



Research article

Anthelmintic medicinal plants in veterinary ethnopharmacology: A network meta-analysis following the PRISMA-P and PROSPERO recommendations



Luigino Calzetta^{a,b,*}, Elena Pistocchini^{b,1}, Antonio Leo^{c,1}, Paola Roncada^c,
Beatrice Ludovica Ritondo^a, Ernesto Palma^{c,**}, David di Cave^d, Domenico Britti^c

^a Department of Experimental Medicine, University of Rome "Tor Vergata", Rome, Italy

^b Unit of Laboratory Medicine, Veterinary Hospital Gregorio VII, Rome, Italy

^c Department of Health Sciences, University of Catanzaro "Magna Graecia", Catanzaro, Italy

^d Department of Clinical Sciences and Translational Medicine, University of Rome "Tor Vergata", Rome, Italy

ARTICLE INFO

Keywords:

Bioinformatics
Ecology
Microbiology
Plant biology
Systematics
Medicinal plants
Drugs
Anthelmintic
Veterinary ethnopharmacology
Livestock

ABSTRACT

Medicinal plants may be effective against helminthic infestation in animals, but to date few studies have investigated the real impact of anthelmintic medicinal plants in veterinary ethnopharmacology.

The aim of this study was to assess the geographical use of anthelmintic medicinal plants in livestock in European Union (EU), and to quantify the anthelmintic efficacy of medicinal plants in comparison with anthelmintic drugs. Surveys on the use of anthelmintic traditional medicinal plants in livestock in the EU were included in the qualitative synthesis. Studies that investigated the efficacy of anthelmintic traditional medicinal plants in animals, compared with negative control and/or anthelmintic drugs, were included in the quantitative synthesis (network meta-analysis).

Twelve surveys (9 in Italy, 2 in Spain, 1 in Austria) reported the use of anthelmintic medicinal plants in livestock living in EU Countries. Data obtained from 256 animals and extracted from 6 studies were included in the network meta-analysis. Medicinal plants and drugs were more effective than negative control (standardized mean difference [SMD]: -0.60 95%CrI -0.88 to -0.31, -0.73 95%CrI -1.08 to -0.38, respectively, $P < 0.001$). Overall, no difference was detected between anthelmintic medicinal plants and anthelmintic drugs, namely albendazole, ivermectin, fenbendazole, and doramectin (SMD: 0.26 95%CrI -0.02 to 0.55, $P > 0.05$). The most effective anthelmintic medicinal plants were *Artemisia absinthium*, *Allium sativum*, and *Duranta erecta*.

There is the strong medical need of performing adequately powered randomized controlled trials in different livestock species aimed to improve the quality of the current evidence concerning the anthelmintic efficacy of medicinal plants compared to that of the currently available antiparasitic drugs.

1. Introduction

Medicinal plants have been extensively used worldwide over history to treat and prevent the occurrence of several diseases, infections, and infestations in domestic animals, prevalently in livestock (Abo-El-Sooud, 2018; Ayrle et al., 2016; Githiori et al., 2005; Suroowan et al., 2017). The scientific interest in veterinary ethnopharmacology has increased since 2000's (Katerere and Luseba, 2010), when a session at the 10th International Congress of International Society for Ethnopharmacology (ISE São Paulo Brazil, 2008) was fully dedicated to different topics of veterinary

ethnopharmacology, including the use of anthelmintic medicinal plants in livestock (Katerere and Luseba, 2010). Unfortunately, to date the role of medicinal plants as anthelmintic strategy in veterinary medicine seems to be of scarce interest for the International Society for Ethnopharmacology (ISE Technische Universität Dresden Germany, 2019).

Certainly, ethnopharmacology may have a rationale in veterinary medicine due to the potential therapeutic efficacy, which is related with low risk of adverse events, reduced microbiological and parasitic resistance, and decreased residues in animal products and environment when compared to chemotherapeutic agents (Abo-El-Sooud, 2018). Despite

* Corresponding author.

** Co-corresponding author.

E-mail addresses: luigino.calzetta@uniroma2.it (L. Calzetta), palma@unicz.it (E. Palma).

¹ The first three authors contributed equally to the manuscript.

some medicinal plants seem to have promising potential activity, to date only few and underpowered studies attempted to investigate the real efficacy profile of anthelmintic herbs in veterinary ethnoparmacology (Abo-El-Sooud, 2018; Githiori et al., 2005; Mayer et al., 2014).

Ethnoveterinary medicine represents an integral part of medical practices in most developing countries (Githiori et al., 2005; Maroyi, 2017), and also in Europe a relevant number of plants used to treat organic livestock have been mapped, with some correspondence between the traditional empirical use and scientific evidence (Mayer et al., 2014).

In the light of this background, the hypothesis of this study is that medicinal plants may have effective anthelmintic activity in animals. Therefore, we have performed a qualitative synthesis of the current literature aimed to provide the geographical distribution of the use of anthelmintic medicinal plants in livestock in European Union (EU). We have also carried out a quantitative synthesis aimed to assess whether medicinal plants may really have in vivo anthelmintic effects in animals, and to compare the efficacy of anthelmintic medicinal plants with that of anthelmintic drugs.

2. Materials and methods

2.1. Search strategy and study eligibility

The protocol of this synthesis of the current literature has been registered in the international prospective register of systematic reviews (PROSPERO, ID: CRD42019126353), and performed in agreement with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses Protocols (PRISMA-P) (Moher et al., 2015), with the flow diagram reported in Figure 1. This study satisfied all the recommended items reported by the PRISMA-P checklist (Moher et al., 2015).

A comprehensive literature search was performed for studies written in English and concerning the use and efficacy of anthelmintic medicinal plants in veterinary medicine.

The studies eligible in the qualitative synthesis (systematic review) were the surveys that investigated the use of anthelmintic medicinal plants in livestock in the area of EU. The EU Countries considered for the qualitative synthesis (systematic review) at the time of studies search were: Austria, Belgium, Bulgaria, Croatia, Cyprus, Czechia, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, United Kingdom.

The studies eligible in the quantitative synthesis (network meta-analysis) were those that investigated the efficacy of anthelmintic medicinal plants in animals, namely both livestock and experimental animals. In this regard, the PICO (Patient problem, Intervention, Comparison, and Outcome) framework was applied to develop the literature search strategy and question, as previously reported (Schardt et al., 2007). Namely, the “Patient problem” included helminthic infestation in animals; the “Intervention” regarded the treatment with anthelmintic medicinal plants; the “Comparison” was performed versus either anthelmintic drugs or negative control, where the negative control could be both untreated animals and animals treated with placebo; the “Outcomes” were the faecal egg count and parasites count at necroscopy.

The search was performed in PubMed (MEDLINE), Scopus, and Web of Science (WOS), in order to provide for relevant studies published up to November 21, 2019. The research string was as follows: (ethno[All Fields] OR (“Tradition”[Journal] OR “tradition”[All Fields]) OR (“ethno”[Journal] OR “Folk”[Journal] OR “folk”[All Fields])) AND (“veterinary”[Subheading] OR “veterinary”[All Fields]) OR (“animals”[MeSH Terms:noexp] OR animal[All Fields]) OR (“livestock”[MeSH Terms] OR “livestock”[All Fields]) OR (“farms”[MeSH Terms] OR “farms”[All Fields] OR “farm”[All Fields]) OR (“sheep, domestic”[MeSH Terms] OR (“sheep”[All Fields] AND “domestic”[All Fields]) OR “domestic sheep”[All Fields] OR “sheep”[All Fields] OR “sheep”[MeSH Terms]) OR (“goats”[MeSH Terms] OR “goats”[All Fields] OR “goat”[All Fields]) OR (“cattle”[MeSH Terms] OR “cattle”[All Fields]) OR (“cattle”[MeSH Terms] OR “cattle”[All Fields] OR “cow”[All Fields]) OR (“swine”[MeSH Terms] OR “swine”[All Fields] OR “pig”[All Fields]) OR calve[All Fields] OR (“poultry”[MeSH Terms] OR “poultry”[All Fields])) AND (“plants”[MeSH Terms] OR “plants”[All Fields] OR “plant”[All Fields]) OR herb[All Fields] OR phyto[All Fields]).

2.2. Study selection

The surveys reporting data concerning the use of anthelmintic medicinal plants in livestock living in EU were selected and included in the qualitative synthesis (systematic review). This selection approach was used to assess in which EU Countries there is scientific documentation of the current use of traditional medicinal plants in livestock.

The studies reporting data concerning the in vivo efficacy of any anthelmintic medicinal plants with respect to the reduction of faecal egg count or parasites count at necroscopy in animals, both livestock and

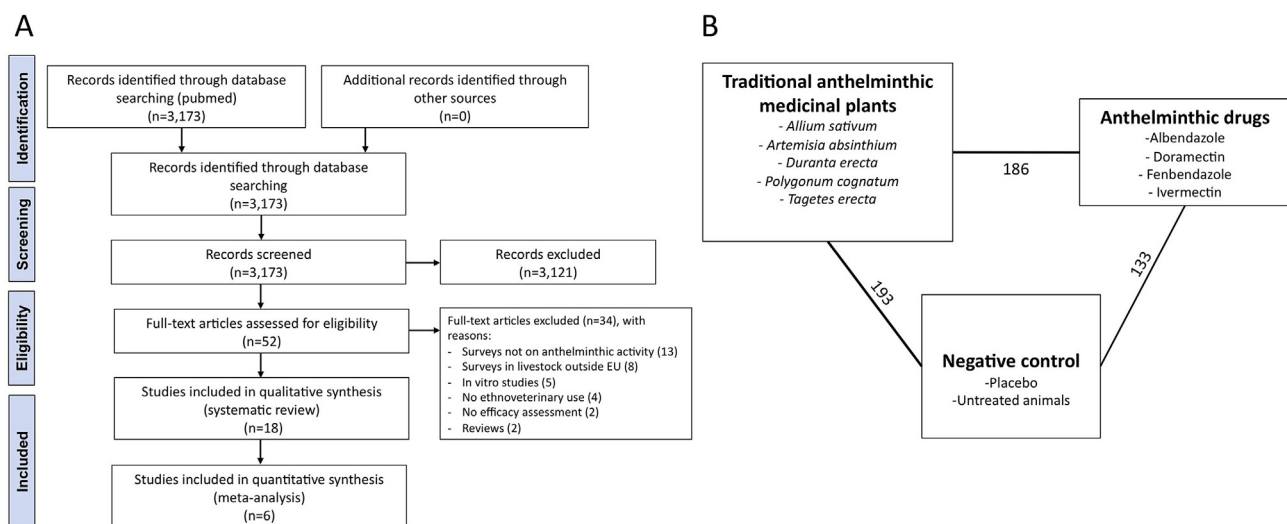


Figure 1. PRISMA flow diagram (A) for the identification of studies included in the network meta-analysis concerning the impact of anthelmintic medicinal plants in veterinary medicine compared to anthelmintic drugs and negative control. Diagram (B) displaying the network of the arms involved in the Bayesian analysis. The area including the treatments are related with the extent of population, and the numbers along the link lines indicate the number of animals comparing pairs of treatments.

experimental animals, were selected and included in the quantitative synthesis (meta-analysis).

Three reviewers independently examined the studies, and any difference in opinion concerning the selection of the studies was resolved by consensus.

2.3. Data extraction

Data from the studies included in the quantitative synthesis were extracted and checked for study characteristics, host animals, parasites, number of analysed subjects, treatments (medicinal plants and comparators [anthelmintic drugs and negative controls]), outcomes, time-points, item to check the consistency with the Animal Research Reporting In Vivo Experiments (ARRIVE) guidelines (Kilkenny et al., 2012), and items to assess the quality of studies. Data were extracted in agreement with Data Extraction for Complex Meta-anALysis (DECiMAL) recommendations (Pedder et al., 2016) and at the time-points eliciting the maximal effect. When needed, arithmetic mean and standard deviation were estimated from the geometric mean, median, range and the sample size as previously described (Hozo et al., 2005).

2.4. Endpoints

The endpoint of the qualitative synthesis (systematic review) was to provide the geographical distribution of the use of anthelmintic medicinal plants in livestock specifically in the EU Countries.

The endpoint of the quantitative synthesis (meta-analysis) was to assess the in vivo anthelmintic efficacy of medicinal plants in animals compared to the effect elicited by anthelmintic drugs and negative control, regardless of the species and the origin of the origin of the medicinal plants.

2.5. Quality of studies, risk bias, and evidence profile

The quality of each study included in the network meta-analysis was assessed by using the SYStematic Review Centre for Laboratory animal Experimentation Risk of Bias (SYRCLE RoB) tool, that is based on the Cochrane Collaboration tool, and has been adjusted for scoring the aspects of bias that play a specific role in animal intervention studies. SYRCLE RoB tool include 10 items to assess the selection, performance, attrition, and reporting bias. For each item yes, no, and unclear risk of bias corresponded to low, high, and unclear risk of bias, respectively (Hooijmans et al., 2014). Three reviewers, with a specific background in the field of quantitative synthesis, independently assessed the quality of the studies, and any difference in opinion concerning the SYRCLE RoB score was resolved by consensus.

The risk of bias in the network meta-analysis was checked via the normalized consistency/inconsistency analysis that permitted to assess whether the outcomes resulting from the consistency and inconsistency models fit adequately with the line of equality, as previously described (Cazzola et al., 2017). The inconsistency of evidence was also assessed by quantifying the inconsistency factor, indicating whether one of the

Table 1. Qualitative synthesis of survey studies that investigated the use of anthelmintic medicinal plants in livestock living in EU Countries.

Author and year	Country	Region	Sub-region	Livestock										
				Bos taurus	Ovis aries	Sus scrofa domesticus	Equus caballus	Oryctolagus cuniculus	Capra aegagrus hircus	Gallus gallus domesticus	Meleagris gallopavo	Equus asinus × Equus caballus	Equus asinus	
(Bullitta et al., 2018)	Italy	Sardinia	NA	X	/	X	X	/	/	/	X	/	/	/
(Vogl et al., 2016)	Austria	Eastern Tyrol	NA	X	X	X	X	/	X	X	/	/	/	/
(Piluzza et al., 2015)	Italy	Sardinia	NA	/	X	/	/	/	/	X	/	/	/	/
(Gonzalez et al., 2011)	Spain	Arribes del Duero	NA	X	X	X	X	X	X	X	/	X	X	X
(Idolo et al., 2010)	Italy	Abruzzi, Lazio and Molise	Alto Sangro, Fucino plane, Lazio and Mainarde	X	X	X	X	X	/	/	/	/	/	/
(Guarino et al., 2008)	Italy	Campania	Sannio	X	/	X	X	X	X	X	/	/	/	X
(Bonet and Valles, 2007)	Spain	Catalonia	NA	X	X	X	X	X	/	X	X	X	X	/
(Bullitta et al., 2007)	Italy	Sardinia	NA	X	X	X	X	X	X	X	X	/	/	/
(Guarrera et al., 2005)	Italy	Basilicata	Maratea	X	X	/	/	X	X	X	/	/	/	/
(Pieroni et al., 2004)	Italy	Basilicata	Dolomiti Lucane	X	X	X	/	X	X	X	/	/	/	/
(Uncini Manganelli et al., 2001)	Italy	Tuscany	NA	X	X	X	X	X	X	X	X	X	X	/
(Guarrera, 1999)	Italy	Marche, Abruzzi, Latium	NA	X	X	X	X	X	/	/	/	/	/	/

NA: not available.

Table 2. Study characteristics.

Author and year	Study design	Host animal	Parasites	Number of analysed animals	Treatments*		Outcomes	Time-point	Consistency with ARRIVE guidelines	
					Medicinal plants	Comparators				
						Anthelmintic drugs				Negative controls
(Guragac Dereli et al., 2019)	In vivo experimental study, active and negative control, 5 arms, parallel-group, randomized	<i>Mus musculus</i>	<i>Syphacia obvelata</i> ; <i>Aspiculuris tetraptera</i>	60	<i>Polygonum cognatum</i> (dose: 100 mg/kg; days of treatment: 1; regimen: single dose administration; vehicle: n-hexane extract, EtOAc extract, MeOH extract)	Doramectin (dose: 0.2 mg/kg; regimen: single dose administration)	CMC suspension	Parasite counts recovered at necropsy	Day 7 post-treatment	NO
(Udobi et al., 2018)	In vivo experimental study, active and negative control, 5 arms, parallel-group, randomized	<i>Mus musculus</i>	<i>Heligmosomoides bakeri</i>	25	<i>Duranta erecta</i> (doses: 250-500-1000 mg/kg; days of treatment: 1; regimen: single dose administration; vehicle: MeOH extract)	Albendazole (dose: 25 mg/kg; regimen: single dose administration)	Untreated	Parasite counts recovered at necropsy	Day 28 post-infection	NO
(Kanojiya et al., 2015)	In vitro and in vivo experimental study, active and negative control, 3 arms, parallel-group	<i>Ovis aries</i>	<i>Haemonchus contortus</i>	45	<i>Allium sativum</i> (dose: 5 g/animal; days of treatment: 1; regimen: single dose administration; vehicle: aqueous extract)	Albendazole (dose: 7.5 mg/kg; regimen: single dose administration)	Untreated	Faecal egg count	Day 21 post-treatment	NO
(Palacio-Landin et al., 2015)	In vitro and in vivo experimental study, active and negative control, 6 arms, parallel-group	<i>Meriones unguiculatus</i>	<i>Haemonchus contortus</i>	42	<i>Allium sativum</i> and <i>Tagetes erecta</i> (dose: 40 mg/mL in 100 µL volume; days of treatment: 1; regimen: single dose administration; vehicle: n-hexane extract, acetone extract)	Fenbendazole (dose: NA; regimen: NA)	Distilled water; tween-20 3%	Parasite counts recovered at necropsy	Day 13 post-infection	NO
(Ayaz et al., 2008)	In vivo experimental study, active and negative control, 3 arms, parallel-group	<i>Mus musculus</i>	<i>Aspiculuris tetraptera</i>	54	<i>Allium sativum</i> (dose: one garlic clove/mouse; days of treatment: 7; regimen: once-daily; vehicle: garlic liquid suspension)	Ivermectin (dose: 0.2 mg/kg; regimen: NA)	NaCl 0.9%	Parasites counts recovered at necropsy	Day 8 post-treatment	NO
(Tariq et al., 2009)	In vitro and in vivo experimental study, active and negative control, 6 arms, parallel-group, randomized	<i>Ovis aries</i>	<i>Haemonchus contortus</i>	30	<i>Artemisia absinthium</i> (doses: 1–2 g/kg; days of treatment: 1; regimen: single dose administration; vehicle: crude aqueous extract, crude ethanolic extract)	Albendazole (dose: 5 mg/kg; regimen: single dose administration)	DMSO 0.5%	Faecal egg counts/g of faeces	Day 15 post-treatment	NO

ARRIVE: Animal Research Reporting In Vivo Experiments; CAE: crude aqueous extract; CEE: crude ethanolic extract; CMC: carboxymethylcellulose; DMSO: dimethyl sulphoxide; EtOAc: ethyl acetate; IM: intramuscular; MeOH: methanol.

* All treatments were administered per os except for ivermectin that was administered IM.

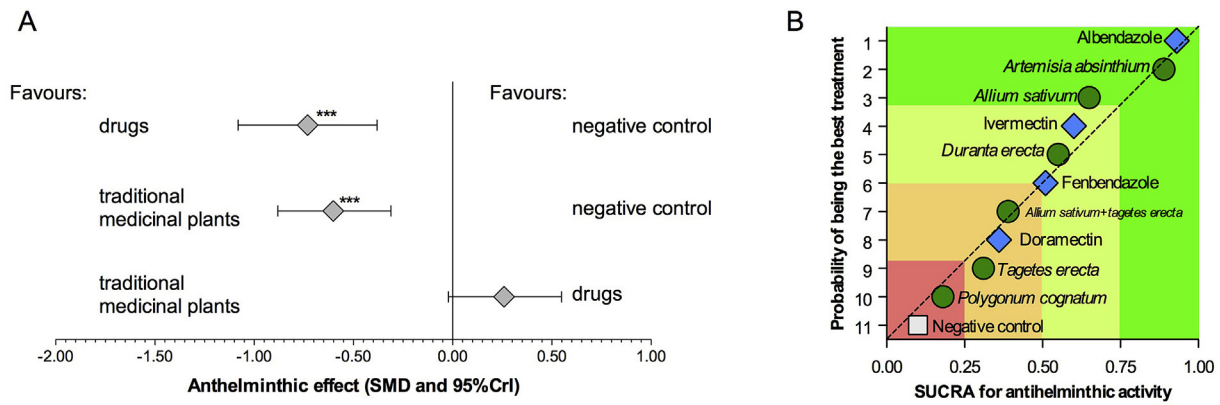


Figure 2. Overall forest plot of network meta-analysis (A) concerning the comparison of anthelmintic efficacy across medicinal plants, drugs and negative control; anthelmintic ranking plot (B) resulting from the network meta-analysis of specific medicinal plants, drugs and negative control in which each treatment was plotted on X-axis according to SUCRA (score of 1 being the most effective) and on Y-axis according to the probability (%) of being the best treatment. ***P < 0.001 vs. comparator. CrI: credible interval; SMD: standardized mean difference; SUCRA: surface under the cumulative ranking curve analysis.

Table 3. Anthelmintic effect of specific medicinal plants compared to negative control. Data are reported as MD because the data resulted from the network meta-analysis of treatment arms that assessed the anthelmintic effect on the same outcome.

Medicinal plants	Anthelmintic effect vs. negative control (MD and 95%CrI)	Outcome
<i>Artemisia absinthium</i>	-514.18 (-591.07, -437.29)***	Faecal egg count
<i>Allium sativum</i>	-71.64 (-86.20, -57.08)***	Parasites count at necropsy
<i>Tagetes erecta</i>	-55.64 (-83.49, -27.80)***	Parasites count at necropsy
<i>Polygonum cognatum</i>	-30.10 (-56.62, -3.58)*	Parasites count at necropsy
<i>Duranta erecta</i>	-10.33 (-20.61, -0.05)*	Parasites count at necropsy

***P < 0.001 and *P < 0.05 vs. negative control.
CrI: credible interval; MD: mean difference.

Author and year	Selection			Performance		Detection		Attrition	Reporting	Other
	Sequence generation	Baseline characteristics	Allocation concealment	Random housing	Blinding	Random outcome assessment	Blinding	Incomplete outcome data	Selective outcome reporting	Other sources of bias
Guragac et al. 2019	?	+	?	+	?	?	?	+	+	+
Udobi et al. 2018	?	+	?	+	?	?	?	+	+	+
Kanojiya et al. 2015	?	-	?	?	?	?	?	+	+	+
Palacio et al. 2015	?	+	?	?	?	?	?	+	+	+
Ayaz et al. 2008	?	+	?	?	?	?	?	+	+	+
Tariq et al. 2008	?	?	?	+	?	?	?	+	+	+

Figure 3. SYRCL RoB assessment for the studies included in the network meta-analysis. ?: unclear risk of bias; +: low risk of bias; -: high risk of bias; RoB: risk of bias.

treatment had a different effect when it was compared with the others (Cazzola et al., 2018).

The quality of the evidence was scored in agreement with the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) system (Guyatt et al., 2011).

2.6. Data synthesis and analysis

A network meta-analysis was performed to determine the anthelmintic effect of medicinal plants compared with anthelmintic drugs and negative control. The network meta-analysis permitted to rank the affect of different

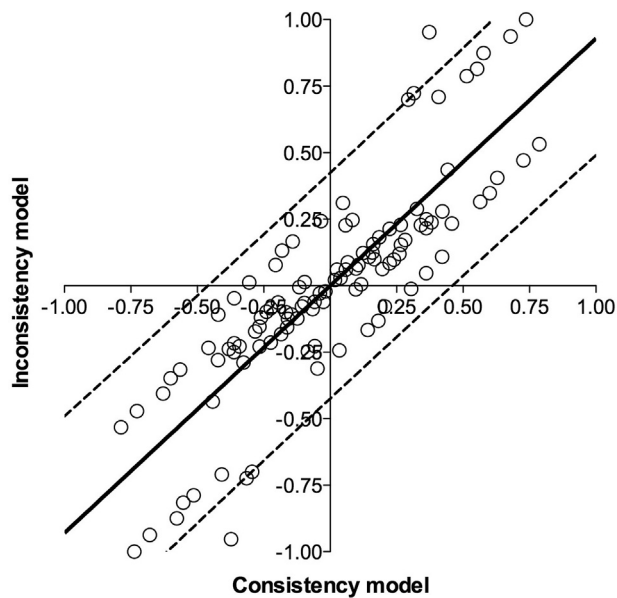


Figure 4. Publication bias assessment via the normalized consistency/inconsistency plot (linear regression and 95% confidence bands) for the comparison of anthelmintic effect across specific medicinal plants, drugs and negative control.

medicinal plants, drugs and negative control with regard to their anthelmintic activity, as previously described (Calzetta et al., 2016). A full Bayesian evidence network was used in the network meta-analysis (chains: 4; initial values scaling: 2.5; tuning iterations: 20.000; simulation iterations: 50.000; tuning interval: 10). The convergence diagnostics for consistency and inconsistency were assessed via the Brooks-Gelman-Rubin method, as previously described (Calzetta et al., 2016).

The output of network meta-analysis was the relative effect (RE) and 95% credible interval (95%CrI). The relative effect (RE) of overall network meta-analysis is reported as standardized mean difference (SMD) = [difference in mean outcome between groups] * [standard deviation of outcome among participants]⁻¹, since the studies assessed the same outcome (anthelmintic effect) by measuring it in a variety of ways (i.e. faecal egg count or parasites count at necropsy). SMD expresses the size of the intervention effect in each study relative to the variability observed in that study (Higgins and Green, 2011b).

Subset network meta-analysis were performed on the anthelmintic effect of specific medicinal plants vs. control by considering the same outcome. In this circumstance the RE was reported as mean difference (MD) (Higgins and Green, 2011a).

The probability that each specific medicinal plant, drug and negative control were the most effective was calculated by counting the proportion of iterations of the chain in which each treatment had the highest RE, and the surface under the cumulative ranking curve analysis (SUCRA), representing the summary of these probabilities, was also calculated (Dobler et al., 2018). The SUCRA is 1 when a treatment is considered to be the best, and 0 when a treatment is considered to be the worst (Cazzola et al., 2017).

The statistical significance was assessed for $P < 0.05$. The GeMTC (van Valkenhoef et al., 2012) software were used for performing the network meta-analysis, GraphPad Prism (CA, US) software to graph the data, and GRADEpro GDT to assess the quality of evidence (Guyatt et al., 2011).

3. Results

3.1. Qualitative synthesis

Twelve studies reported data from survey studies that investigated the use of anthelmintic medicinal plants in livestock living in EU

Countries (Bonet and Valles, 2007; Bullitta et al., 2007, 2018; Gonzalez et al., 2011; Guarino et al., 2008; Guarrera, 1999; Guarrera et al., 2005; Idolo et al., 2010; Pieroni et al., 2004; Piluzza et al., 2015; Uncini Manganelli et al., 2001; Vogl et al., 2016). Of these studies 9, 2 and 1 investigations were conducted in Italy, Spain, and Austria, respectively. Details on specific regions, sub-regions, and livestock species are shown in Table 1.

3.2. Quantitative synthesis

3.2.1. Studies and population characteristics

Data obtained from 256 animals (48.05% in the anthelmintic medicinal plants arm, 24.61% in the anthelmintic drugs arm, and 27.34% in the negative control arm) were extracted from 6 studies (Ayaz et al., 2008; Guragac Dereli et al., 2019; Kanojiya et al., 2015; Palacio-Landin et al., 2015; Tariq et al., 2009; Udobi et al., 2018). The species used in the in vivo experimental settings were *Mus musculus* (Ayaz et al., 2008; Guragac Dereli et al., 2019; Udobi et al., 2018), *Meriones unguiculatus* (Palacio-Landin et al., 2015), and *Ovis aries* (Kanojiya et al., 2015; Tariq et al., 2009). All the studies investigated the impact of anthelmintic medicinal plants, anthelmintic drugs and negative control on gastrointestinal nematodes. The period of treatment ranged from 1 day to 1 week.

Although all the studies included in this quantitative synthesis enrolled animals used for experimental purpose, none of the studies have been performed in agreement with the ARRIVE guidelines (Kilkenny et al., 2012). Detailed studies design, hosts, parasites, treatments, outcomes, and time-points are reported in Table 2.

3.2.2. Network meta-analysis

The overall network meta-analysis indicated that the anthelmintic effect of medicinal plants and drugs was significantly ($P < 0.001$) more effective than that elicited by negative control (SMD: -0.60 95%CrI -0.88 to -0.31, -0.73 95%CrI -1.08 to -0.38; respectively), and no significant difference ($P > 0.05$) was detected between anthelmintic medicinal plants and anthelmintic drugs (SMD: 0.26 95%CrI -0.02 to 0.55) (Figure 2A).

The results of the overall network meta-analysis were confirmed by the SUCRA ranking, in which both albendazole, *Artemisia absinthium*, and *Allium sativum* were located in the upper ranking quartile, as well as *Duranta erecta* resulted to have the same anthelmintic efficacy of ivermectin and fenbendazole (third quartile in SUCRA ranking), followed by doramectin, *Allium sativum* plus *Tagetes erecta*, and *Tagetes erecta* (second quartile in SUCRA ranking), and finally *Polygonum cognatum* and negative control (first quartile in SUCRA ranking). Overall, the most effective anthelmintic medicinal plants were *Artemisia absinthium*, *Allium sativum*, and *Duranta erecta* (Figure 2B).

The results of the subset network meta-analysis were performed on the anthelmintic effect of specific medicinal plants vs. negative control by considering the same outcome are reported in Table 3.

3.2.3. Quality of studies, risk bias, and evidence profile

The studies included in the quantitative synthesis may have been affected by a certain level of selection bias and performance bias, although 4 studies adequately reported the baseline characteristics of animals and 3 studies indicated that the housing was randomly assigned. Conversely, it was unclear for all the studies whether there was any form of detection bias. In any case, no attrition bias and reporting bias was detected, as well as other source of bias. Detailed findings resulting from the SYRCLC RoB tool are reported in Figure 3.

The level of uncertainty resulting from the SYRCLC RoB tool was confirmed by the normalized consistency/inconsistency analysis resulting from the network comparison across specific medicinal plants, drugs and negative control, in which a certain number of points did not fit adequately with the line of equality, as confirmed by the linear regression model (goodness of fit: R^2 0.71; slope 0.93 95% confidence intervals 0.82

to 1.04) (Figure 4), and by a significant ($P < 0.05$) inconsistency factor in the evidence cycle including albendazole, *Allium sativum*, *Allium sativum* plus *Tagetes erecta*, *Artemisia absinthium*, fenbendazole, and negative control. Conversely, no significant ($P > 0.05$) inconsistency factor was detected in the evidence cycles including albendazole, *Allium sativum*, *Allium sativum* plus *Tagetes erecta*, *Duranta erecta*, fenbendazole, and negative control, or *Allium sativum*, *Allium sativum* plus *Tagetes erecta*, fenbendazole, and negative control, or *Allium sativum* plus *Tagetes erecta*, fenbendazole, *Tagetes erecta* and negative control.

The GRADE analysis indicated moderate quality of evidence (+++) for the overall network meta-analysis concerning the comparison across medicinal plants, drugs, and negative control with respect to the comparison of their anthelmintic effect.

4. Discussion

The qualitative synthesis of the current literature regarding the geographical distribution of the use of anthelmintic medicinal plants in livestock indicates that, across the EU Countries, the most investigations were performed in Italy (9 survey studies), followed by Spain (2 survey studies), and Austria (1 survey study). No further papers are currently available for the remaining EU Countries but, considering that it is extensively recognized that the absence of evidence is not evidence of absence (Alderson, 2004; Altman and Bland, 1996), we cannot exclude that medicinal plants are, or have been, used in further area of EU for anthelmintic purpose in livestock.

Such an effort in performing numerous survey studies moved generally from the empirical knowledge that Mediterranean farmers in EU traditionally used plants sourced locally to treat their animals (Bullitta et al., 2018; Uncini Manganelli et al., 2001). Nevertheless, only in 2014 there was an attempt to systematically report the studies concerning the ethnoveterinary of medicinal plants to treat organic livestock use in EU (Mayer et al., 2014). Although the paper of Mayer and colleagues was of interest (Mayer et al., 2014), the qualitative synthesis of literature that they provided was not performed in agreement with the recommendation available at that time (PRISMA statement) for the development and reporting of systematic review (Moher et al., 2009), and no quantitative synthesis (meta-analysis) was performed.

Considering this background, we have conducted a quantitative synthesis in agreement with the PRISMA-P and PROSPERO recommendations (Moher et al., 2015) concerning the current literature on the anthelmintic effect of medicinal plants in veterinary medicine. The full Bayesian network approach used in our study permitted not only to assess the anthelmintic effect of medicinal plants, but also and most importantly to compare the anthelmintic effect of medicinal plants with the efficacy of anthelmintic drugs.

The results of our study shows that the overall anthelmintic effect of the medicinal plants included in the network meta-analysis was significantly greater compared to negative control and, as expected, that also anthelmintic drugs were more effective than negative control. Surprisingly, our analysis suggests that the overall efficacy of anthelmintic medicinal plants was similar to that of anthelmintic drugs. These findings are further confirmed by the rank resulting from the SUCRA concerning each specific treatment arm, in which *Artemisia absinthium*, *Allium sativum*, and *Duranta erecta*, that were the most effective anthelmintic medicinal plants, reached the same efficacy of albendazole, ivermectin and fenbendazole (two upper quartiles).

Indeed, the result of this quantitative synthesis should draw the attention of the scientific community: here we provide for the first time the evidence of a potential new paradigm that the infestation by nematodes in animals can be effectively treated with the extracts of medicinal plants. We can further extend this concept by hypothesizing that the gastrointestinal helminthic infestation could be naturally controlled in livestock grazing in pastures rich in medicinal plants such as *Artemisia*

absinthium and, thus, reduce the use of anthelmintic drugs. This is certainly of interest for breeding organic livestock, and might also help to reduce the potential environmental risk due to the extensive use of anthelmintic agents (Lumaret et al., 2012).

The last report concerning the environmental risks of medicinal products (Mudgal et al., 2013) has highlighted that the most extensive use of avermectins is in the control of livestock parasites. This evidence supports the fact that the global antiparasitic veterinary market represents 28% of the global EU veterinary medicine marketing, that is equivalent to 1.2 billion Euros (Shoop and Soll, 2002). It is impressive that, since its market introduction in the early 1980s, ivermectin has become the most widely used antiparasitic drug with over 5 billion doses sold worldwide until 2000s (Shoop and Soll, 2002). Such an extensive and uncontrolled use of antiparasitic agents has raised relevant concerns, supported by scientific evidence, regarding the parasitic resistance and the environmental impact related with the use of avermectins (Laing et al., 2017; Lumaret et al., 2012). Therefore, considering that the extracts of *Artemisia absinthium*, *Allium sativum*, and *Duranta erecta* seem to be as effective as ivermectin, a drug ranked in EU in the top four ecotoxic substances and for which parasitic resistance is increasing (Boxall et al., 2003; Kools et al., 2008), shifting the antiparasitic therapy towards the use of anthelmintic medicinal plants could reduce the residue of macrocyclic lactones in terrestrial and aquatic environments and prevent future resistance (Lumaret et al., 2012; Tremblay and Wratten, 2002).

Indeed this study has limitations, the most important of which is the moderate quality of evidence resulting from the GRADE analysis, a method that provides explicit criteria for rating the quality of evidence by assessing study design, risk of bias, imprecision, inconsistency, indirectness, and magnitude of effect (Guyatt et al., 2011). This means that we are moderately confident in the effect estimate: the true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different. In other words, further investigations are likely to have an important impact on the 95%CrI and may change the RE reported in this research (Balslem et al., 2011). Certainly, performing animal research in agreement with the ARRIVE guidelines (Kilkenny et al., 2012) influences the quality of each single study and, thus, may improve the quality of evidence of meta-analyses (Vesterinen et al., 2013). Furthermore, across the studies included in the network meta-analysis, only two were carried out by enrolling livestock (Kanojija et al., 2015; Tariq et al., 2009), whereas the most were performed in rodents (Guragac Dereli et al., 2019; Palacio-Landin et al., 2015; Tariq et al., 2009; Udobi et al., 2018). Unexpectedly, data from SUCRA shown in Figure 2B indicate that the combination of *Allium sativum* plus *Tagetes erecta* was less effective than the extract of *Allium sativum* alone, suggesting for a potential antagonistic interaction between the extracts of *Allium sativum* and *Tagetes erecta*.

5. Conclusion

This study provides the evidence that medicinal plants are as effective as drugs against helminthic infestation in animals, and that *Artemisia absinthium*, *Allium sativum*, and *Duranta erecta* are the most effective anthelmintic medicinal plants. The findings of this study suggest that there is the strong medical need of performing adequately powered randomized controlled trials in different livestock species (cattle, pigs, sheep, and horses), and eventually in pets (dogs and cats), aimed to improve the quality of the current evidence concerning the anthelmintic efficacy of medicinal plants compared to that of the currently available antiparasitic drugs. Finally, but not less important, the future randomized controlled trials should be designed to provide also information on the pharmacological interaction across the extracts of different anthelmintic medicinal plants that may lead either to synergistic or antagonistic effect (Calzetta et al., 2018).

Declarations

Author contribution statement

Luigino Calzetta: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Elena Pistocchini, Antonio Leo: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Paola Roncada, Domenico Britti: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

Beatrice Ludovica Ritondo: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Ernesto Palma, David di Cave: Analyzed and interpreted the data; Wrote the paper.

Funding statement

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

References

- Abo-El-Sooud, K., 2018. Ethnoveterinary perspectives and promising future. *Int. J. Vet. Sci. Med.* 6, 1–7.
- Alderson, P., 2004. Absence of Evidence is Not Evidence of Absence. *British Medical Journal Publishing Group*.
- Altman, D.G., Bland, J.M., 1996. Absence of evidence is not evidence of absence. *Aust. Vet. J.* 74, 311.
- Ayaz, E., Turel, I., Gul, A., Yilmaz, O., 2008. Evaluation of the anthelmintic activity of garlic (*Allium sativum*) in mice naturally infected with *Aspiculuris tetraptera*. *Recent Pat. Anti-Infect. Drug Discov.* 3, 149–152.
- Ayrle, H., Mevissen, M., Kaske, M., Nathues, H., Gruetzner, N., Melzig, M., Walkenhorst, M., 2016. Medicinal plants—prophylactic and therapeutic options for gastrointestinal and respiratory diseases in calves and piglets? A systematic review. *BMC Vet. Res.* 12, 89.
- Balshem, H., Helfand, M., Schunemann, H.J., Oxman, A.D., Kunz, R., Brozek, J., Vist, G.E., Falck-Ytter, Y., Meerpohl, J., Norris, S., Guyatt, G.H., 2011. GRADE guidelines: 3. Rating the quality of evidence. *J. Clin. Epidemiol.* 64, 401–406.
- Bonet, M.A., Valles, J., 2007. Ethnobotany of Montseny biosphere reserve (Catalonia, Iberian Peninsula): plants used in veterinary medicine. *J. Ethnopharmacol.* 110, 130–147.
- Boxall, A.B.A., Kolpin, D.W., Halling-Sorensen, B., Tolls, J., 2003. Peer Reviewed: Are Veterinary Medicines Causing Environmental Risks? *ACS Publications*.
- Bullitta, S., Piluzza, G., Viegi, L., 2007. Plant resources used for traditional ethnoveterinary phytotherapy in Sardinia (Italy). *Genet. Resour. Crop Evol.* 54, 1447–1464.
- Bullitta, S., Re, G.A., Manunta, M.D.I., Piluzza, G., 2018. Traditional knowledge about plant, animal, and mineral-based remedies to treat cattle, pigs, horses, and other domestic animals in the Mediterranean island of Sardinia. *J. Ethnobiol. Ethnomed.* 14, 50.
- Calzetta, L., Matera, M.G., Cazzola, M., 2018. Pharmacological mechanisms leading to synergy in fixed-dose dual bronchodilator therapy. *Curr. Opin. Pharmacol.* 40, 95–103.
- Calzetta, L., Rogliani, P., Ora, J., Puxeddu, E., Cazzola, M., Gabriella Matera, M., 2016. LABA/LAMA combination in COPD: a meta-analysis on the duration of treatment. *Eur. Respir. Rev. Offic. J. Eur. Respir. Soc.* 1–11.
- Cazzola, M., Calzetta, L., Barnes, P.J., Criner, G.J., Martinez, F.J., Papi, A., Gabriella Matera, M., 2018. Efficacy and safety profile of xanthines in COPD: a network meta-analysis. *Eur. Respir. Rev. Offic. J. Eur. Respir. Soc.* 27.
- Cazzola, M., Rogliani, P., Calzetta, L., Hanania, N.A., Matera, M.G., 2017. Impact of mucolytic agents on COPD exacerbations: a pair-wise and network meta-analysis. *COPD* 14, 552–563.
- Dobler, C.C., Wilson, M.E., Murad, M.H., 2018. A pulmonologist's guide to understanding network meta-analysis. *Eur. Respir. J.* 52.
- Githiori, J.B., Höglund, J., Waller, P.J., 2005. Ethnoveterinary plant preparations as livestock dewormers: practices, popular beliefs, pitfalls and prospects for the future. *Anim. Health Res. Rev.* 6, 91–103.
- Gonzalez, J.A., Garcia-Barriuso, M., Amich, F., 2011. Ethnoveterinary medicine in the Arribes del Duero, western Spain. *Vet. Res. Commun.* 35, 283–310.
- Guarino, C., De Simone, L., Santoro, S., 2008. Ethnobotanical Study of the Sannio Area. Campania, southern Italy.
- Guarrera, P.M., 1999. Traditional antihelmintic, antiparasitic and repellent uses of plants in Central Italy. *J. Ethnopharmacol.* 68, 183–192.
- Guarrera, P.M., Salerno, G., Caneva, G., 2005. Folk phytotherapeutic plants from Maratea area (Basilicata, Italy). *J. Ethnopharmacol.* 99, 367–378.
- Guragac Dereli, F.T., Ilhan, M., Kozan, E., Kupeli Akkol, E., 2019. Effective eradication of pinworms (*Syphacia obvelata* and *Aspiculuris tetraptera*) with *Polygonum cognatum* Meissn. *Exp. Parasitol.* 196, 63–67.
- Guyatt, G., Oxman, A.D., Akl, E.A., Kunz, R., Vist, G., Brozek, J., Norris, S., Falck-Ytter, Y., Glasziou, P., DeBeer, H., Jaeschke, R., Rind, D., Meerpohl, J., Dahm, P., Schunemann, H.J., 2011. GRADE guidelines: 1. Introduction-GRADE evidence profiles and summary of findings tables. *J. Clin. Epidemiol.* 64, 383–394.
- Higgins, J.P.T., Green, S., 2011a. *Cochrane Handbook for Systematic Reviews of Interventions*. Version 5.1.0 [updated March 2011]. Available from: https://handbook-5-1.cochrane.org/chapter_9/9_2_3_1_the_mean_difference_or_difference_in_means.htm. The Cochrane Collaboration 9.2.3.1 The mean difference (or difference in means).
- Higgins, J.P.T., Green, S., 2011b. *Cochrane Handbook for Systematic Reviews of Interventions*. Version 5.1.0 [updated March 2011]. Available from: https://handbook-5-1.cochrane.org/chapter_9/9_2_3_2_the_standardized_mean_difference.htm. The Cochrane Collaboration 9.2.3.2 The standardized mean difference.
- Hooijmans, C.R., Rovers, M.M., de Vries, R.B., Leenaars, M., Ritskes-Hoitinga, M., Langendam, M.W., 2014. SYRCLE's risk of bias tool for animal studies. *BMC Med. Res. Methodol.* 14, 43.
- Hozo, S.P., Djulbegovic, B., Hozo, I., 2005. Estimating the mean and variance from the median, range, and the size of a sample. *BMC Med. Res. Methodol.* 5, 13.
- Idolo, M., Motti, R., Mazzoleni, S., 2010. Ethnobotanical and phytomedicinal knowledge in a long-history protected area, the Abruzzo, Lazio and molise national park (Italian Apennines). *J. Ethnopharmacol.* 127, 379–395.
- ISE São Paulo Brazil, 2008. 10th International Congress of International Society for Ethnopharmacology (Joint congress with the XX Symposium of Brazilian Medicinal Plants), São Paulo, Brazil, 16–19 September, 2008. Scientific program available at: <http://www.plantasmedicinai.unifesp.br/en/prog2.html>.
- ISE Technische Universität Dresden Germany, 2019. 19th International Congress of International Society for Ethnopharmacology, Technische Universität Dresden, Germany, 12–14 June, 2019. Scientific program available at: https://tu-dresden.de/mn/ressourcen/dateien/internationale/veranstaltung-19th-international-kongress-of-etnopharmacology/190603_program.pdf?lang=de.
- Kanojiya, D., Shanker, D., Sudan, V., Jaiswal, A.K., Parashar, R., 2015. Assessment of in vitro and in vivo anthelmintic potential of extracts of *Allium sativum* bulb against naturally occurring ovine gastrointestinal nematodiosis. *Vet. Q.* 35, 200–206.
- Katerere, D.R., Luseba, D., 2010. *Ethnoveterinary Botanical Medicine: Herbal Medicines for Animal Health*. CRC Press.
- Kilkenny, C., Browne, W.J., Cuthi, I., Emerson, M., Altman, D.G., 2012. Improving bioscience research reporting: the ARRIVE guidelines for reporting animal research. *Vet. Clin. Pathol.* 41, 27–31.
- Kools, S.A., Boxall, A., Moltmann, J.F., Bryning, G., Koschorreck, J., Knacker, T., 2008. A ranking of European veterinary medicines based on environmental risks. *Integr. Environ. Assess. Manag.* 4, 399–408.
- Laing, R., Gillan, V., Devaney, E., 2017. Ivermectin - old drug, new tricks? *Trends Parasitol.* 33, 463–472.
- Lumaret, J.P., Errouissi, F., Floate, K., Rombke, J., Wardhaugh, K., 2012. A review on the toxicity and non-target effects of macrocyclic lactones in terrestrial and aquatic environments. *Curr. Pharmaceut. Biotechnol.* 13, 1004–1060.
- Maroyi, A., 2017. Diversity of use and local knowledge of wild and cultivated plants in the Eastern Cape province, South Africa. *J. Ethnobiol. Ethnomed.* 13, 43.
- Mayer, M., Vogl, C.R., Amorena, M., Hamburger, M., Walkenhorst, M., 2014. Treatment of organic livestock with medicinal plants: a systematic review of European ethnoveterinary research. *Forsch. Komplementmed.* 21, 375–386.
- Moher, D., Liberati, A., Tetzlaff, J., Altman, D.G., Group, P., 2009. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *BMJ* 339, b2535.
- Moher, D., Shamseer, L., Clarke, M., Ghersi, D., Liberati, A., Petticrew, M., Shekelle, P., Stewart, L.A., Group, P.-P., 2015. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. *Syst. Rev.* 4, 1.
- Mudgal, S., Toni, A.D., Lockwood, S., Salés, K., Backhaus, T., Sorensen, B.H., 2013. Study on the environmental risks of medicinal products, Final report prepared for executive agency for health and consumers. *BIO Intell. Serv.*
- Palacio-Landin, J., Mendoza-de Gives, P., Salinas-Sanchez, D.O., Lopez-Arellano, M.E., Liebano-Hernandez, E., Hernandez-Velazquez, V.M., Valladares-Cisneros, M.G., 2015. In vitro and in vivo nematocidal activity of *Allium sativum* and *Tagetes erecta* extracts against *Haemonchus contortus*. *Turk. Parazitoloji Derg.* 39, 260–264.
- Pedder, H., Sarri, G., Keeney, E., Nunes, V., Dias, S., 2016. Data extraction for complex meta-analysis (DECIMAL) guide. *Syst. Rev.* 5, 212.
- Pieroni, A., Howard, P., Volpato, G., Santoro, R.F., 2004. Natural remedies and nutraceuticals used in ethnoveterinary practices in inland southern Italy. *Vet. Res. Commun.* 28, 55–80.
- Piluzza, G., Virdis, S., Serrallutzu, F., Bullitta, S., 2015. Uses of plants, animal and mineral substances in Mediterranean ethno-veterinary practices for the care of small ruminants. *J. Ethnopharmacol.* 168, 87–99.

- Schardt, C., Adams, M.B., Owens, T., Keitz, S., Fontelo, P., 2007. Utilization of the PICO framework to improve searching PubMed for clinical questions. *BMC Med. Inf. Decis. Mak.* 7, 16.
- Shoop, W., Soll, M., 2002. Ivermectin, abamectin and eprinomectin. In: *Macrocyclic Lactones in Antiparasitic Therapy*. CABI Publishing Oxon, UK, pp. 1–29.
- Suroowan, S., Javeed, F., Ahmad, M., Zafar, M., Noor, M.J., Kayani, S., Javed, A., Mahomoodally, M.F., 2017. Ethnoveterinary health management practices using medicinal plants in South Asia—a review. *Vet. Res. Commun.* 41, 147–168.
- Tariq, K.A., Chishti, M.Z., Ahmad, F., Shawl, A.S., 2009. Anthelmintic activity of extracts of *Artemisia absinthium* against ovine nematodes. *Vet. Parasitol.* 160, 83–88.
- Tremblay, L.A., Wratten, S.D., 2002. Effects of Ivermectin in Dairy Discharges on Terrestrial and Aquatic Invertebrates. New Zealand Department of Conservation.
- Udobi, M.I., Nzeakor, T.A., Eke, I.G., Ezech, I.O., Onyeabor, A., Idika, I.K., Nwosu, C.O., 2018. Evaluation of the anthelmintic potential of *Duranta erecta* L. (Verbenaceae) fruits used in Nigerian ethnomedicine as a vermifuge. *J. Ethnopharmacol.* 216, 57–62.
- Uncini Manganelli, R.E., Camangi, F., Tomei, P.E., 2001. Curing animals with plants: traditional usage in Tuscany (Italy). *J. Ethnopharmacol.* 78, 171–191.
- van Valkenhoef, G., Lu, G., de Brock, B., Hillege, H., Ades, A.E., Welton, N.J., 2012. Automating network meta-analysis. *Res. Synth. Methods* 3, 285–299.
- Vesterinen, H.M., Johnson, P.I., Koustas, E., Lam, J., Sutton, P., Woodruff, T.J., 2013. In support of EHP's proposal to adopt the ARRIVE guidelines. *Environ. Health Perspect.* 121, A325-A325.
- Vogl, C.R., Vogl-Lukasser, B., Walkenhorst, M., 2016. Local knowledge held by farmers in Eastern Tyrol (Austria) about the use of plants to maintain and improve animal health and welfare. *J. Ethnobiol. Ethnomed.* 12, 40.