# ORIGINAL PAPER

# Observations on effects of a neem seed extract (MiteStop®) on biting lice (mallophages) and bloodsucking insects parasitizing horses

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Abstract The hair of 300 horses belonging to short hair and long hair races had been routinely treated during the last 3 years with a neem seed extract (MiteStop®) in order to kill mallophages (e.g., specimens of the genus Werneckiella). It was found that in all cases, a hidden infestation with these biting lice had existed, which became visible when the product (diluted 1:20 with tap water) was brushed onto the hair. The mallophages left the body surface and became visible as a fine "wooly looking" layer at the tips of the hair. Furthermore, this treatment stopped the forming of dandruff of the skin of the horses, which, in case of heavy mallophage infestations, had looked like being powdered. Another interesting result of the treatment was reported by the riders. They described that the product had a considerable repellent effect on bloodsucking tabanids, mosquitoes, ceratopogonids, simuliids, as well as on licking flies. This repellency effect

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H. Mehlhorn (⊠) Department of Parasitology, Heinrich Heine University Düsseldorf, Düsseldorf, Germany e-mail: Heinz.Mehlhorn@uni-duesseldorf.de was noted to last for up to 7 days if the horses were not washed.

# Introduction

The name of the specimens of the insect order Mallophaga comes from the Greek words for "wool eater," although they do not eat wool but live on the surface of the skin of mammalian animals and on the skin as well as among the feathers of birds, feed on skin scales and feather coverings, and/or even lick blood-being very host specific (Zlotorzycka et al. 1974). With respect to their living sites, there are two basic groups ("hairlings" and "featherlings"), which parasitize either the hair of mammalians or the feathers of birds. With respect to the arrangement of their chewing mouthparts (mandibles) and their paired, rather short antennae, there are two groups (suborders): Amblycera (they possess antennae mostly with five segments each lying in a groove along the lateral side of the broad head, and their mandibles bite horizontally), while in the case of the suborder Ischnocera, the antennae with 3-5 segments remain clearly visible, and the mandibles bite vertically. Different species of both the hairlings and featherlings belong either to the suborders Amblycera or Ischnocera.

The most important mallophage of horses—*Werneckiella equi equi*—belongs to the order Ischnocera and is found worldwide, since horses were distributed onto each continent as means of transport since thousands of years and with them traveled their parasites and other agents of diseases (Murray 1957; Arundel 1985; Moreby 1978; Perris 1995; Rommel 2000; Mehlhorn et al. 1993; Mehlhorn 2008; Wright 1999; Larsen et al. 2005; Mencke et al. 2004, 2005; Foil and Foil 1986). The recently finally accepted

species Werneckiella equi (Price et al. 2003) has many synonyms in older literature and was placed before in the genera Damalinia, Trichodectes, or Bovicola. The mallophages of mammalian hosts and thus W. equi are mostly hidden at the bases of hair, so that mass reproduction is only seen at the late state when skin and/or hair have already severe damage. Therefore, the prevention of such large populations is needed, which grow mostly during winter after infestations were acquired during summer on the meadow by contact with other infested hosts or by using the same equipment (e.g., equipment or blankets; Jones and DiPietro 1996). In cases in which animals have further hidden diseases, the population of the hairlings may reach more than one million per horse or cow. Therefore, regular schemes of treatment seem reasonable to prevent severe scabies-like skin appearances (focal alopecia, scaling, crusts, excoriations, exudations, hair loss; Larsen et al. 2005; Fadok 1984) and/or even transmission of the RNA viruses of infectious anemia worldwide in endemic regions. In general, the treatment of horses against mallophages is done by body washing with typical insecticides (pyrethroids, carbamates, fipronil, triflumuron, avermectines, imidacloprid, etc.; Rommel 2000; Eckert et al. 2008; Mencke et al. 2004, 2005; Larsen et al. 2005; Lloyd and Hayes 2002; Polozowski et al. 2001; Lowden et al. 2007; Sorrell et al. 2010), which in general requires a second treatment due to the fact that the nits glued to the hair are not or poorly affected. The present paper reports the effects of a biological insecticide based on a neem seed extract (MiteStop®) that has been shown to have a broad range of efficacy against many species of ticks, mites, and insects (Schmahl et al. 2010).

## Materials and methods

## Product

MiteStop<sup>®</sup> is a concentrate of a neem seed extract developed by the university spin-off company Alpha-Biocare (Düsseldorf, Germany). It is classified as a biocide of the EU class 18. This concentrated water-free extract was freshly diluted 1:20 with tap water just prior to use (Schmahl et al. 2010).

#### Horses

Three-hundred horses belonging to long and short hair races were treated each several times per year during the last 3 years with the product beginning in springtime until early December, when most of the outdoor bloodsuckers had disappeared in nature.

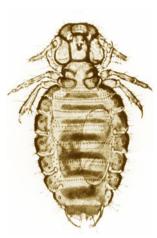
#### Application

The product was freshly diluted prior to use: 100 ml of the product was placed in a bowl and covered by about 2 l of tap water. After some stirring with a brush or a sponge, this 2 l of ready-to-use solution was brought onto the hair, so that the whole body was wet from the upper side of the hooves to the back, head, and face. Afterwards, it was controlled what happened. The horse owners reported their observations to the distributor and ordered further material. The family owned horses of the authors and those of neighboring riding stables were personally treated and inspected by the authors. In heavy infestations, the treatment was repeated twice at 1-week intervals, which were noted due to appearance of numerous dead mallophages at the tips of the hair after the first treatment.

#### Results

During wintertime when the horses were in stables, the number of mallophages increased—apparently due to an infestation by body contacts with other horses in the late phase of the summer and after the last treatments or by sharing riding equipment with other horses. All horses of the present study were kept under good conditions; but nevertheless, they were found to be more or less intensely infested. This was diagnosed by intense combing/brushing at several places of the body.

Although in some publications it is claimed that mallophages mainly occur at special places (such as along the head, neck, and lateral sides, but not at the lower limbs, tail, and ears), the present study showed their presence at merely all portions of the body. However, clearly visible dermatological symptoms such as alopecia, scaling, excoriations, exudations, crusts, lesions, etc., which might attract licking flies (and thus potential transmitters of agents of diseases), were scarce even in heavily infested horses. On the other hand, mallophageinfested places of the fur led to skin reactions such as local trembling when these places are touched. This was apparently a sign of pruritus or peculiar sensitivity at these places. When inspecting the hair of the horses, in general, different developing stages (nits, larvae, adults) of the species W. equi equi were observed (Fig. 1). This species belongs to the order of Ischnocera (= from Greek: with tiny horns = antennae). These adult stages were characterized by their typical broad head with a rounded anterior front and one pair of eyes at the head as well as by their large spiracles at the lateral sides of the segments of the abdomen. The mallophages measured about 1.6 mm in the case of the rare males and 1.8-2 mm in females, while their heads reached a diameter of 0.3 mm being nearly as broad as the broadest region of the dorsalFig. 1 Diagrammatic representation of a female *Werneckiella* mallophage containing an egg



ventrally flattened, but if fed, the ton-like swollen abdominal body consists of eight visible segments. Embryological studies indicated that there had been ten anlagen of full abdominal segments, of which the first was reduced, while segments 8 and 9 were apparently fused, so that only eight remained active (Soulsby 1986).

The above-described treatment with  $MiteStop^{\mathbb{R}}$  led to the observation that about 1 h after the application of the product to the hair, the tips of the latter were spotted with remnants of the bodies of the mallophages which apparently

had migrated from the body surface to the tips of the hair before being killed. Due to this effect, the hair appeared "wooly twisted." When using a dry brush, the chitinous carcasses of the mallophages could be combed down. When they were collected on a towel, they appeared very dry. When combing the mallophages down in heavy infestations, a large amount of dandruff was also removed. It was noted by the researchers and by the different rider groups that even a single treatment reduced considerably the formation of dandruff, which was even more reduced if the treatment was repeated twice at intervals of 1 week. Apparently, the treatment stopped also the development of the larvae in the nits, since in treated horses, it took months until a considerable infestation was noted again. This fact is remarkable, since the normal development inside the eggs takes only 5-10 days (Arundel 1985), being followed by a 2-week period to reach the adult stages (Martini 1946; Mehlhorn 2008), which live for about 1–2 months. The

they glue to the bases of hair. Practically all users reported that the application of the product MiteStop<sup>®</sup> led, in addition to its insecticidal activity, to a considerable repellency of tabanids, other bloodsucking insects, ticks, and even flies, when application of the product was done in midsummer prior to a ride.

females produce mostly in total about 100 eggs which

Table 1 Use of insecticides in the treatment against horse mallophages

Active compounds	Dose	Way of application	Control of effects	Authors
Combination of permethrin and pyriproxifen	2% permethrin and 0.05% pyriproxyfen in a spray solution	Sprayed onto fur until wet, repeated after 15 days	Brushing of hair, fur control; no stages found after the second treatment	Sorrell et al. 2010
Triflumuron	2.5 mg triflumuron per kg body weight (=1 ml product per 10 kg body weight)	Brought onto the back line between the poll and the highest point of the rump as a continuous strip by help of a syringe	Hair control at 10 partings of the fur at days 44 and 71 p.t.	Lowden et al. 2007
Amitraz	0.025% Spray	Spray	Control of hair	Eckert et al. 2008
Cyhalothrin	0.4 mg/kg	Pour on		
Deltamethrin	0.75 mg/kg	Pour on		
Flumethrin	2.00 mg/kg	Pour on		
Doramectin	0.5 mg/kg	Pour on		
Ivermectin	0.5 mg/kg	Pour on		
Moxidectin	0.5 mg/kg	Pour on		
Imidacloprid	8 ml of the 10% Advantage <sup>®</sup> Spot on repeated on day 28	1×4 ml along the mane, 1×4 ml along the trunk (one side)	Louse check on days 2, 14, 28, and 56 p.t.	Mencke et al. 2005
Neem seed extract (MiteStop®)	1:20 tap water-diluted solution	2 l of the freshly prepared product are brought onto fur by help of a brush until fur is wet	Brushing the hair, cutting off hair to check for motile stages and nits at days 1, 10, and 15	Present study

The typical insecticides are mostly not registered for horses, but for cattle. Therefore, the veterinarian has to use them "off label" *p.t.* post treatment

It was furthermore reported that this protection was persistent at least for 3 days; in some cases, even repellency for 1 week was reported.

In addition, there were observations that treated horses were apparently not attacked by the so-called autumn mites (the larvae of the mite species *Neotrombicula autumnalis*), which suck lymph just above the hooves and thus introduce numerous severely itching wounds. Several other users also reported that in those cases, when horses had lesions along the upper side of the hooves—apparently due to infections with fungi and/or bacteria—healing occurred during the first week after treatment.

## Discussion

Animals on the meadow are attacked by a large variety of bloodsucking or licking ectoparasites belonging to the groups of ticks, mites, and insects. These ectoparasites, often occurring in huge numbers, may parasitize for a few minutes (temporarily) such as mosquitoes and flies, stay some days such as ticks, or even permanently such as bloodsucking lice (Anoplura) or biting lice (Mallophaga) (Hansen and Londershausen 2008; Mehlhorn et al. 1993, 2001; Rommel 2000; Eckert et al. 2008). While insects flying from one host to the other or ticks with a regular change of hosts (as all temporary ectoparasites) have developed the potential to transmit agents of diseases, permanent parasites such as lice are less often vectors of parasites, bacteria, or viruses (Mehlhorn 2008; Aspöck 2010). However, mass infestations with ectoparasites may introduce severe clinical signs of different types of dermatosis due to their biting and/or bloodsucking activities. These diseases were connected with loss of blood, restlessness, skin pain, itching, exudations, and/or lesions mostly leading to considerable loss of weight and/or loss of general fitness, which may make them more easy victims of severe infectious diseases, while the latter would run much more smoothly in non-infested animals.

The mallophages of the present study (*W. equi equi*) are underestimated parasites of horses, since they are much more common than believed. The reasons for this neglect are that they are even as adults very tiny (1.6–2 mm) and thus are poorly seen with the naked eye, that they stay close to the skin, and that they have a fast grip with their legs (each with one claw) at the hair, so that even brushing will not remove them easily from the hair. In contrast to *Werneckiella equi asini*—the mallophage of donkeys—*W. equi equi* develops only a few males in a population. This led to the fact that the females are apparently also able to lay parthenogenetically unfertilized eggs, from which also fertile females develop. As a consequence of these abilities, the mallophages of horses remain very often undetected even in mass infestations (which often occur under bad containment conditions of the horses). This led to the fact that the industry did not care intensely for the development of peculiar anti-lice products for horses. Therefore, true insecticides being used in other animals were adapted in doses and in the way of treatment for horses (Table 1). Especially phoxim and imidacloprid but also permethrin and triflumuron had been intensively tested to be useful for horses (Mencke et al. 2004, 2005; Larsen et al. 2005; Hanssen et al. 1999; Sorrell et al. 2010; Lowden et al. 2007). Applications of other insecticides (used and registered for cattle) are also reported in these papers and in the textbooks of (Rommel 2000) and Eckert et al. (2008). However, all these true insecticides can only be obtained by prescription of a veterinarian. This is apparently an obstacle, which prevents riders to treat this rather inapparent but in any way important dermatosis.

The neem seed extract (MiteStop®), which has a broad range of efficacies (Schmahl et al. 2010), is on the other hand freely available in horse supply shops and has, in addition, the advantages that it repels biting insects and ticks and apparently has reducing effects on wounds along the hooves. Furthermore, the positive effect of a treatment can be seen soon after the treatment, since apparently the compound makes the mallophages crawl onto the tips of the hair just before they die. Since 10% of the active ingredient of MiteStop® is also included in shampoos against human head lice (Abdel-Ghaffar and Semmler 2007; Heukelbach et al. 2006; Abdel-Ghaffar et al. 2010), the efficacy of MiteStop® against the bloodsucking lice of horses (Haematopinus asini) is also given, although in the present study, there was no search for these parasites, which might introduce considerable blood loss in case that they occur in larger numbers.

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