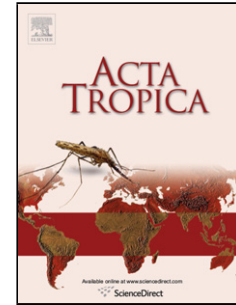


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Invited Review

Control of biting lice, Mallophaga – A review

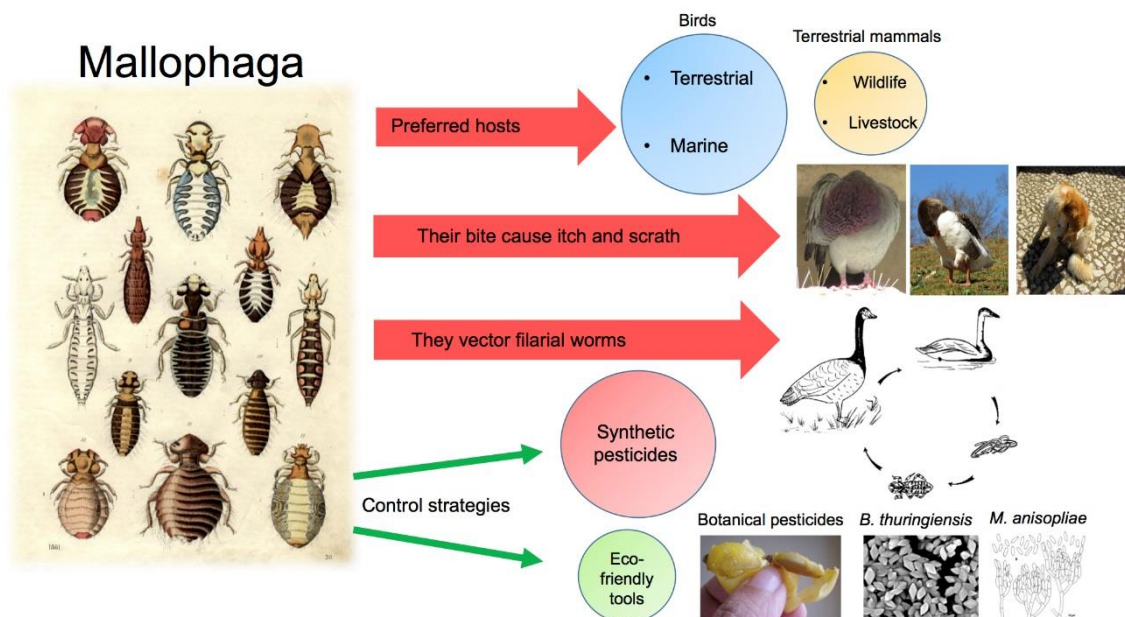
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Graphical abstract



Highlights

- Mallophaga are common parasites of terrestrial and marine birds, as well as livestock and pets
- We reviewed current knowledge on the various strategies available for Mallophaga control
- Their control is still anchored to the employ of synthetic insecticides used to control primary pests of livestock
- Entomopathogenic fungi and *Bacillus thuringiensis* preparations have been also employed
- Botanical pesticides included products from two Meliaceae, neem and *Carapa guianensis*

Abstract

The chewing lice (Mallophaga) are common parasites of different animals. Most of them infest terrestrial and marine birds, including pigeons, doves, swans, cormorants and penguins. Mallophaga have not been found on marine mammals but only on terrestrial ones, including livestock and pets. Their bites damage cattle, sheep, goats, horses and poultry, causing itch and scratch and arousing phthiriasis and dermatitis. Notably, Mallophaga can vector important parasites, such as the filarial heartworm *Sarconema eurycerca*. Livestock losses due to chewing lice are often underestimated, maybe because farmers notice the presence of the biting lice only when the infestation is

too high. In this review, we examined current knowledge on the various strategies available for Mallophaga control. The effective management of their populations has been obtained through the employ of several synthetic insecticides. However, pesticide overuse led to serious concerns for human health and the environment. Natural enemies of Mallophaga are scarcely studied. Their biological control with predators and parasites has not been explored yet. However, the entomopathogenic fungus *Metarhizium anisopliae* has been reported as effective *in vitro* and *in vivo* experiments against *Damalinia bovis* infestation on cattle. Furthermore, different *Bacillus thuringiensis* preparations have been tested against Mallophaga, the most effective were *B. thuringiensis* var. *kurstaki*, *kenyae* and *morrisoni*. Lastly, plant-borne insecticides have been evaluated against Mallophaga. Tested products mainly contained bioactive principles from two Meliaceae, *Azadirachta indica*, and *Carapa guianensis*. High efficacy of neem-borne preparations was reported, leading to the development of several products currently marketed. Overall, our review highlighted that our knowledge about Mallophaga vector activity and control is extremely patchy. Their control still relied on the employ of chemical pesticides widely used to control other primary pests and vectors of livestock, such as ticks, while the development of eco-friendly control tool is scarce. Behavior-based control of Mallophaga, using pheromone-based lures or even the Sterile Insect Technique (SIT) may also represent a potential route for their control, but our limited knowledge on their behavioral ecology and chemical communication strongly limit any possible approach.

Keywords: bird lice; biting lice; biopesticide; eco-friendly control; Phthiraptera

Introduction

The Mallophaga, commonly known as chewing lice, constitute an order that includes around 2500 species of insects. The name Mallophaga comes from Greek *mallos* = wool, hair; *phagein* = feeding by gnawing (Mehlhorn et al. 2012). They are ectoparasites of birds and, to a lesser extent, of mammals. Rarely, they can be found on stenothermic insects (Séguy 1944; Saxena and Agarwal 1983). The Mallophaga order is composed of three suborders, Amblycera, Ischnocera, and Rhyncophthirina (Masutti and Zangheri 2001). The species included in the two most representative suborders, Amblycera and Ischnocera, show opposite behaviors. Amblycera are nimble and agile, while Ischnocera are slow and sluggish, and rarely abandon their hosts. The chewing lice cannot live more than 3-6 days without the host (Grandi 1951).

Birds can harbor many Mallophaga genus, major ones include *Lipeurus*, *Cuclotogaster* and *Menacanthus*. All of them generally live on poultry, even if minor genera, such as *Gonioides* and *Menopon*, can live on pigeons, doves, ducks, game birds, and even birds of prey (Emerson 1954; Ash 1960; Clay 1951, 1976; Hill and Tuff 1978; Price and Clayton 1983; Urquhart et al. 1987) (Figure 1). Mallophaga are also known as “pollen lice”, since one of the most common species, *Menopon gallinae* Linnaeus lives at the base of chicken feathers (Masutti and Zangheri 2001). Young birds are generally the most suffering subjects. Otherwise, also adults can be very pained. They are not able to rest, often their weight goes down day by day. Often, depression in eggs production is recorded because of heavy infestations (Urquhart et al. 1987).

Mallophaga are common also on marine birds (Cheng 1976). Generally, penguins host two genera of Ischnocera, *Austrogonoides* and *Nesiotinus*. They live in

penguins' feather coat that is water repellent and contains air around the body to prevent heat loss. Therefore, insects can live always in a terrestrial environment and they do not need adaptations for marine life (Cheng 1976). On the other hand, pelicans can harbor both Ischnocera and Amblycera species, with special reference to *Pectinopygys* spp. for the first suborder, *Colpocephalum* sp. and *Piagetiella* sp. for the second one. The genus *Pectinopygus* is also hosting by cormorants. Shags usually harbor also Amblycera, such as *Eidmaniella* sp. (Cheng 1976). Similarly, gulls can be parasitized by Ischnocera (*Quadriceps* sp. and *Saemundssonina* sp.), as well as Amblycera (*Actornithophilus* sp. and *Austromenopon* sp.). Furthermore, many other marine birds usually host chewing lice, such as puffins, terns, albatrosses, and gannets (Cheng 1976), while Mallophaga have been not reported on marine mammals (Cheng 1976).

The suborder Ischnocera includes several parasites of terrestrial mammals (Emerson and Price 1983; Mey 1988; Masutti and Zangheri 2001). A good example is *Trichodectes canis* Degeer, which causes cutaneous crust and hair loss in dogs. Especially, this species can be found in dog breeds that possess long ears (e.g., basset and spaniel). The ears are a suitable habitat for the multiplication of the pest (Urquhart et al. 1987). In cats, the same symptoms are caused by *Felicola subrostratus* Burmeister. This pest is typical of longhaired breeds, because they have more difficulties in grooming than shorthairs (Urquhart et al. 1987). *Damalinia (Bovicola) bovis* Linnaeus lives on ungulates, and arouses cutaneous irritations, itch, and hair removal in various parts of the body (Masutti and Zangheri 2001). It prefers the top of the head, neck and shoulders because these places are very difficult to reach by cattle tooth. The intense pruritus can cause self-inflicted injury by scratching (Urquhart et al. 1987). *Damalinia (Bovicola) ovis* Schrank is the cause of phthiriasis in sheep. It can

infest the whole body of the animal and for this reason it is called the “body louse” (Urquhart et al. 1987). *D. ovis* is not the only species that infest sheep. *Linognathus pedalis* Osborn, also called the “foot louse”, prefers the lower region of sheep hind legs. However, *D. ovis* is more active than this pest. Both are susceptible to high temperature and high percentage of humidity (Urquhart et al. 1987). Likewise, *Damalinia (Bovicola) caprae* Gurlt arouses phthiriasis and wool loss in goats. Equines are parasitized by *Werneckiella equi* Linnaeus, which causes itch and dermatitis (Masutti and Zangheri 2001).

Generally, the typical habitat of Mallophaga is difficult to reach by birds’ beak and mammals’ tooth, making their removal extremely difficult for the selected host. For instance, *Tetrophthalamus* sp. lives inside the membranous sac of the pelican’s lower jaw, while *Neocolpocephalum flavescens* Nitzsch penetrates inside the barrel of bird feathers (Grandi 1951).

The Mallophaga diet is mainly composed of necrotic fragments, epidermal peeling, hairs, feathers, egg corion, exuviae and sebaceous secretions from their hosts (Grandi 1951; Urquhart et al. 1987). The hematophagy is not typical for these insects, but sometimes it has been observed, particularly in the *Amblycera* species (Grandi 1951; Urquhart et al. 1987).

The reproduction can be sexual or thelytokous parthenogenesis, it depends on species. The eggs, very large, can reach one third of the length of female body and they can be hatching in mass or alone. Generally, the eggs, that are commonly called “nits”, are glued to host feathers or hairs (Grandi 1951; Urquhart 1987). During their life span, about one month, females lay among 200-300 operculate eggs (Urquhart et al. 1987). The postembryonic development takes place in three nymphal instars during a 2–3-

week period, and with the third molting insects reach the adult stage. The time that occurs to have the whole cycle from nit to adult is about 2-3 weeks (Grandi 1951; Urquhart et al. 1987).

Mallophaga do not result very dangerous for vertebrates in which they live. Otherwise, they can open entryways for many microorganisms, such as pathogenic bacteria and tapeworms. Infested animals try to get rid of these insects making dust bathing and brushing off ant's nests (Grandi 1951). Generally, heavily infested animals result subjected to anemia, and only heavy infestations can have strong economic impacts on livestock productions. Otherwise, the economic losses due to Mallophaga are often underestimated (Campbell et al. 2001). Animals diet can define the level of infestation. A low-protein alimentation breaks down cattle resistance to parasites (Campbell et al. 2001). The infestation level can be also determinate by the season. Intensive farming favors infection among animals and the situation gets worse in winter (Masutti and Zangheri 2001) and spring and decreases in summer (Arundel and Sutherland 1988). This fluctuation in Mallophaga populations may be due to high summer temperatures, solar radiation, heavy rainfall and to sharing effects for livestock animals (Arundel and Sutherland 1988).

Mallophaga as vectors of filarasis and fowl cholera

The filarial heartworm *Sarconema eurycerca* Wehr, belongs to the Dipletalonematidae family, and parasitized waterfowls (Seegar et al. 1976). An intermediate host for the life cycle of this nematode is the chewing lice *Trinoton anserinum* Fabricius that plays the role of heartworm vector (Seegar et al. 1976). A

dissection of 89 Mallophaga collected from different individuals of *Cygnus olor* Gmelin, the mute swan, and *Cygnus columbianus columbianus* Ord, the whistling swan, showed that in the midgut of the 66% of *T. anserinum* a fresh blood meal was found. This has been surprising since historically, it has been thought that chewing lice did not feed routinely on blood, but only occasionally. Therefore, they were not deeply considered as potential vectors of parasites and pathogens, leading to underestimated losses due to their feeding activity (Seegar et al. 1976). Furthermore, researchers found microfilariae larvae in 39 of the 89 *T. anserinum* above mentioned. More than one developmental stage of *S. eurycerca* was found in the 62% of infected chewing lice examined. Microscopic analysis illustrated that infective nematode larvae were very active, moving back and forth between lice head and thorax (Seegar et al. 1976). These results highlight that *T. anserinum* is a natural vector of the filarial heartworm.

Later, Bartlett and Anderson (1987) studied the development of *Pelecitus fulicaeatrae* Diesing in the third stage in the amblyceran chewing louse *Pseudomenopon pilosum* Scopoli, as well as the biting-lice mediated transmission of this avian filarioid worm in coots. The microfilariae and developing first-stage larvae have been detected in nymphs and adults of *P. pilosum*, while third-stage larvae have been found only in adult insects (Bartlett and Anderson 1987). Lastly, Bartlett (1993) reported that Amblycera and Ischnocera can act as vectors of *Eulimdana* spp. (Nematoda: Filarioidea) in charadriiform birds, pointing out very short reproductive periods in adult worms.

Besides the ability of Mallophaga to vector filarial worms, it should be also mentioned that live and virulent *Pasteurella multocida*, the agent of avian or fowl cholera, was found in the gut of *Menacanthus stramineus* Nitzsch and *M. gallinae* fed on the blood of hens affected with fowl cholera; it has been observed that *P. multocida*

appeared in the gut of Mallophaga seven hours after experimental infection of hens on which they were feeding (Derylo 1970).

Control of Mallophaga

Cattle

Mallophaga control is possible using different kinds of insecticides, including topically applied formamidines, organophosphates and synthetic pyrethroids (White et al. 2007). Current knowledge about the efficacy of different insecticides tested against Mallophaga species is summarized in Table 1. Campbell et al. (2001) tested the efficacy of different insecticidal formulations on *D. bovis* control, including permethrin (DeLice®), a pyrethroid that was used both pour-on and spray, with or without its synergist piperonyl butoxide (PBO). They achieved good results in Mallophaga control, but only if the applications are more than one. On the other hand, other insecticides, such as Eprinex®, Ivomec®, Dectomax®, Cydectin® and Phoenectin®, all endectocides, have been found very efficient with only one application (Campbell et al. 2001).

Against *D. bovis*, it was also tested the efficacy of doramectin, an insecticide derived from ivermectin. Rooney et al. (1999) demonstrated that doramectin is very efficient if used as pour-on formulation against a variety of cattle parasites. It also exhibits a good residual efficacy (Lloyd et al. 2001). The persistent efficacy is a very important factor in the selection of the treatment against parasites infestation. In fact, it is fundamental to prevent the re-infestation of treated cattle, that can be caused by local

management and cattle processing practices (Lloyd et al. 2001). In this study, doramectin was administered topically at the dose of 500 µg/kg body weight. The results suggest that this insecticide had a period of residual efficacy more or equal to 63 days. The duration of egg stage in *D. bovis* was lower than 63 days; it has been also noted that doramectin could provide effective control of the emerging nymphs (Lloyd et al. 2001).

White et al. (2007) investigated the efficacy of spinosad used topically for *D. bovis* control; the efficacy of spinosad was compared to commercially available coumaphos and cyfluthrin. Spinosad had a very low mammalian toxicity and a strong insecticide effect against ectoparasites of economic importance to the beef and dairy industries (Davey et al. 2001; Kirst et al. 2002). In the study by White et al. (2007), animals were divided in four groups. The group A was formed by cattle that received 0.04% of spinosad applied topically as a whole body spray at a volume of 1.9 L per head. The animals of group B were treated with 2.5% of pour-on spinosad applied along the dorsal mid-line at the rate of 4 mL per 50 kg body weight. The cattle of the group C were topically treated with 0.03% of coumaphos applied as whole body spray, at a volume of 1.9 L per head. The animals belonged to Group D received the application of cyfluthrin 1 % applied as a pour-on at a maximum volume of 2.0 mL per 25-90 kg body weight. Group E was composed by untreated cattle; it was used as the negative control group (White et al. 2007). Results showed that a single treatment with spinosad 0.04% applied topically is highly efficient ($\geq 99\%$) against *D. bovis* for at least 8 weeks. The treatment with coumaphos used topically was less efficient, providing only 3-4 weeks of control. Spinosad pour-on application provided excellent control results, for up to 8

weeks; this control level is quite the same to the synthetic pyrethroid cyfluthrin (White et al. 2007).

Goats and sheep

Mallophaga of *Damalinia* genus represent a considerable problem for small stock farmers. Researchers have reported that the sheep chewing louse, *D. ovis* is not host specific and it can parasitize both sheep and goats. *D. caprae* instead can be hosted only by goats (Hallam 1985). These insects can lead to reduced wool production and decrease in quality of sheep and goat wool (Fourie et al. 1995). Usually, *Damalinia* sp. control is effectuated by spraying insecticides on the goats' body. This practice could be problematic in cold and windy days because predisposes animals to pneumonia (Bates et al. 2001). The control treatments are generally more effective immediately after shearing (Medley and Drummond 1963; Chamberlain and Hopkins 1971).

Flumethrin, a synthetic pyrethroid, is known for its good acaricidal action on cattle ticks. It has been reported as very effective on two Mallophaga species, *D. bovis* and *D. ovis* (Garg et al. 1998). The effectiveness of this pyrethroid was tested also against *D. caprae*, which naturally parasitizes goats (Garg et al. 1998). The animals were treated with flumethrin at a dose rate of 1 mg/kg body weight. The product was applied pour-on as one continuous strip all along the mid-dorsal line of goats. The results demonstrated that flumethrin had a very good insecticide action also on *D. caprae*, protecting treated goats for a period of almost 42 days. A similar protection period has been reported in sheep against *D. ovis*, and in cattle against *D. bovis* (Garg et al. 1998). No adverse effects have been found on treated goats (Garg et al. 1998).

Furthermore, Fourie et al. (1995) investigated the efficacy of the Insect Growth Regulator (IGR) diflubenzuron (625 g/1000 L water), against the body louse *D. limbata* on Angora goats. Results showed that with only one treatment the number of *D. limbata* nymphal stages per goat was reduced of about 90 % from week 6 to week 16 post treatment in animals constantly exposed to *D. limbata* re-infestation. In the same conditions, at week 24, the efficacy of diflubenzuron was still 88.5 %. On goats kept in quarantine conditions, the efficacy of this IGR was 100% at week 4 (Fourie et al. 1995). Therefore, diflubenzuron at this concentration can be considered as highly effective in eradicating *D. limbata* population from Angora goats kept in quarantine (Fourie et al. 1995).

Dogs

Dogs can be parasitized by *T. canis*, particularly if they are very old or very young, or if they are strongly debilitated (Pollimer et al. 2002). Infested dogs often suffer by alopecia and skin irritation. *T. canis* can be also a vector of dog pathogens, such as *Dipylidium caninum* Linnaeus. Pollimer et al. (2002) investigated the efficacy of fipronil against *T. canis*, both in laboratory and in field (Pollimer et al. 2002). Laboratory assays were conducted with different topical treatments evaluating the efficacy of Frontline® Spray, Frontline® Spot-On, and Frontline® Plus. No live *T. canis* lice were found on dogs that were subjected to the treatments above from days 2 to 63 (Pollimer et al. 2002). In the field, three different treatments, Bolfo® collar, Frontline® Spray, and Frontline® Spot-On, were evaluated. Their efficacy on day 2 were 98.5%, 99.6% and 98.3%, respectively. No health problems were found at the end

of both assays (Pollimer et al. 2002). It has been highlighted that Frontline® used topically is an optimal insecticide against *T. canis*, in addition the authors confirmed the efficacy of fipronil in dog pediculosis control (Pollimer et al. 2002).

Resistance to chemical insecticides

Since 1986, scientists become aware of a plausible resistance to synthetic pyrethroids (SP) manifested by chewing lice. In many cases, poor technique management explained the failures of treatments, but in other cases did not (Boray et al. 1988). Johnson et al. (1992) studied the efficacy of synthetic pyrethroid formulations against different strains of *D. ovis* with known SP resistance factor. Some Mallophaga strains were susceptible to the pour-on treatment, while others were resistant. This fact can be due to the annual applications of pour-on pyrethroids aimed at chewing lice eradication (Johnson et al. 1992). The problem of SP resistance concerns only the pour-on formulations, even though this treatment deposits more insecticide than a plunge or a shower dip (Johnson et al. 1990). This suggests that the spread and the rate of insecticide is determinant for the selection of resistance strains and for the failure of the treatment (Johnson et al. 1992). To the best of our knowledge, little knowledge is still available on this issue. Additional researchers are urgently required, as recently done for other important groups of arthropod vectors (see Hemingway and Ranson 2000; Benelli 2015a; Naqqash et al. 2016 for reviews).

Eco-friendly control of Mallophaga

Historically, the control of Mallophaga species mostly relied to the employ of synthetic pesticides (James 1999). However, nowadays is known that the use of chemicals should be reduced for many reasons. Insecticides may harm human and animal health, and they can produce residuals inside animal's body and products (Mehlhorn 2008). Furthermore, chemicals may have harmful impact on the environment and they can cause insecticides resistance (Briggs et al. 2006; Naqqash et al. 2016). In this scenario, research on the development of eco-friendly control tools against Mallophaga and other livestock ectoparasites is a priority.

However, natural enemies of Mallophaga are scarcely studied. To the best of our knowledge, their biological control with predators and parasites has not been explored yet. In the following sections, we discuss current knowledge available on the efficacy of different bio-pesticides, including *Bacillus thuringiensis* Berliner preparations and entomopathogenic fungi, e.g., *Metarhizium anisopliae* (Metchnikoff) Sorokin, as well as botanical pesticides, in the fight against Mallophaga.

Bacillus thuringiensis

Current knowledge about the toxicity of different *B. thuringiensis*-based preparations against Mallophaga species is summarized in Table 1. The first report concerning the efficacy of *B. thuringiensis* preparations on the poultry lice was by Hoffman and Gingrich (1968), showing that *B. thuringiensis* used as a wettable powder was efficient against the shaft lice, *M. gallinae*, the wing lice, *Lipeurus caponis* Linnaeus, and the chicken body lice, *M. stramineus*. Later, the insecticidal activity of the δ -endotoxin of *B. thuringiensis* var. *kurstaki* was assessed on four species of chewing

lice belonging to the *Damalinia* genus, i.e. *D. bovis*, *D. limbata*, *D. ovis*, and *Damalinia crassipes* Redow (Gingrich et al. 1974).

Moreover, Lonc et al. (1986) focused on the efficacy of two *B. thuringensis* formulations, Dipel and Bacilan, against *M. gallinae* and *E. stramineus*. The results of this *in vitro* study suggested that both chewing lice species were susceptible to the two formulations. Dipel was efficient at low concentration, with a LD₅₀ of 2.2% against *E. stramineus* and 2.9% against *M. gallinae*. On the other hand, Bacilian was efficient at higher concentrations, with a LD₅₀ of 2.9% and 3.2%, respectively (Lonc et al. 1986). Dipel formulation at 10% led to 100% of chewing lice mortality in 5-6 h from the contact with the preparation. The same result has been also achieved with Bacilan formulation, but only after 8-9 h. When Dipel preparations were tested at concentration lower than 10%, a second treatment was needed to reach the same level of efficacy. In this scenario, both Dipel and Bacilan can be recommended for chewing lice control (Lonc et al. 1986).

Lonc and Lachowicz (1987), conducted *in vitro* assays to evaluate the *M. gallinae* susceptibility to 10 subspecies of *B. thuringensis* (subsp. *thuringiensis*, subsp. *kurstaki*, subsp. *aizawai*, subsp. *tolworthi*, subsp. *pakistani*, subsp. *israelensis*, subsp. *kenyae*, subsp. *galleriae*, subsp. *morrisoni*, and susp. *finitimus*). Results suggested that the most efficient subspecies were *B. thuringensis* subsp. *kurstaki*, *finitimus*, *kenyae* and *morrisoni*. For *B. thuringensis* var. *kurstaki*, *kenyae* and *morrisoni*, after 20 h from the start of the experiments, *M. gallinae* mortality was higher than 90%. On the other hand, the *B. thuringensis* subspecies *galleriae* and *aizawai* showed the lowest efficacy (Lonc and Lachowicz 1987).

Drummond et al. (1992) tested the efficacy of 22 *B. thuringensis* strains against the sheep louse, *D. ovis*. Only 4 of them resulted toxic. They were *B. thuringensis* var. *kurstaki*, var. *toumanoffi*, var. *thuringensis* and var. *indiana*. The most toxic strain was *B. thuringensis* var. *kurstaki*, which caused $93 \pm 3\%$ mortality after 72 hours at 34°C and 70% R.H. (Drummond et al. 1992) (Table 1).

Entomopathogenic fungi

The use of entomopathogenic fungi is widely spread in biological control (Gillespie and Moorhouse 1989). Approximately 750 species of fungi are known to be pathogens of arthropods (Kirk et al. 2001). They result suitable for Integrated Pest Management programs, due to their low toxicity to vertebrates and high specificity (Briggs et al. 2006). *M. anisopliae* is an entomopathogenic fungus widely used against crop pests in Asia and Africa (Green Guard™ and Green Muscle®) (Arthurs and Thomas 2001), as well against arthropod vectors of public health importance, including some species of mosquitoes (Scholte et al. 2007; Amerasan et al. 2016), ticks (Kaaya and Hassan 2000; Gindin et al. 2002) and mites (Brooks et al. 2004). To the best of our knowledge, only a study focused on the potential of *M. anisopliae* against Mallophaga species.

Briggs et al. (2006) tested the efficacy of *M. anisopliae* against *Damalinia bovis* (L.) infestation on cattle (Table 1). Results suggest that this fungus is effective both *in vitro* and *in vivo* experiments. At 1×10^8 conidia ml⁻¹ mean infection was of 71%, if tested *in vitro*, and 73% if tested *in vivo* (Briggs et al. 2006). However, more studies are necessary in relation to find a formulation of the fungal spores that can best penetrate

the hair coat of cattle (Briggs et al. 2006). Furthermore, little is known about the possible presence of inhibitory skin secretions and competitive skin microflora (Briggs et al. 2006).

Herbal formulations

In the last two decades, a huge number of herbal preparations, including plant extracts, essential oils, and selected pure constituents, have been tested against more than 400 species of arthropod vectors of high economic importance (e.g. Leung 1985; Brown 1996; Oladimeji et al. 2000; Schmutterer 2002; Fajimi and Taiwo 2005; Mehlhorn et al. 2005, 2006, 2010; Amer and Mehlhorn 2006; Heukelbach et al. 2006; Abdel-Ghaffar and Semmler 2007; Kim et al. 2007; Athanasiadou et al. 2007; Bäumler 2007; Abdel-Ghaffar et al. 2008a,b, 2009; Mehlhorn 2008; Khater et al. 2009; Michaelakis et al. 2009; Khater et al. 2013; Benelli 2015b; Pavela and Benelli 2016a,b; Tabari et al. 2017).

Some of the plant-borne components have a very strong activity against arthropod pests and vectors (Schmahl et al. 2010). However, most of the current research on plant-based insecticides focused on their toxicity on major arthropod vectors, mainly mosquitoes (see reviews by Benelli 2015b; Pavela 2015; Benelli and Mehlhorn 2016) and ticks (see reviews by Benelli et al. 2016 and Pavela et al. 2016), while a strictly limited number of studies focused on Mallophaga control. Our literature survey showed that tested products mainly contained bioactive principles from two Meliaceae species, the neem tree, *Azadirachta indica* A. Juss (Meliaceae), and *Carapa guianensis* Aublet (Table 1).

Azadirachta indica extracts

The extracts of *A. indica*, usually known as neem tree, offer many possibilities of protection against pests and parasites that can threaten human and animal's health (Mulla and Su 1999; Schmutterer 2002; Lundh et al. 2005; Semmler et al. 2009; Seddiek et al. 2013; Benelli et al. 2017). The neem seeds contain more than 300 compounds, including important bioactive principles, such as azadirachtin A, salannin, nimbin, and nimbolide (Kraus 2002; Morgan 2004; Nicoletti et al. 2016). US EPA certified the use of cold-pressed neem oil as insecticide, reporting its repellent and antifeedant activities (EPA 2012). It can also affect ecdysteroid and juvenile hormone synthesis, leading to mortality in arthropod pests and vectors (Meurant et al. 1994; Sayah 2002; Benelli et al. 2016), including blood-sucking lice of humans (*Pediculus humanis capis* Linnaeus) and dogs (*Linognathus setosus* Olfers) (Mehlhorn et al. 2012).

Concerning the toxicity of neem-borne preparations against Mallophaga, Habluetzel et al. (2007) studied the efficacy of the botanical insecticide Neem Azal®, which contains 34% of azadirachtin A, against a Mallophaga hosted by goats, *D. limbata*. At the time of the experiments, this insecticide was diluted with tap water to have treatment solutions at concentrations of 650 ppm or 125 ppm of azadirachtin (Habluetzel et al. 2007). Results suggest that Neem Azal® at a concentration of 650 ppm reduced *D. limbata* density of 76-96% from weeks 2 to 18 post-treatment. At the lower concentration tested an insects' reduction of 60-92% was recorded from weeks 2 to 14 (Habluetzel et al. 2007). These results agreed with those reported in New Zealand (Heath et al. 1995) and Australia (Guerrini 2000) for *D. ovis* hosted by sheep.

Furthermore, results suggest that Neem Azal® treatments influence the oviposition and oogenesis of *D. limbata* females. *Damalina* ovaries resulted morphologically modified. Loss in cellular structure was observed. It can suggest a direct cytotoxic effect of neem extracts (Habluetzel et al. 2007).

Schmahl et al. (2010) investigated the effects of neem seed extract diluted with tap water against *W. equi* (Table 1). One-hundred heavy infested horses were washed with a 1:20 water diluted solution. After drying, many dead Mallophaga became visible and they were brushed away (Schmahl et al. 2010). The treated horses grazing in the meadow were immune by *W. equi* for at least 4 h (Schmahl et al. 2010).

Furthermore, Mehlhorn et al. (2012) focused on the effect of two neem seed preparations, Mite Stop® and Wash Away Dog against the chewing lice of dogs, *T. canis*. Large dogs were treated with Mite Stop® diluted at a concentration of 1:33 with tap water. Small dogs were treated with Wash Away Dog containing 10% of neem seed extract and 90% of fine shampoo (Mehlhorn et al. 2012). In both cases, the products had been left onto dogs' hair for 20 min, then they were washed away with normal tap water. The results suggest that only one application of these products can be enough to kill both the motile stage and those developing inside eggs (Mehlhorn et al. 2012).

The Mite Stop® formulation was also tested against the chicken chewing lice, *M. gallinae*, *L. caponis* and *Columbicola* sp. It was diluted at a concentration of 1:33 in tap water and used as spray (Al-Quaraisy et al. 2012). Three days after treatments a screening was conducted to detect living insects. Chickens that showed additional infestations were treated again dipping them into the same prepared until they were completely wet. Results suggest that one hour after the dipping treatment no live

nymphs and adults were found on chickens. The same results were recorded four weeks after treatments, either if the test was repeated or not (Al-Quaraisy et al. 2012).

Carapa guianensis extracts

The extracts of Andiroba tree, *C. guianensis* Aubl., has been reported as promising to formulate products against *F. subrostratus*, a species parasitizing domestic cats. Indeed, De Barros et al. (2012), conducted *in vitro* experiments to evaluate the efficacy of *C. guianensis* seed oil at different concentrations. No living chewing lice were found post-treatment with concentrations of 50 or 100% of *C. guianensis* seed oil during the first hour of the test. Same results were recorded at the concentrations of 25 and 10% of the product at the end of the third hour from the treatment (De Barros et al. 2012). The andiroba seed oil showed interesting proprieties to control Mallophaga infestations. However, further studies to confirm this potential are necessary, with special reference *in vivo* experiments at lower doses in real field conditions.

Camphor oil

The lousicidal activity of camphor oil has been tested on the slender pigeon louse, *Columbicola columbae* Linnaeus, comparing its toxicity with d-phenothrin and deltamethrin (Khater et al. 2014). Pigeons were sprayed with 8% camphor oil + Tween 80, 9% d-phenothrin, or 0.005% deltamethrin (50 mg/L or 1 ml/L). The prevalence of lice infestations was 85% (340 out of 400, 550 ± 50 louse/pigeon, range of infestation: 100–800). *In vivo* assays showed that *C. columbae* infestation was eliminated seven

days post-treatment with camphor oil (8%), 14 days post-treatment with d-phenothrin (9%), and 21 days post-treatment with deltamethrin (0.005%). While temporary coughing, sneezing, and ocular inflammations without dermatitis were observed among pigeons sprayed with deltamethrin, camphor oil showed a promising potential for the development of a new and safe product for controlling *C. columbae* (Khater et al. 2014).

Conclusions

The Mallophaga order is composed of insects that infest many animal species, including livestock and pets. In the past, economic losses due to chewing lice were very underrated. Nowadays, it has been confirmed that Mallophaga can be vectors of important parasites, such as the filarial heartworm, *Sarconema eurycerca*. For this reason, effective, reliable and eco-friendly control methods are urgently required. Classic control programs rely to synthetic insecticides to fight chewing lice. Results suggest that their efficacy can be so strong. However, these pesticides are often toxic to human and animal health, and to the environment. They also rapidly lead to the development of resistance on targeted pest populations.

On the other hand, our literature survey experience a severe lack of knowledge about potential eco-friendly methods of Mallophaga control. Indeed, the natural enemies of Mallophaga are scarcely studied. Their biological control with predators and parasites has not been explored yet. Among biocontrol agents, the entomopathogenic fungus *M. anisopliae* has been reported as effective *in vitro* and *in vivo* experiments on *D. bovis* infestation on cattle. Moreover, different *Bacillus thuringiensis* strains have been tested against Mallophaga, and the most effective ones were *B. thuringiensis* var. *kurstaki*,

kenyae and *morrisoni*. As regard to green acaricides, few herbal formulations have been tested on Mallophaga, they mainly contained bioactive principles from two Meliaceae, *A. indica*, and *C. guianensis*. While the latter was found effective at rather high concentrations, high efficacy of neem-borne herbal preparations was reported, leading to the development of several products currently marketed.

Overall, our review highlighted that our knowledge about Mallophaga vector activity and control is extremely patchy. Their control still relied to the employ of chemical pesticides widely used to control other primary pests and vectors of livestock, such as ticks, while the development of eco-friendly control tool is scarce. Behavior-based control of Mallophaga, using pheromone-based lures or even the Sterile Insect Technique may also represent a potential route for their control, but our limited knowledge on their behavioral ecology and chemical communication strongly limit any possible approach. In this framework, a careful improvement of mass-rearing techniques of these insects can be also extremely helpful to boost basic research on them (Saxena and Agarwal 1983).

Conflict of Interest

The Authors declare no competing interests.

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Captions

Figure 1. (a) Mallophaga species from "*Buch Der Welt*" by Carl Hoffmann, 1863. Two examples of Mallophaga infesting birds of prey: (b) *Craspedorhynchus* sp., and (c) *Degeeriella fulva* Giebel, C. (photo courtesy of Dr. Pavel Krásenský, www.macrophotography.cz).

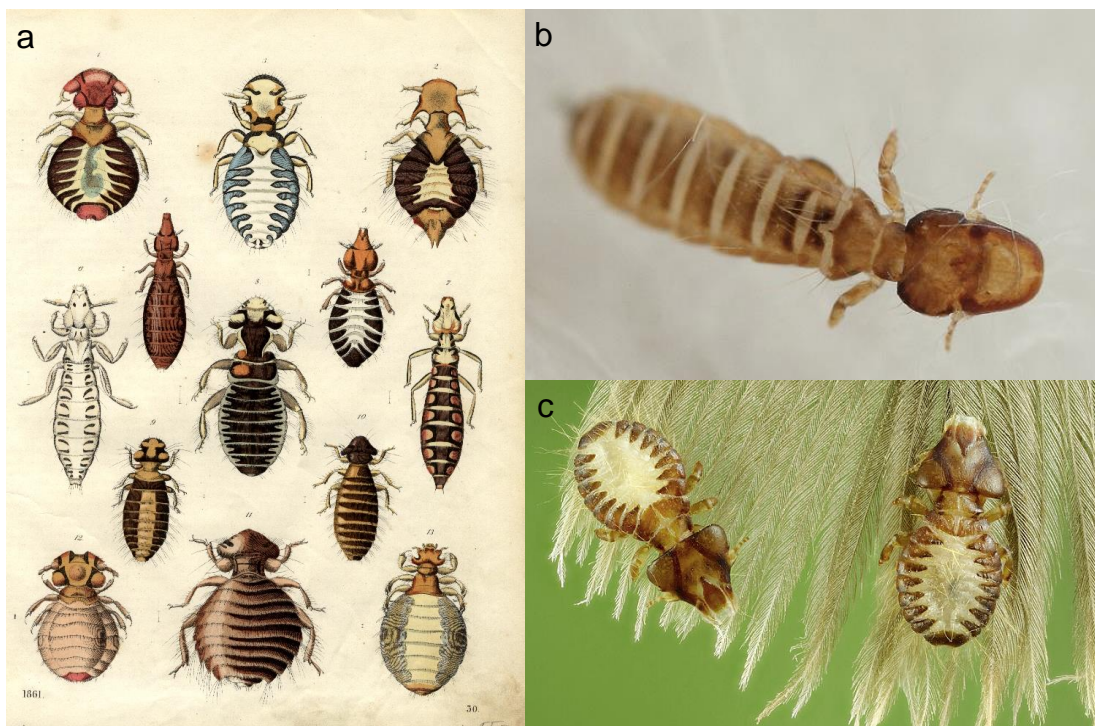


Table 1. Current knowledge about the efficacy of different insecticides tested against Mallophaga species.

Target	Treatment	Efficacy	References
<i>Menopon</i> <i>gallinae</i>	Dipel solution 10%	100% mortality after 5-6 h LC ₅₀ = 2.9%	Lonc et al. (1986)
<i>Eomencanthus</i> <i>stramineus</i>	Dipel solution 10%	100% mortality after 5-6 h LC ₅₀ = 2.2 %	Lonc et al. (1986)
<i>Menopon</i> <i>gallinae</i>	Bacilian solution 10%	100% mortality after 8-9 h LC ₅₀ = 3.2%	Lonc et al. (1986)
<i>Eomencanthus</i> <i>stramineus</i>	Bacilian solution 10%	100% mortality after 8-9 h LC ₅₀ = 2.9%	Lonc et al. (1986)
<i>Menopon</i> <i>gallinae</i>	<i>Bacillus</i> <i>thuringensis</i> var. <i>kurstaki</i> 23 x 10 ⁶ spores/ml	93% mortality after 20 h	Lonc and Lachowicz (1987)
<i>Menopon</i> <i>gallinae</i>	<i>Bacillus</i> <i>thuringensis</i> var. <i>finitimus</i> 23 x 10 ⁶ spores/ml	89% mortality after 20 h	Lonc and Lachowicz (1987)
<i>Menopon</i> <i>gallinae</i>	<i>Bacillus</i> <i>thuringensis</i> var. <i>kenyae</i> 23 x 10 ⁶	91% mortality after 20 h	Lonc and Lachowicz (1987)

		spores/ml	
<i>Menopon</i>	<i>Bacillus</i>	63% mortality after 20 h	Lonc and
<i>gallinae</i>	<i>thuringensis</i> var.		Lachowicz
	<i>thuringensis</i> 23 x		(1987)
	10 ⁶ spores/ml		
<i>Menopon</i>	<i>Bacillus</i>	74% mortality after 20 h	Lonc and
<i>gallinae</i>	<i>thuringensis</i> var.		Lachowicz
	<i>aizawai</i> 23 x 10 ⁶		(1987)
	spores/ml		
<i>Menopon</i>	<i>Bacillus</i>	77% mortality after 20 h	Lonc and
<i>gallinae</i>	<i>thuringensis</i> var.		Lachowicz
	<i>tolworthi</i> 23 x 10 ⁶		(1987)
	spores/ml		
<i>Menipon</i>	<i>Bacillus</i>	71% mortality after 20 h	Lonc and
<i>gallinae</i>	<i>thuringensis</i> var.		Lachowicz
	<i>pakistani</i> 23 x 10 ⁶		(1987)
	spores/ml		
<i>Menopon</i>	<i>Bacillus</i>	74% mortality after 20 h	Lonc and
<i>gallinae</i>	<i>thuringensis</i> var.		Lachowicz
	<i>israeliensis</i> 23 x		(1987)
	10 ⁶ spores/ml		
<i>Menopon</i>	<i>Bacillus</i>	53% mortality after 20 h	Lonc and
<i>gallinae</i>	<i>thuringensis</i> var.		Lachowicz
	<i>galleriae</i> 23 x 10 ⁶		(1987)

		spores/ml	
<i>Menopon</i> <i>gallinae</i>	<i>Bacillus</i> <i>thuringensis</i> var. <i>morrisoni</i> 23 x 10 ⁶ spores/ml	92 % mortality after 20 h	Lonc and Lachowicz (1987)
<i>Damalinia</i> <i>ovis</i>	<i>Bacillus</i> <i>thuringensis</i> var. <i>kurstaki</i> strain WB3S-16	LC ₅₀ = 0.131 mg	Drummond et al. (1992)
<i>Damalinia</i> <i>ovis</i>	<i>Bacillus</i> <i>thuringensis</i> var. <i>kurstaki</i> strain HD-263	LC ₅₀ = 0.253 mg	Drummond et al. (1992)
<i>Damalinia</i> <i>ovis</i>	<i>Bacillus</i> <i>thuringensis</i> var. <i>kurstaki</i> strain HD-1	LC ₅₀ = 0.523 mg	Drummond et al. (1992)
<i>Damalinia</i> <i>ovis</i>	<i>Bacillus</i> <i>thuringensis</i> var. <i>kurstaki</i> strain HD-721	LC ₅₀ = 2.601 mg	Drummond et al. (1992)
<i>Damalinia</i> <i>limbata</i>	Diflubenzuron 500-625 g/1000 L water	90% mortality from week 6 to 16 (no quarantine) 85.5 mortality at week 24 (no quarantine)	Fourie et al. (1995)

100% mortality at week 4 (quarantine)			
<i>Damalinia caprae</i>	Flumethrin pour-on 1 mg/kg wt.	100% mortality for almost 42 days	Garg et al. (1998)
<i>Damalinia bovis</i>	Permethrin 1.0% EC+5% PBO 3 ml/45.4 kg wt.	77.3% mortality at week 8	Campbell et al. (2001)
<i>Damalinia bovis</i>	Permethrin 1.0% EC+1% PBO 15 ml/45.4 kg wt.	97% mortality at week 8	Campbell et al. (2001)
<i>Damalinia bovis</i>	Eprinex® 5 mg/ml A.I., 1 ml/10 kg wt. Eprinomectin pour-on ^a	100% mortality at week 8	Campbell et al. (2001)
<i>Damalinia bovis</i>	Ivomec® 5 mg/ml A.I., 1 ml/10 kg wt. Ivermectin pour-on ^a	100% mortality at week 8	Campbell et al. (2001)
<i>Damalinia bovis</i>	Phoenectin® 5 mg/ml A.I., 1 ml/10 kg wt. pour-on ^{a,b}	100% mortality at week 8	Campbell et al. (2001)

<i>Damalinia</i>	Dectomax®	100% mortality at week 8	Campbell
<i>bovis</i>	5 mg/ml A.I., 1 ml/10 kg wt. Doramectin pour- on ^a		et al. (2001)
<i>Damalinia</i>	Cyductin®	100% mortality at week 8	Campbell
<i>bovis</i>	5 mg/ml A.I., 1 ml/10 kg wt. Moxidectin pour- on ^a		et al. (2001)
<i>Damalinia</i>	Pirimiphos-methyl	92.6% mortality at week 8	Campbell
<i>bovis</i>	27% ½ oz/ animal		et al. (2001)
<i>Damalinia</i>	Pirimiphos-methyl	94% mortality at week 8	Campbell
<i>bovis</i>	27% 1 oz/animal		et al. (2001)
<i>Damalinia</i>	Permethrin 8.5%	95% mortality at week 4	Campbell
<i>bovis</i>	water-base spray 2.5 ml in 2.5 l H ₂ O		et al. (2001)
<i>Damalinia</i>	Same rate as	95% mortality at week 4	Campbell
<i>bovis</i>	above but 10 ml in 2 l H ₂ O		et al. (2001)
<i>Damalinia</i>	7.4% Permethrin +	59.4% mortality at week 4	Campbell

<i>bovis</i>	7.4% PBO 2.5 ml in 2 l H ₂ O		et al. (2001)
<i>Damalinia bovis</i>	Permethrin 1% pour-on 2 ml/45.4 kg wt.	76.3% mortality at week 4	Campbell et al. (2001)
<i>Damalinia bovis</i>	Permethrin 10% pour-on 15 ml/45.4 kg wt.	79.17% mortality at week 4	Campbell et al. (2001)
<i>Damalinia bovis</i>	Permethin S 1% P + 1% PBO 15 ml/ 45.4 kg wt.	96.2% mortality at week 4	Campbell et al. (2001)
<i>Damalinia bovis</i>	Permethrin synergized 1% P + 1% PBO 15 ml/45.4 kg wt.	99.8% mortality at week 4	Campbell et al. (2001)
<i>Damalinia bovis</i>	Permethrin 5% pour-on 3 ml/45.4 kg wt.	62% mortality at week 8	Campbell et al. (2001)
<i>Damalinia bovis</i>	Permethrin 5% pour-on 3 ml/45.4 kg wt. ^c	85% mortality at week 8	Campbell et al. (2001)
<i>Damalinia bovis</i>	Pirimiphos-methyl 27% pour-on 20 ml/animal	99.5% mortality at week 8	Campbell et al. (2001)

<i>Damalinia</i>	Pirimiphos-methyl	80.5% mortality at week 8	Campbell
<i>bovis</i>	27% pour-on		et al.
	20 ml/animal ^c		(2001)
<i>Damalinia</i>	Pirimiphos-methyl	100% mortality at week 8	Campbell
<i>bovis</i>	27%		et al.
	10 ml/45.4 kg wt.		(2001)
<i>Damalinia</i>	Lambda	100% mortality at week 8	Campbell
<i>bovis</i>	cyhalothrin 1.0%		et al.
	microencapsulated		(2001)
	10 ml/head		
	<272 kg wt.,		
	15 ml/head		
	>272 kg wt.		
<i>Damalinia</i>	Lambda	100% mortality at week 8	Campbell
<i>bovis</i>	cyhalothrin 1.0%		et al.
	pour-on		(2001)
	10 ml/head		
	<272 kg wt.,		
	15 ml/head		
	>272 kg wt.		
<i>Damalinia</i>	Tetrachlorvinphos	100% mortality at week 8	Campbell
<i>bovis</i>	10% pour-on		et al.
	15 ml/45.4 kg wt.		(2001)
<i>Damalinia</i>	Tetrachlorvinphos	100% mortality at week 8	Campbell

<i>bovis</i>	10% pour-on		et al.
	15 ml/45.4 kg wt. ^c		(2001)
<i>Damalinia</i>	Tetrachlorvinphos	100% mortality at week 8	Campbell
<i>bovis</i>	10% pour-on		et al.
	7.5 ml/45.4 kg wt.		(2001)
<i>Damalinia</i>	Tetrachlorvinphos	100% mortality at week 8	Campbell
<i>bovis</i>	10% pour-on		et al.
	7.5 ml/45.4 kg wt. ^c		(2001)
<i>Damalinia</i>	Diflubenzuron	100% mortality at week 8	Campbell
<i>bovis</i>	3.0%		et al.
	3 ml/45.4 kg wt.		(2001)
<i>Damalinia</i>	Diflubenzuron	100% mortality at week 8	Campbell
<i>bovis</i>	3.0%		et al.
	4.5 ml/45.4 kg wt.		(2001)
<i>Damalinia</i>	Diflubenzuron	100% mortality at week 8	Campbell
<i>bovis</i>	3.0% + permethrin		et al.
	3%		(2001)
	3 ml/45.4 kg wt.		
<i>Damalinia</i>	Diflubenzuron	100% mortality at week 8	Campbell
<i>bovis</i>	3.0% + permethrin		et al.
	3% 4.5ml/45.4 kg		(2001)
	wt.		
<i>Damalinia</i>	Doramectin pour-	Period of residual efficacy \geq 63 days	Lloyd et al.
<i>bovis</i>	on 500 μ g/kg wt.		(2001)

<i>Trichodectes canis</i>	Frontline® Spray (lab) 0.25% (w/v) fipronil	100% mortality from week 2 to 63	Pollimer et al. (2002)
<i>Trichodectes canis</i>	Frontline® Spot-On (lab) 10 % (w/v) fipronil	100% mortality from week 2 to 63	Pollimer et al. (2002)
<i>Trichodectes canis</i>	Frontline® Plus (lab) 10% (w/v) fipronil + 9% (S)-methoprene	100% mortality from week 2 to 63	Pollimer et al. (2002)
<i>Trichodectes canis</i>	Frontline® Spray (field) 6 ml/kg	99.6 % on day 2 100% on day 28	Pollimer et al. (2002)
<i>Trichodectes canis</i>	Frontline® Spot-On (field) as per label	98.3% on day 2 100% on day 42	Pollimer et al. (2002)
<i>Trichodectes canis</i>	Bolfo® Collar (field) 45 g propoxur	98.5% on day 2	Pollimer et al. (2002)
<i>Damalinia bovis</i>	<i>Metarhizium anisopliae</i> 1 x 10 ⁸ conidia ml ⁻¹	Mean infection of 71% (<i>in vitro</i>) Mean infection of 73% (<i>in vivo</i>)	Briggs et al. (2006)
<i>Damalinia bovis</i>	Spinosad topical spray 25 g/L diluted with water	≥ 99% mortality for at least 8 weeks	White et al. (2007)

	to 0.04 % active ingredient		
<i>Damalina bovis</i>	Spinosad pour-on 25 g/L at 2 mg/kg wt.	Excellent control for up 8 weeks	White et al. (2007)
<i>Damalina bovis</i>	Coumaphos 0.03% topical treatment in a volume 1.9L/head	Excellent control for 3-4 weeks	White et al. (2007)
<i>Damalina bovis</i>	Cyfluthrin 1% pour-on in a volume of 2.0 mL per 25-90 kg wt.	Excellent control for up 8 weeks	White et al. (2007)
<i>Damalinea limbata</i>	Neem Azal® 650 ppm	76-96% mortality from week 2 to 18	Habluetzel et al. (2007)
<i>Damalinea limbata</i>	Neem Azal® 250 ppm	60-92% mortality from week 2 to 14	Habluetzel et al. (2007)
<i>Werneckiella equi</i>	Mite Stop® 1:20 diluted with tap water	After 3-4 days, Mallophaga infestation were highly reduced. A second treatment at week 4 was needed to prevent the infestation the next year	Schmahl et al. (2010)
<i>Trichodectes</i>	Mite Stop® 1:33	One application killed both the motile stages	Mehlhorn

<i>canis</i>	diluted with tap water (treatment for large dogs)	and eggs	et al. (2012)
<i>Trichodectes canis</i>	Wash Away Dog 10% neem seed extract + 90% fine shampoos (treatment for small dogs)	One application killed both the motile stages and eggs	Mehlhorn et al. (2012)
<i>Menopon gallinae, Lipeurus caponis, Columbicola sp.</i>	Mite Stop® 1:33 diluted with tap water	100% mortality at week 4	Al- Quraishy et al. (2012)
<i>Felicola subrostratus</i>	<i>Carapa guianensis</i> seed oil (doses: 10-25%, 50- 100%)	100% mortality in the first hour (dose: 50- 100%) 100% mortality in the third hour (dose: 10- 25%)	de Barros et al. (2012)
<i>Columbicola columbae</i>	Camphor oil	<i>In vivo</i> tests showed that <i>C. columbae</i> infestation was eliminated 7 days post- treatment with camphor oil (8%).	Khater et al. (2014)
<i>Columbicola</i>	d-phenothrin	<i>In vivo</i> tests showed that <i>C. columbae</i>	Khater et

<i>columbae</i>		infestation was eliminated 14 days post-treatment with d-phenothrin (9%).	al. (2014)
<i>Columbicola columbae</i>	Deltamethrin	<i>In vivo</i> tests showed that <i>C. columbae</i> infestation was eliminated 21 days post-treatment with Deltamethrin (0.005%).	Khater et al. (2014)

^a The five currently registered endectocides (macrocyclic lactones) for cattle lice control, West Central Research and Extension Center, University of Nebraska, North Platte, NE (Campbell et al. 2001).

^b Approved by Department of Health and Human Services, Food and Drug Administration as ivermectin, a generic copy of Merial ivermectin (Federal Register 1998, p. 160).

^c Two applications, the second 14 days after the first.