suspension	larvicidal	(Sinott et al. 2012)
	Bacillus thuringiensis var. *israelensis*	spore crystal suspension	larvicidal	(Sinott et al. 2012)
	Bacillus thuringiensis var. *osvaldocruzi*	spore crystal suspension	larvicidal	(Sinott et al. 2012)
	Clonostachys candelabrum	7 metabolites (Five roselipins, linoleic acid, and aurantiogliocladin)	diacylglycerol acyl transferase 2	(Ayers et al. 2010)
	Duddingtonia flagrans	chlamydospores	larvicidal	(Larsen 2000; Waghorn et al. 2003; Waller 2006)
	Monacrosporium salinum	secondary metabolites	larvicidal	(Liu et al. 2015)
	Monacrosporium thaumasium	secondary metabolites	larvicidal	(Vilela et al. 2013)

Figure 3 Fodder trees boost livestock health and increase pastoral sustainability. Acacia nilotica (left) and Salix spp. (right) trees contain condensed tannins and other polyphenols with established anti-parasitic properties. Livestock grazing in more natural pasture systems (e.g., rangelands, wood pasture) naturally consume the pods and bark (respectively) of these trees when ill. Planting more trees with anthelmintic properties could help control livestock endoparasites, reduce inputs (water, fertilizer) needed to feed livestock, and provide environmental benefits like soil stabilization and flood control. All images are available in the Public Domain (CC0).

using a variety of solvents (e.g., ethanol, acetone, chloroform, methanol, water). Each solvent will cause plant cells to release different categories of metabolites based on polarity and hydrophilicity. When combined with metabolomic analysis of each fraction, this approach is a good way to identify highly active components. However, these approaches, specifically fractionation, may overlook the synergistic role of metabolites in killing parasites. If screening of fractions is performed, combining all fractions as one treatment could be a way to quickly assess any potential synergistic activity.

4. In Vivo *tests*: Plants showing anthelmintic activity *in vitro* may not show the same activity *in vivo*. While a number of studies slaughter livestock used in *in vivo* experiments, there are more humane alternatives. Fecal egg counts can be conducted weekly or monthly during a grazing experiment (Taylor et al. 2009). No livestock are harmed in the process and vets can check livestock weekly to ensure those receiving herbal/plant-based therapeutics did not contract a life-threatening parasitic infection. In addition to conducting fecal egg counts, the number of eggs and/or larvae in soil cores and on grass samples can also be performed to establish whether there are changes in the abundance of parasites where animals are grazing depending upon treatment type (e.g., synthetic anthelmintic, bioactive forages, herbal supplement, etc.) (Lopes et al. 2016).

Implications for Agriculture and Conservation

The potential role of plants with potential anthelmintic properties has important ramifications for agriculture and conservation. First, a greater emphasis could be placed on cultivating these plants. For example, in semi-natural ecosystems, grazing activities could center around when these plants are in flower. In more sedentary agricultural systems, these plants could be included in pasture and/or meadow seed mixes. Second, many plants used within ethnoveterinary systems are indigenous and may be under threat. Some plants traditionally given to livestock (or naturally grazed) to control parasites are considered "weeds" that are removed to achieve conservation objectives (Lans et al. 2007). For example, conservation groups often cut down willow from wet pastures and juniper from chalk grasslands in the UK. Conservation activities could promote the active use of these plants to sustain local populations.

Future Directions

What role should ethnobiological research play in the development of new anthelmintics? As this short perspective piece has shown, ethnobotanical and ethnozoological research has shed light on the vast array of plants which could potentially be added to pastures and feed supplements to naturally prevent and control parasitic infection. Plants could thus provide a sustainable alternative to traditional synthetic anthelmintics. However, further interdisciplinary and rigorous research on plant-based anthelmintics is needed. We need to establish the natural availability of secondary metabolites in specific ecosystems (e.g., pastures, rangelands) and specific plants. We also need to establish whether the metabolomic composition of these plants changes over time. To prevent needless replication of research and to make this data accessible to end-users (e.g., farmers), such research should be made publicly available. Future research could concentrate on identifying species which could be used as anthelmintic fodder trees. These would provide perennial forage and may be suitable to regions experiencing high levels of aridity and/or flooding (Figure 3). For example, in the Middle East the pods of acacia trees (e.g., *Acacia nilotica*) contain saponins and proanthocyanins (Abdel-Farid et al. 2014). These trees are drought-resistant and provide a good source of forage when other grasses, forbs, and herbs have disappeared. In the UK, willow trees (*Salix* spp.) contain high levels of salicylic acid and proanthocyanidins (Agnolet et al. 2012), and cattle roaming natural pastures often actively consume the bark of the tree when ill (French 2017). Because these trees are also good for mitigating flooding, planting more of them would have both economic and environmental effects.

More research could also investigate whether aqueous solutions made from plant crude extracts could be applied to highly infected pastures to reduce parasitic load. Finally, further interdisciplinary research on parasite ecology and livestock health should record and integrate local ecological knowledge into regional programs for controlling parasite outbreaks. For example, farmers and graziers can provide information on how changes in the weather (e.g., increased flooding) and animal husbandry practices (e.g., over-stocking) may increase/decrease parasitic infection in livestock. This information could be analyzed along with environmental, climatological, and hydrological information in geographical information systems (GIS). While perhaps optimistic, further interdisciplinary research into the bioactivity of plants traditionally used to manage parasites—and incorporation of these plants into current agricultural systems—could reduce the rise of livestock anthelmintic resistance globally.

Notes

[1] These include: Sustainable Control of Parasites in Sheep (SCOPS) (http://www.scops.org.uk/); the Responsible Use of Medicines in Agriculture Alliance (RUMA) (http://www.ruma.org.uk/); the Antiparasitic Resistance Management Strategy (ARMS) of the FDA in the USA; and the World Health Organization Global Action Plan (GAP) on antimicrobial resistance (http://www.who.int/antimicrobial-resistance/en/).

Declarations

Permissions: Not applicable.

Sources of Funding: None declared.

Conflicts of Interest: None declared.

References Cited

Abdel-Farid, I.B., M.G. Sheded, and E. A. Mohamed. 2014. Metabolomic Profiling and Antioxidant Activity of Some *Acacia* Species. *Saudi Journal of Biological Science* 21:400–408. DOI:10.1016/j.sjbs.2014.03.005.

Acevedo-Ramírez, P.M. del C., J.A. Figueroa-Castillo, R. Ulloa-Arvizú, L.G. Martínez-García, A. Guevara-Flores, J.L. Rendón, R.O. Valero-Coss, P.M. Gives, and H. Quiroz-Romero. 2015. Proteolytic Activity of Extracellular Products from *Arthrobotrys musiformis* and their Effect *In Vitro* Against *Haemonchus contortus* Infective Larvae. *Veterinary Record Open 2*, e000103. DOI:10.1136/vetreco-2014-000103.

Agnolet, S., S. Wiese, R. Verpoorte, and D. Staerk. 2012. Comprehensive Analysis of Commercial Willow Bark Extracts by New Technology Platform: Combined Use of Metabolomics, High-Performance Liquid Chromatography–Solid-Phase Extraction–Nuclear Magnetic Resonance Spectroscopy and High-Resolution Radical Scavenging Assay. *Journal of Chromatography A* 1262: 130–137. DOI:10.1016/j.chroma.2012.09.013.

Al-Tabini, R., K. Al-Khalidi, and M. Al-Shudiefat. 2012. Livestock, Medicinal Plants, and Rangeland Viability in Jordan's Badia: Through the Lens of Traditional and Local Knowledge. *Pastoralism Research, Policy, and Practice* 2:4. DOI:10.1186/2041-7136-2-4.

Asami, D., J. Hong, D. Barrett, and A. Mitchell. 2003. Comparison of the Total Phenolic and Ascorbic Acid Content of Freeze-Dried and Air-Dried Marionberry, Strawberry, and Corn Grown Using Conventional, Organic, and Sustainable Agricultural

Practices. *Journal of Agricultural Food Chemistry* 51:1237–1241. DOI:10.1021/jf020635c.

Athanasiadou, S., and I. Kyriazakis. 2004. Plant Secondary Metabolites: Antiparasitic Effects and their Role in Ruminant Production Systems. *Proceedings of the Nutritional Society* 63:631–639. DOI:10.1079/PNS2004396.

Ayers, S., D.L. Zink, K. Mohn, J.S. Powell, C.M. Brown, G. Bills, A. Grund, D. Thompson, and S. B. Singh. 2010. Anthelmintic Constituents of *Clonostachys candelabrum*. *Journal of Antibiotics* 63:119–122. DOI:10.1038/ja.2009.131.

Bartha, S.G., C.L. Quave, L. Balogh, and N. Papp. 2015. Ethnoveterinary Practices of Covasna County, Transylvania, Romania. *Journal of Ethnobiology and Ethnomedicine* 11: 35. DOI:10.1186/s13002-015-0020-8.

Beinart, W., and K. Brown. 2013. African Local Knowledge and Livestock Health: Traditional, Environmental & Biomedical Approaches in South Africa. James Currey, Oxford, UK.

Boik, J.C., R.A. Newman, and R. J. Boik. 2008. Quantifying Synergism/Antagonism Using Nonlinear Mixed-Effects Modeling: A Simulation Study. Statistical Medicine 27:1040–1061. DOI:10.1002/sim.3005.

Buckingham, S.D., F.A. Partridge, and D. B. Sattelle. 2014. Automated, High-Throughput, Motility Analysis in *Caenorhabditis elegans* and Parasitic Nematodes: Applications in the Search For New Anthelmintics. *International Journal of Parasitology, Drugs, and Drug Resistance* 4:226–232. DOI:10.1016/j.ijpddr.2014.10.004.

Burke, J.M., A. Wells, P. Casey, and R.M. Kaplan. 2009. Herbal Dewormer Fails to Control Gastrointestinal Nematodes in Goats. *Veterinary Parasitology* 160:168–170. DOI:10.1016/j.vetpar.2008.10.080.

Cappello, M., R.D. Bungiro, L.M. Harrison, L.J. Bischof, J.S. Griffitts, B.D. Barrows, and R. V. Aroian. 2006. A Purified *Bacillus thuringiensis* Crystal Protein with Therapeutic Activity Against the Hookworm Parasite *Ancylostoma ceylanicum*. *Proceedings of the National Academy of Sciences* 103:15154–15159. DOI:10.1073/pnas.0607002103.

Cooke, A.S., E.R. Morgan, and J.A.J. Dungait. 2017. Modelling the Impact of Targeted Anthelmintic Treatment of Cattle on Dung Fauna. *Environmental Toxicology and Pharmacology* 55:94–98. DOI:10.1016/j.etap.2017.07.012.

Cowan, M.M.1999. Plant Products as Antimicrobial Agents. *Clin. Microbiol. Rev.* 12:564–582.

de Macedo, M., S. Clais, E. Lanckacker, L. Maes, E. Lima, and P. Cos. 2015. Anti-Infective Agents: The Example of Antibacterial Drug Leads. In *Ethnopharmacology*, edited by M. Heinrich and A. Jäger, pp. 111–123. Wiley-Blackwell, Chichester, West Sussex.

de Torres, C., M.C. Díaz-Maroto, I. Hermosín-Gutiérrez, and M.S. Pérez-Coello. 2010. Effect of Freeze-Drying and Oven-Drying on Volatiles and Phenolics Composition of Grape Skin. *Analytica Chimica Acta* 660:177–182. DOI:10.1016/j.aca.2009.10.005.

Demeler, J., N. Krüger, J. Krücken, V.C. von der Heyden, S. Ramünke, U. Küttler, S. Miltsch, M.L. Cepeda, M. Knox, J. Vercruysse, P. Geldhof, A. Harder, and G. von Samson-Himmelstjerna. 2013. Phylogenetic Characterization of β-Tubulins and Development of Pyrosequencing Assays for Benzimidazole Resistance in Cattle Nematodes. *PLoS one* 8:e70212. DOI:10.1371/journal.pone.0070212.

Duke, J.A. 1992. *Handbook of Phytochemical Constituent Grass, Herbs, and Other Economic Plants*. CRC Press, Boca Raton, FL.

Falbo, M.K., V.T. Soccol, I.E. Sandini, J.H. Novakowiski, and C. R. Soccol. 2015. Effect of Spraying *Arthrobotrys conoides* Conidia on Pastures to Control Nematode Infection in Sheep. *Semina: Ciências Agrárias* 36:239–252. DOI:10.5433/1679-0359.2015v36n1p239.

Fayer, R. 2000. Global Change and Emerging Infectious Diseases. *Journal of Parasitology* 86: 1174–1181. DOI:10.1645/0022-3395(2000)086[1174:GCAEID]2.0.CO;2.

Fox, N.J., P.C.L. White, C.J. McClean, G. Marion, A. Evans, and M.R. Hutchings. 2011. Predicting Impacts of Climate Change on *Fasciola hepatica* Risk. *PLoS one* 6:e16126. DOI:10.1371/journal.pone.0016126.

French, K.E. 2017. Species Composition Determines Forage Quality and Medicinal Value of High Diversity Grasslands in Lowland England. *Agriculture, Ecosystems, and the Environment* 241:193–204. DOI:10.1016/j.agee.2017.03.012.

Garvis, S., A. Munder, G. Ball, S. de Bentzmann, L. Wiehlmann, J.J. Ewbank, B. Tümmler, and A. Filloux. 2009. *Caenorhabditis elegans* Semi-Automated Liquid Screen Reveals a Specialized Role for the Chemotaxis Gene cheB2 in *Pseudomonas aeruginosa* Virulence. *PLoS Pathogens* 5:e1000540. DOI:10.1371/journal.ppat.1000540.

Githiori, J.B., J. Höglund, P.J. Waller, and R. Leyden Baker. 2003. Evaluation of Anthelmintic Properties of Extracts from Some Plants Used as Livestock Dewormers by Pastoralist and Smallholder Farmers in Kenya Against *Heligmosomoides polygyrus* Infections in Mice. *Veterinary Parasitology* 118:215–226. DOI:10.1016/j.vetpar.2003.10.006.

Hoste, H., F. Jackson, S. Athanasiadou, S.M. Thamsborg, and S.O. Hoskin. 2006. The Effects of Tannin-Rich Plants on Parasitic Nematodes in Ruminants. *Trends in Parasitology* 22:253–261. DOI:10.1016/j.pt.2006.04.004.

Hotez, P.J., J.M. Bethony, D.J. Diemert, M. Pearson, and A. Loukas. 2010. Developing Vaccines to Combat Hookworm Infection and Intestinal Schistosomiasis. *Nature Reviews in Microbiology* 8:814–826. DOI:10.1038/nrmicro2438.

Hu, Y., and R.V. Aroian. 2012. Bacterial Pore-Forming Proteins as Anthelmintics. *Invertebrate Neuroscience* 12:37–41. DOI:10.1007/s10158-012-0135-8.

Jr, J.F.U., Y. Hu, M.M. Miller, U. Scheib, Y.Y. Yiu, and R. V. Aroian. 2013. *Bacillus thuringiensis*-Derived Cry5B Has Potent Anthelmintic Activity against *Ascaris suum*. *PLOS Neglected Tropical Diseases* 7:e2263. DOI:10.1371/journal.pntd.0002263.

Kaminsky, R., P. Ducray, M. Jung, R. Clover, L. Rufener, J. Bouvier, S.S. Weber, A. Wenger, S. Wieland-Berghausen, T. Goebel, N. Gauvry, F. Pautrat, T. Skripsky, O. Froelich, C. Komoin-Oka, B. Westlund, A. Sluder, and P. Mäser. 2008. A New Class of Anthelmintics Effective Against Drug-Resistant Nematodes. *Nature* 452:176–180. DOI:10.1038/nature06722.

Kaplan, R.M. and A.N. Vidyashankar. 2012. An Inconvenient Truth: Global Worming and Anthelmintic Resistance. Veterinary Parasitology Special Issue: Novel Approaches to the Control of Helminth Parasites of Livestock 186:70–78. DOI:10.1016/j.vetpar.2011.11.048.

Karesh, W.B., A. Dobson, J.O. Lloyd-Smith, J. Lubroth, M.A. Dixon, M. Bennett, S. Aldrich, T. Harrington, P. Formenty, E.H. Loh, C.C. Machalaba, M.J. Thomas, and D.L. Heymann. 2012. Ecology of Zoonoses: Natural and Unnatural Histories. *The Lancet* 380:1936–1945. DOI:10.1016/S0140-6736(12)61678-X.

King, K.C., R.B. Stelkens, J.P. Webster, D.F. Smith, and M.A. Brockhurst. 2015. Hybridization in Parasites: Consequences for Adaptive Evolution, Pathogenesis, and Public Health in a Changing World. *PLOS Pathog* 11:e1005098. DOI:10.1371/journal.ppat.1005098.

Landau, S.Y., H. Muklada, A. Abu-Rabia, S. Kaadan, and H. Azaizeh. 2014. Traditional Arab Ethno-Veterinary Practices in Small Ruminant Breeding in Israel. *Small Ruminant Research* 119:161–171. DOI:10.1016/j.smallrumres.2014.01.004.

Lans, C., N. Turner, T. Khan, G. Brauer, and W. Boepple. 2007. Ethnoveterinary Medicines Used for Ruminants in British Columbia, Canada. *Journal of Ethnobiology and Ethnomedicine* 3:11. DOI:10.1186/1746-4269-3-11.

Larsen, M. 2000. Prospects for Controlling Animal Parasitic Nematodes by Predacious Micro Fungi. *Parasitology* 120:S121–S131.

Liu, W., Y. Han, B. Wang, L. Sun, M. Chen, K. Cai, X. Li, M. Zhao, C. Xu, Q. Xu, L. Yi, H. Wang, D. Xie, X. Li, J. Wu, J. Yang, S. Wei, D. Li, C. Chen, T. Zheng, Q. Li, and J. Peng. 2015. Isolation, Identification, and Characterization of the Nematophagous Fungus *Monacrosporium salinum* from China. *Journal of Basic Microbiology* 55:992–1001. DOI:10.1002/jobm.201400909.

Lopes, S.G., L.B.G. Barros, H. Louvandini, A.L. Abdalla, and L.M. Costa Junior. 2016. Effect of Tanniniferous Food from *Bauhinia pulchella* on Pasture Contamination with Gastrointestinal Nematodes from Goats. *Parasitology Vectors* 9:102. DOI:10.1186/s13071-016-1370-3.

Marcellino, C., J. Gut, K.C. Lim, R. Singh, J. McKerrow, and J. Sakanari. 2012. WormAssay: A Novel Computer Application for Whole-Plate Motion-Based Screening of Macroscopic Parasites. *PLoS Neglected Tropical Diseases* 6. DOI:10.1371/journal.pntd.0001494.

Nabukenya, I., C. Rubaire-Akiiki, D. Olila, K. Ikwap, and J. Höglund. 2014. Ethnopharmacological Practices by Livestock Farmers in Uganda: Survey Experiences from Mpigi and Gulu Districts. *Journal* *of Ethnobiology and Ethnomedicine* 10:9. DOI:10.1186/1746-4269-10-9.

Numa, C., J.R. Verdú, C. Rueda, and E. Galante. 2012. Comparing Dung Beetle Species Assemblages between Protected Areas and Adjacent Pasturelands in a Mediterranean Savanna Landscape. *Rangeland Ecology and Management* 65:137–143. DOI:10.2111/REM-D-10-00050.1.

Patz, J.A, T.K. Graczyk, N. Geller, and A.Y. Vittor. 2000. Effects of Environmental Change on Emerging Parasitic Diseases. *International Journal for Parasitology* 30:1395–1405.

Piluzza, G., L. Sulas, and S. Bullitta. 2014. Tannins in Forage Plants and their Role in Animal Husbandry and Environmental Sustainability: A Review. *Grass Forage Science* 69:32–48. DOI:10.1111/gfs.12053.

Ridsdill-Smith, T.J. 1993. Effects of Avermectin Residues in Cattle Dung on Dung Beetle (Coleoptera: Scarabaeidae) Reproduction and Survival. *Veterinary Parasitology* 48:127-37.

Sabatelli, L. 2010. Effect of Heterogeneous Mixing and Vaccination on the Dynamics of Anthelmintic Resistance: A Nested Model. PLOS ONE 5:e10686. DOI:10.1371/journal.pone.0010686.

Semenza, J.C. and B. Menne. 2009. Climate Change and Infectious Diseases in Europe. *Lancet Infectious Diseases* 9:365–375. DOI: 10.1016/S1473-3099(09)70104-5.

Shalaby, H.A. 2013. Anthelmintics Resistance: How to Overcome it? *Iranian Journal of Parasitology* 8:18–32.

Silva, F. dos S., U.P. Albuquerque, L.M. Costa Júnior, A. da S. Lima, A.L.B. do Nascimento, and J.M. Monteiro. 2014. An Ethnopharmacological Assessment of the Use of Plants Against Parasitic Diseases in Humans and Animals. *Journal of Ethnopharmacology* 155:1332–1341. DOI:10.1016/j.jep.2014.07.036.

Sinott, M.C., N.A. Cunha Filho, L.L.D. Castro, L.B. Lorenzon, N.B. Pinto, G.A. Capella, and F.P.L. Leite. 2012. *Bacillus* spp. Toxicity Against *Haemonchus contortus* Larvae in Sheep Fecal Cultures. *Experimental Parasitology* 132:103–108. DOI:10.1016/j.exppara.2012.05.015.

Smith, J.F., K.T. Jagusch, L.F.C. Brunswick, and R.W. Kelly. 1979. Coumestans in Lucerne and Ovulation in Ewes. *New Zealand Journal of Agricultural Research* 22:411–416. DOI:10.1080/00288233.1979.10430768.

Soelberg, J. and A.K. Jäger. 2016. Comparative Ethnobotany of the Wakhi Agropastoralist and the Kyrgyz Nomads of Afghanistan. *Journal of Ethnobiology and Ethnomedicine* 12:2. DOI:10.1186/s13002-015-0063-x.

Spratt, D.M. 1997. Australian and New Zealand Societies for Parasitology Scientific Meeting Endoparasite Control Strategies: Implications for Biodiversity of Native Fauna. *International Journal of Parasitology* 27:173–180. DOI: 10.1016/S0020-7519(96)00147-6.

Storey, B., C. Marcellino, M. Miller, M. Maclean, E. Mostafa, S. Howell, J. Sakanari, A. Wolstenholme, and R. Kaplan. 2014. Utilization of Computer Processed High Definition Video Imaging for Measuring Motility of Microscopic Nematode Stages on a Quantitative Scale: "The Worminator." *International Journal of Parasitology, Drugs, and Drug Resistance* 4:233–243. DOI:10.1016/j.ijpddr.2014.08.003.

Strong, L. 1993. Overview: The Impact of Avermectins on Pastureland Ecology. *Veterinary Parasitology* 48:3–17. DOI:10.1016/0304-4017(93)90140-I.

Taylor, C.M., Q. Wang, B.A. Rosa, S.C.-C. Huang, K. Powell, T. Schedl, E.J. Pearce, S. Abubucker, and M. Mitreva. 2013. Discovery of Anthelmintic Drug Targets and Drugs Using Chokepoints in Nematode Metabolic Pathways. *PLOS Pathology* 9:e1003505. DOI:10.1371/journal.ppat.1003505.

Taylor, M.A., J. Learmount, E. Lunn, C. Morgan, and B.H. Craig. 2009. Multiple Resistance to Anthelmintics in Sheep Nematodes and Comparison of Methods Used for Their Detection. Small Ruminant Research Special Issue: Keynote Lectures of the 7th International Sheep Veterinary Congress 86:67–70. DOI:10.1016/j.smallrumres.2009.09.020

Traversa, D. and G. von Samson-Himmelstjerna. 2016. Anthelmintic Resistance in Sheep Gastro-Intestinal Strongyles in Europe. Small Ruminant Research Special Issue: Advances in Sheep and Goats Research: A Holistic Approach. Selected Papers from SIPAOC 2014 Meeting, Italy 135:75–80. DOI:10.1016/j.smallrumres.2015.12.014.

Vatta, A.F. and A.L.E. Lindberg. 2006. Managing Anthelmintic Resistance in Small Ruminant

Livestock of Resource-Poor Farmers in South Africa: Review Article. *Journal of South African Veterinary Association* 77:2–8. DOI:10.4102/jsava.v77i1.331.

Vieira, M.E.D., C. Costa, A.C. Silveira, and M.D. Arrigoni. 2001. Saponins and Tannins in Twenty-Eight Alfalfa (*Medicago sativa L.)* Cultivates Grown in Botucatu SP. *Brazilian Journal of Animal Science* 30: 1432–1438. DOI:10.1590/S1516-35982001000600007.

Vilela, V.L.R., T.F. Feitosa, F.R. Braga, J.V. de Araújo, S.C. de Lucena, E.S. Dantas, A.C.R. Athayde, and W.W. Silva. 2013. Efficacy of *Monacrosporium thaumasium* in the Control of Goat Gastrointestinal Helminthiasis in a Semi-Arid Region of Brazil. *Parasitology Research* 112: 871–877. DOI:10.1007/s00436-012-3078-4.

Waghorn, T.S., D.M. Leathwick, L.-Y. Chen, and R.A. Skipp. 2003. Efficacy of the Nematode-Trapping Fungus *Duddingtonia flagrans* against Three Species of Gastro-Intestinal Nematodes in Laboratory Faecal Cultures from Sheep and Goats. *Veterinary Parasitology* 118: 227–234. DOI:10.1016/j.vetpar.2003.09.018.

Waller, P.J. 2006. From Discovery to Development: Current Industry Perspectives for the Development of Novel Methods of Helminth Control in Livestock. *Veterinary Parasitology* 139:1–14. DOI:10.1016/j.vetpar.2006.02.036.

Waller, P.J., G. Bernes, S.M. Thamsborg, A. Sukura, S.H. Richter, K. Ingebrigtsen, and J. Höglund. 2001. Plants as De-Worming Agents of Livestock in the Nordic Countries: Historical Perspective, Popular Beliefs, and Prospects for the Future. *Acta Veterinaria Scandanavia* 42:31. DOI:10.1186/1751-0147-42-31.

Wei, J.-Z., K. Hale, L. Carta, E. Platzer, C. Wong, S.-C. Fang, and R.V. Aroian. 2003. *Bacillus thuringiensis* Crystal Proteins that Target Nematodes. *Proceedings of the National Academy of Sciences* 100:2760–2765. DOI:10.1073/pnas.0538072100.

Williams, A.R., C. Fryganas, A. Ramsay, I. Mueller-Harvey, and S.M. Thamsborg. 2014. Direct Anthelmintic Effects of Condensed Tannins from Diverse Plant Sources Against *Ascaris suum*. *PLOS ONE* 9, e97053. DOI:10.1371/journal.pone.0097053.

Wink, M. 2010. Introduction. In *Functions and Biotechnology of Plant Secondary Metabolites*, edited by M. Wink, pp. 1–20. Blackwell Publishing Ltd., Oxford, UK.

Wink, M. and O. Schimmer. 2010. Molecular Modes of Action of Defensive Secondary Metabolites. In *Functions and Biotechnology of Plant Secondary Metabolites*, edited by M. Wink, pp. 21–161. Blackwell Publishing Ltd., Oxford, UK.