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ORIGINAL PAPER

Effects of a neem seed extract (MiteStop®) on mallophages (featherlings) of chicken: in vivo and in vitro studies

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Abstract Mallophages of birds (featherlings) are mostly very tiny and can even as adults better be recognized by their movements than by their elongate body shape when using just the naked eye. Since some species (e.g., the "shaft louse" *Menopon gallinae*, the elongate feather louse *Lipeurus caponis*, or *Columbicola* sp.) may pierce the pulp of feathers or the skin by their biting or scratching mandibles and thus lick the excreted blood, they may be extremely dangerous especially to young birds, even if they only feed by nibbling along the feather surface and/or eat epidermal debris. The present paper reports on the successful treatment of different races of fowls being severely infested with both above cited species. This in vivo treatment was done either by a short dipping of the whole fowl into the 1:33 dilution (with tap water) of a neem seed extract (MiteStop®) or by spraying them with the freshly diluted product. It was seen that the dead mallophages dropped down from the feathers as soon as they were dry again. As a precaution, a second treatment was done by some owners 1 week after the first one in order to eliminate all stages, which eventually might have hatched from untouched nits during the time interval between the two treatments. When controlling the treated fowls 4 weeks after the treatment, in no case (treated once or twice), living motile stages were diagnosed indicating the high efficacy of this nontoxic neem seed extract. When treating in vitro cutoff feathers contaminated with *L. caponis*, it was seen under the stereomicroscope, that the mallophages tried to run away from the 1:33 water-diluted active compound indicating that there is also a repellent effect. Treated *L. caponis* stopped leg movements within 3 min and died on their feathers within 1–20 min. Then, the last slight trembling movements of their legs and convulsions of their intestine stopped finally.

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Introduction

Biting lice (Mallophaga) are found on mammals as well as on birds, mostly invisibly hidden in the hair or in the plumage (Zlotorzycka et al. 1974; Argawal and Saxena 1979; Marshall 1981; Martini 1946; Nelson and Murray 1971; Trivedi et al. 1991; Soulsby 1986; Mehlhorn et al. 1993; Eckert et al. 2008; Rommel 2000; Al-Quraishy et al. 2011). With their biting mouthparts (mandibles), all developmental stages of the mallophages (three nymphs and the female and male adults) feed on the skin, eat epidermal debris, nibble on hair or feathers, and in some cases, they may also pierce or scratch the skin so that small droplets of blood are exudated from the skin being finally licked by the mallophages. Two large suborders are described among the members of the order Mallophaga (Soulsby 1986; Lucius and Frank-Loos 1997; Mehlhorn 2008):

– Amblycera: Their mandibles bite horizontally, their antennae (one pair) lay in grooves along the head, and their heads have two pairs of eyes (ommatidia). Species of this group may either parasitize on mammals or on birds, e.g, *Menopon gallinae*, the so-called shaft louse, is found on poultry, while *Trimenopon hispidum* occurs, e.g., on guinea pigs.
– Ischnocera: Their mandibles bite vertically, they possess one pair of eyes, and their antennae are tiny but are visible on the head's surface. Species of this group may either live on mammals or birds, e.g., *Lipeurus caponis* (the so-called wing louse) is found on birds, while, e.g., *Werneckiella equi* parasitizes on donkeys and horses or *Trichodectes canis* occurs on dogs (Al-Quraishy et al. 2011).

However, depending on their hosts, both groups of mallophages have developed similar peculiar adaptations that guarantee a strong holdfast on their host. All species of birds have developed two claws or structures of similar function, while all species of mammals possess only one claw, since it is apparently easier to clutch at the rather smooth mammal hair than at the rather stiff central shafts or lateral branches of the feathers of birds (Soulsby 1986).

Fig. 1 Light micrograph of a specimen of *L. caponis*

Fig. 2 *M. gallinae*: light micrograph of an unfed stage. Note the somewhat triangular head

Birds that are parasitized by mallophages often show changes in behavior. They stop grooming themselves and are restless and scratch with their claws at the breast or at their lateral body often introducing wounds. This leads to the effect that they feed less than uninfested birds, grow much slower, and eventually lay fewer eggs. Although the external aspect of infested chicken appears rather unchanged, a large number of mallophages may live on their skin and/or in the fine feathers. In those cases, where huge numbers of mallophages occur and where the specimens pierce the skin and lick blood, anemia and loss of fitness may occur that makes them more sensible to infections with other agents of diseases. This altogether may lead—especially in young chicken—to death. Therefore, observation of the chicken is an important precautionary measure that prohibits explosive outbreaks with enormous damages and economical losses in egg production and/or meat growth. However, infestations of chicken in those stables, where they stay only for a short time, are rare or reach mostly only a rather limited amount. Nevertheless, a constant control is helpful, especially when chicken are kept on the floor, so that body contacts are common and benefit the transmission of mallophages. On the other side, the means for treatment are also limited (Eckert et al. 2008; Hansen and Londershausen 2008), since typical products exclusively for chicken are in many countries not available or scarce in others (such as phoxim in the product of ByeMite®). The present study deals with the effects of a special nontoxic neem seed extract, which might be used as

Fig. 3 *Columbicola* sp.: adult stage on a feather

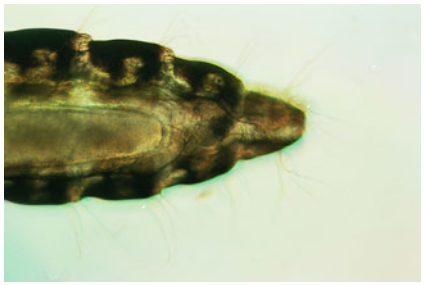

Fig. 4 *Columbicola* sp.: light micrograph of the terminal end of a female on the feather of a duck (note the cigarillo-like egg in the uterus)

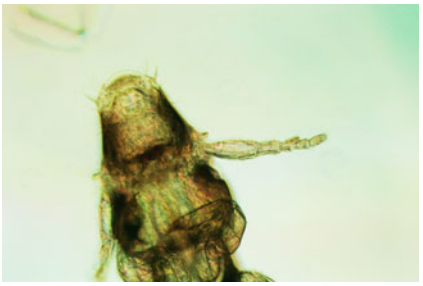

Fig. 6 Light micrograph of the head of *Columbicola* sp. Note the five segments of the antennae

prophylaxis besides treatment of diagnosed cases. This product (MiteStop®) had been proven efficacious against a broad spectrum of mites, ticks, and insects including the mallophages of horses and dogs (Schmahl et al. 2010; Al-Quraishy et al. 2011; Mehlhorn et al. 2011).

Materials and methods

Different chicken owners with low numbers of chickens (the largest farm held 180 specimens) complained that their chicken showed disturbances in behavior accompanied by an apparent itching and structural defects along their plumage. Inspections of the feathers showed that there occurred stages of the red bird mite, *Dermanyssus gallinae*. In many cases, mallophages were also seen, which had damaged considerably in some cases the skin and the feathers. Therefore, treatment was started using the neem seed extract MiteStop®, which had been proven to be highly effective against the mites and several other pests (Abdel-Ghaffar et al. 2009; Locher et al. 2010a, b; Schmahl et al. 2010). Since most of the mites did not stay on the chickens, but stayed hidden somewhere in the stable, the concentrate MiteStop® was 1:33 diluted in tap water and sprayed onto the chickens as well as on possible hiding sites of the mites. Three days after the treatment, the chickens were checked for living mites. In cases of chickens with additional or separate infestations with mallophages of the *Columbicola* sp., *M. gallinae*, and *L. caponis*, the chickens were dipped for seconds into the freshly prepared (1:33 with tap water diluted) MiteStop® solution until they were completely wet. After drying, the chickens were inspected for the occurrence of surviving mallophages by spreading the feathers and by inspection with the help of a magnifying glass.

Fig. 5 Light micrograph of the posterior end of a male of *Columbicola* sp. on a feather of a duck. Note the slightly visible (protruded) "penis apparatus" at the terminal end of the abdomen

In vitro studies

In order to follow under the microscope the killing efficacy of the MiteStop® product, several infested feathers were cut off from the plumage of chicken and/or ducks and transported in a closed plastic tube from the poultry yard to the institute. There, the feathers with the mallophages (*Columbicola* sp., *M. gallinae*, or *Philopterus thuringiacus*) were placed under the microscope and covered by a tiny droplet of the freshly prepared 1:33 water-diluted product. Then, it was observed for the next 20 min.

Results

The in vivo studies were executed with the following species that occurred on the naturally infested poultry:

– *L. caponis* (Fig. 1): The adults that were found on the chickens appeared long and slender reaching a length of around 2.5 mm in the female stage and about 2.0 mm in males.

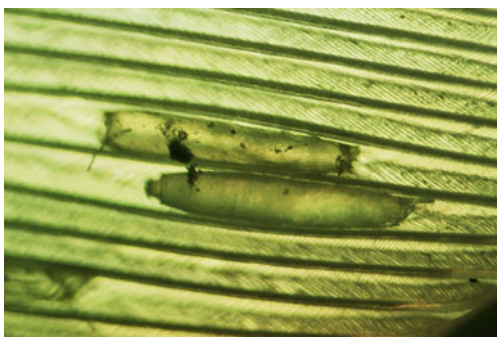

Fig. 7 Light micrograph of two nits of *Columbicola* sp. Note that one is still covered by an operculum (cover) with protruding tubules. The other nit is empty

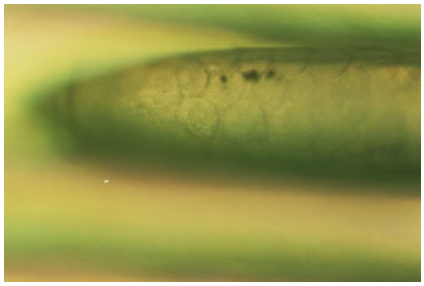

Fig. 8 Light micrograph of the surface of nits showing a typical design

– *M. gallinae* (Fig. 2): The about 2-mm-sized adults of this species, which occur on fowls, ducks, and pigeons, move rather rapidly.

The in vitro studies were done with nymphs and adults of the following species:

– *L. caponis* (see above),
– *M. gallinae* (see above),
– *Columbicola* sp.: These mallophages were found on the feathers of ducks (Figs. 3, 4, 5, 6, 7, 8, 9, and 10) with a length of about 2 mm. They move very quickly. Their eggs inside the uterus as well when glued as nits at the feathers appeared slender and cigarillo-like. Their operculum cover was provided with short protruding tubules on the surface of the operculum. The surface of the nits was characterized by a mosaic-like pattern.
– *P. thuringiacus* (Fig. 11): Some 1.6-mm long specimens were collected from a feather that apparently belonged formerly to a titmouse bird species.

In vivo treatment

When dipping (just in–out) the infested birds completely into the 1:33 tap water-diluted MiteStop® solution, it was noted that after drying (1 h) the feathers, all motile stages (nymphs and adults) were dead. Also, the control check 4 weeks after the treatment did not show motile stages. Therefore,

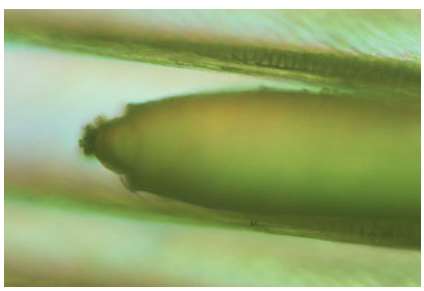

Fig. 9 Upper portion of a nit of *Columbicola* sp. Note that the protruded operculum has tubelike protrusions

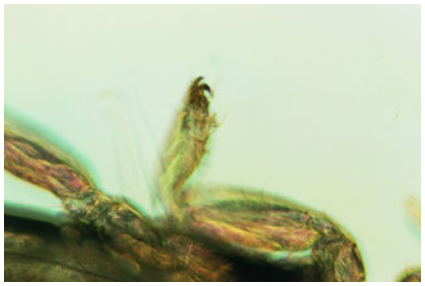

Fig. 10 Light micrograph of the claws at a leg. These claws may be close which allows clutching at fine branches of the hair. Two claws are typical for the feet of mallophages of birds, while mallophages of mammals have only one claw

apparently, the developmental stages in the nits had been killed during the treatment, too, either the treatment was repeated or not.

The consecutive microscopic inspection of the in vitro treatment of the mallophages on cutoff or dropped down feathers showed that the adult mallophages of the species *L. caponis*, *M. gallinae*, *Columbicola* sp., and *P. thuringiacus* reacted practically identical when coming into contact with the 1:33 water-diluted product MiteStop®. If a droplet of this dilution was placed on a feather close to a featherling, this specimen ran away at high speed. If a small droplet of the product was placed directly onto such a mallophage, the parasite tried to get away from the wet region. However,

Fig. 11 Scanning electron micrograph of *P. thuringiacus* on a feather of a titmouse bird. Note the typical head and the long bristles running backwards from the first segment of the abdomen

even if the mallophage succeeded in leaving the product-covered spot of the hair, its movements stopped at a distance of about 5 mm and its legs started to have uncoordinated movements, while the intestine showed contractions. About 10 min after the covering of the mallophage by the diluted product, the slowed down movements of the legs had stopped completely as well as the contractions of the intestine, so that 1 h after the treatment at the latest, all treated specimens were dead, while the controls showed even 2 days after the beginning of the experiments full fitness when being kept in the feathers in a closed plastic tube at a room temperature of 22°C.

Discussion

Mallophages, i.e., biting lice are often neglected by the owners of rearing facilities, since even high-graded infestations, which occur by body contacts between poultry or when a hen visits the same nest for egg deposition do not introduce clearly visible symptoms of the disease. However, since the mallophages feed on the plumage, on the skin, and even may pierce the skin to lick blood, poultry is harmed directly by low food uptake, less numerous egg laying, or by slow growth or indirectly affected, when these wounds in the skin are superinfected by bacteria or when the immune system is not adequately effective in cases of attacks of agents of infectious diseases. Therefore, infestations with mallophages are of considerable importance, especially when other ectoparasites such as ticks or red poultry mites (*D. gallinae*) are present at the same time in a stable. Thus, it is strongly recommended to inspect the stables at regular intervals for the occurrence of such ectoparasites that may harm the health of the poultry and decrease the economic revenue of the owners of the poultry.

In case of the occurrence of such ectoparasites, an efficacious treatment of the stables and of the chicken/poultry is legally needed. Since there exist only a few ectoparasitocidal products that are officially/legally registered for the use in poultry, the situation turns out to be difficult. The reason is that most chemical insecticides being registered for other animals would require a considerable waiting time for the meat consumption of treated poultry and afford the discard of eggs being laid in the stable during the spray action. Often, a withdrawal of food is needed during the treating period. In addition, in the past, resistances have been developed against most of the older insecticides, e.g., phoxim has today only a reduced efficacy against the red bird mite, *D. gallinae* (Abdel-Ghaffar et al. 2009). On the other hand, the biological compound MiteStop® based on a neem seed extract has a very high and broad efficacy against a wide spectrum of mites, ticks, and insects that molest animals and humans (Heukelbach et al. 2006; Abdel-Ghaffar et al. 2008a, b; Abdel-Ghaffar and Semmler 2007; Schmahl et al. 2010; Semmler et al. 2009; Abdel-Ghaffar et al. 2009; Locher et al. 2010a; b). Just recently, it was shown that this product is not only able to kill general pests of animals and humans but also acts very specifically against the biting lice of horses (Al-Quraishy et al. 2011) and against beetles from the families Tenebrionidae and Dermestidae, the larvae of which may enter the plumage of poultry and feed on tiny feathers or on skin debris (Walldorf et al. 2011). The efficacy of MiteStop® on biting lice is not astonishing, since it was shown that the active compound in MiteStop® eliminates human blood-sucking lice (*Pediculus humanus capitis*, Abdel-Ghaffar et al. 2010) and blood-sucking lice of dogs (*Linognathus setosus*, Mehlhorn et al. 2011).

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