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Lousicidal efficacy of camphor oil, *d*-phenothrin, and deltamethrin against the slender pigeon louse, *Columbicola columbae*

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Deltamethrin

Abstract The slender pigeon louse, *Columbicola columbae*, is an annoying ectoparasite of pigeons. The aims of present work were to study the prevalence of lice infestations among pigeons in Gharbia governorate, Egypt, and to compare the lousicidal efficacy of camphor oil (CAM) to those of *d*-phenothrin (DPH) and deltamethrin (DMT) against *C. columbae*. Pigeons were classified into four groups (25 pigeons each). Birds were sprayed with 8% CAM and few drops of Tween 80, 9% DPH, 0.005% DMT (50 mg/L or 1 ml/L), and the control group was sprayed with distilled water and few drops of Tween 80. The prevalence of lice infestations was 85% (340 out of 400, 550 ± 50 louse/bird, and the range of infestation was 100–800). All *in vitro* treated lice with 1% CAM and DPH were died within an hour post treatment and the lethal values were 0.25% and 0.28%, respectively. The lethal time values were 6.50 and 2.30 min post-treatment with 0.004% CAM and DPH, respectively. The *in vivo* treatments indicated that the louse infestations were almost completely eliminated 7 days post-treatment with CAM and DPH and 14 days PT with DMT. Temporary coughing, sneezing, and ocular inflammations without dermatitis were observed among birds sprayed with DMT. CAM has a potential for the development of a new and safe product for controlling poultry lice.

1. Introduction

Pigeons are infested with a variety of ectoparasites [1]. The slender pigeon louse, *Columbicola columbae* Linnaeus, found primarily in the feathers on the undersides and upper wings of pigeons [2]. A female can potentially lay up to 9 eggs per day every 3–5 days. It proceeds through three nymphal instars to reach their final adult stage within a month. The adult life span of *C. columbae* is 4–7 weeks [3].

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C. columbae feeds by chewing on the feathers of their pigeon hosts. Nonetheless, these lice also have the ability to move from the wings to the body to feed on the fluff of the basal parts of the feathers found there [3]. Like other mallophages, *C. columbae* may also pierce or scratch the skin so that small droplets of blood are exudated from the skin being finally licked by the mallophages and skin lesions may become sites of secondary infection. Heavy infestations by mallophages often show changes in birds' behavior, drop of egg production, 10–20% [4], and meat growth as well as death, especially in young birds [5].

Lice are permanent ectoparasites; consequently, their control is much easier than control of other temporary ectoparasites. Most chemical insecticides adversely affect health of poultry [6] and induce insecticidal residues in meat and eggs [7,8]. In addition, resistances have been developed against insecticides [9,10]. It is unwise to use pesticides on a bird's plumage as preening birds eat their lice. Consequently, side effects of chemical insecticides have prompted a search for new alternatives.

Synthetic pyrethroids, such as *d*-phenothrin, and deltamethrin, are pesticides derived from naturally occurring pyrethrins, taken from pyrethrum of dried *Chrysanthemum* flowers. They are chemically designed to be more toxic with lower break down times and are formulated with synergists increasing potency and compromising the body's ability to detoxify pesticide [11].

Essential oils have been long used for their insecticidal activity against many species of insects, including lice. Camphor, *Cinnamomum camphora* (Family: *Lauraceae*), has long been valued for its great medicinal uses. It is used as a room freshener and food disinfectant. Furthermore, camphor-soot paste is used in eye make-up [12]. Some Egyptian essential oils, including camphor, showed potentials, for the first time as far as we know, for the development of new, speedy and safe insecticide, and insect repellents [13,14].

Despite severe clinical signs and death of highly infested birds, the data about epidemiology and control of *C. columbae* are very few in Egypt [15,16]. To the best of our knowledge, there are only few trials, worldwide, for controlling *Columbicola* spp. [5,16,17]. Therefore, the aims of present work were to study the prevalence of the slender pigeon louse infestation in Gharbia governorate, Egypt, and to compare the lousicidal efficacy of camphor oil to those of *d*-phenothrin and deltamethrin against *C. columbae*. As far as we know, the currently applied materials had been tried for the first time against pigeon lice infestations.

2. Materials and methods

2.1. Pigeons and lice

Different owners of the domestic pigeons, *Columba livia domestica* (the largest farm held 300 specimens) in Gharbia governorate, Egypt, complained that their pigeon showed lowered growth and disturbances in behavior accompanied by apparent itching and damaged skin and feathers.

Feather inspections revealed infestations with chewing lice. Four hundred pigeons were examined for the intensity and prevalence of lice infestations in different localities in Gharbia governorate. Lice from naturally infested flocks of pigeon, were identified according to Adams et al. [18]. Lice were used for the *in vitro* bioassays. Naturally infested pigeons with lice were used for the *in vivo* treatments.

2.2. Tested substances

(1) CAM: camphor oil, El-Kabtian Company, Egypt. The oil is authorized by the Egyptian Ministry of Health for different human uses.
(2) DPH: 0.4% *d*-phenothrin (Item®, Mash Co., Egypt). It is an anti-lice shampoo and authorized in Coryne, Monaco, France. It is also authorized by the Ministries of Health in France and Egypt for treatment of adults and eggs of the human head louse, *Pediculus humanus var capitis*.
(3) DMT: deltamethrin 5% (Butox® 50 EC), Intervet Company, Cairo, Egypt. Each ml contains 50 mg deltamethrin.

2.3. Testing for in vitro pediculicidal activity

In vitro immersion tests were carried out to evaluate the efficacy of the tested substances against lice infesting pigeons. Preliminary experiments were conducted to determine suitable experimental parameters, such as dilution factors for tested substances and the duration of their exposure to lice.

The method used to assess the pediculicidal activity was done according to Khater et al. [19] with slight modification. Lice (per replicate) were dipped in a warped filter paper (9-cm diameter). The finalized concentration–response bioassay was carried out as follows. Ten lice were immersed for 60 s in 100 ml solution from each concentration of tested chemical and the solution was continuously stirred during the process. Each test substance was diluted in water to different concentrations. The applied concentrations were 0.004%, 0.03%, 0.06%, 0.25%, 0.5%, and 1% for both CAM and DPH. A few drops of Tween 80 were added as an emulsifier in case of CAM.

The immersed lice then held in the lower half of a 9-cm glass Petri dish. After 30 min, the liquid had spread out and no excess moisture was left in the dish. Two control groups were treated with distilled water only and with Tween 80 and distilled water. Four replicates were used for each concentration (40 lice for each concentration). Bioassays were performed at 27 ± 2 °C and 75 ± 5% relative humidity. Lice were examined, under a stereoscope, at different time intervals (1, 4, and 24 h post-treatment). Death of lice was defined as the lack of limb movement, and failure to respond when the legs were stroked with a forceps [13,19]. The number of fatalities was recorded.

2.4. Testing for in vitro lethal time (LT)

The time–response bioassay was similar to the standard concentration–response bioassay with the following exception; lice were exposed to a single concentration for each trial. The mortality was initially assessed 1 h after being subjected to test materials, followed by mortality assessment at 2, 4, and 24 h. The used concentrations were 0.04%, 0.03%, 0.06%, 0.25%, and 0.5% for both CAM and DPH.

2.5. Experimental design and treatment strategy

One hundred pigeons were randomly divided into four equal groups (25 birds/each, and each group was divided into five subgroups) and used for the *in vivo* treatments; birds were placed in separate premises without contact among groups. The number of louse/bird (naturally infested) was counted on day 0, just before treatment. The birds were sprayed with the freshly prepared solutions (the lethal concentrations (95), i.e. 95% of the sample population die from exposure, according to our *in vitro* results) until they were completely wet and all parts of the body having adequate contact with the insecticide solutions.

The first group (Gr1) was sprayed with 8% CAM and few drops of Tween 80. The second group (Gr2) was sprayed with 9% DPH. The third group (Gr3) was sprayed with 0.005% DMT (50 mg/L or 1 ml/L), the recommended dose by the manufacture and according to Khater al. [19]; therefore, we did not make *in vitro* trials for DMT. The fourth group (Gr4) was sprayed with distilled water and few drops of Tween 80.

Sprayed birds were inspected visually for the presence of surviving mallophages on day zero before medication and days 1, 7, 14, and 28 post-treatments by spreading the feathers and by inspection with the help of a magnifying glass. The total number of live lice (nymphs and adults) on the left side of each bird was counted and multiplied by two, according to Kinsey et al. [20]. The efficacy of each drug was determined on the basis of reduction percentage; present control, of lice infestations [13,17,19]. Birds and spraying operators were observed daily for any abnormal health observations and skin irritations.

2.6. Statistical analysis

The mortality data were subjected to Probit transformation followed by regression analysis to determine the lethal concentration, LC, and lethal time, LT, values as well as the slope of the regression lines by a computer, using POLO–PCO. Biological data were subjected to analysis of variance (ANOVA) by Duncan multiple range test, according to Duncan [21] using SPSS program (SPSS v.10, SPSS Inc., Chicago, IL, USA). The reduction (%) of lice on pigeons was calculated according as the following equation:

$$\text{Lice reduction } \% = (\text{Pre-treatment count} - \text{post-treatment } count)/\text{Pre-treatment } count \times 100$$

3. Results

The examined birds were found parasitized, exclusively, by the mallophages, *C. columbae*. It had been found that 340 out of 400 pigeons were infested with *C. columbae*. The prevalence of lice infestations was found to be 85% and the intensity of infestation was 550 ± 50 louse/bird and the range of infestation was 100–800. Heavy infested birds showed changes in behavior, such as restlessness, increased preening activity, scratching signs in the breast or lateral body often introducing wounds, and structural defects along their plumage.

In vitro larval immersion bioassays revealed that the lousicidal efficacy of the applied materials increased as the concentration and the exposure time increased. One hour post-treatment, 100% lousicidal efficacy was achieved by 1% CAM and DPH and the LC50 values were 0.25% and 0.28% for CAM and DPH, respectively (Tables 1 and 2). With regard to the time–response observations, the LT50 values were 6.50 and 2.30 min after treatment with 0.004% CAM and DPH, respectively (Table 3).

The *in vivo* treatments indicated that the reduction percentage of lice infestations post-treatments with CAM, DPH, and DMT were 82.64%, 79.61%, and 48.11%, 1 day post-treatment and 99.83%, 98.83%, and 82.33%, respectively, 7 days post-treatments. All lice in Grs. 1–3 were died 21 days post-treatments. On the other hand, lice infestations were increased in the control group (Table 4).

Table 1 *In vitro* mortality of the pigeon louse, *Columbicola columbae*, after treatment with camphor oil and *d*-phenothrin.

Conc. (%)	Time post-treatment (h) 1		2		3		4		24	
	D	%	*D*	%	*D*	%	*D*	%	*D*	%
CAM										
0.004	4	10	6	15	15	37.5	15	37.5	32	80
0.030	6	15	7	17.5	17	42.5	17	42.5	35	87.5
0.060	7	17.5	8	20	25	62.5	26	65	40	100
0.250	8	20	16	40	33	82.5	34	85	40	100
0.500	25	62.5	25	62.5	35	87.5	36	90	40	100
1.000	40	100	40	100	40	100	40	100	40	100
Control 1	0	0	0	0	1	2.5	1	2.5	2	5
Control 2	0	0	0	0	0	0	1	2.5	3	7.5
DPH										
0.004	4	10	7	17.5	28	70	40	100	40	100
0.030	5	12.5	12	30	31	77.5	40	100	40	100
0.060	6	15	19	47.5	33	82.5	40	100	40	100
0.250	8	20	32	80	34	85	40	100	40	100
0.500	23	57.5	35	87.5	37	92.5	40	100	40	100
1.000	40	100	40	100	40	100	40	100	40	100
Control 1	0	0	0	0	1	2.5	2	5	3	7.5
Control 2	0	0	0	0	1	2.5	1	2.5	3	7.5

Table 2 Sensitivity of lice to camphor oil and *d*-phenothrin.

	Time post-treatment (h)							
	Camphor oil					*d*-Phenothrin		
	1	2	3	4	24	1	2	3
LC10	0.020	0.009	0.005	0.001	0.00001	0.0197	0.004	0.000
LC50	0.251	0.177	0.019	0.173	0.0004	0.283	0.048	0.001
LC70	0.754	0.594	0.081	0.071	0.002	0.844	0.140	0.006
LC80	1.467	1.234	0.198	0.167	0.004	1.634	0.266	0.029
LC90	3.692	3.406	0.683	0.549	0.0147	4.084	0.650	0.234
LC95	7.911	7.870	1.898	1.462	0.042	8.701	1.358	1.311
LC99	33.043	37.879	12.888	9.195	0.298	35.963	5.411	33.078
Slope[a]	1.098 ± 139	0.999 ± 0.127	0.819 ± 120	0.853 ± 0.122	0.799 ± 0.213	1.106 ± 0.141	1.135 ± 0.132	0.486 ± 0.127

LC = lethal concentration.
[a] Slope of the regression lines.

Table 3 Lethal time values of camphor oil and *d*-phenothrin.

	Time post-treatment (h)									
	Camphor oil					*d*-Phenothrin				
	0.004	0.03	0.06	0.25	0.5	0.004	0.03	0.06	0.25	0.5
LT10	0.989	0.842	0.932	0.817	0.160	1.317	1.143	0.987	0.779	0.347
LT50	6.497	4.944	2.775	1.937	0.834	2.301	2.068	1.829	1.477	0.881
LT70	14.040	10.202	4.336	2.758	1.642	2.892	2.636	2.354	1.919	1.289
LT80	22.375	15.812	5.680	3.415	2.474	3.321	3.053	2.742	2.248	1.624
LT90	12.704	29.036	8.260	4.592	4.370	4.022	3.742	3.389	2.800	2.235
LT95	72.826	47.962	11.252	5.866	6.984	4.711	4.427	4.037	3.357	2.918
LT99	198.214	122.692	20.098	9.284	16.845	6.339	6.067	5.604	4.717	4.777

LT = lethal time.

After spraying with CAM and DPH, spraying operators and pigeons did not show respiratory signs or inflammation on the eyes and/or skin, with the exception that few pigeons suffered from temporary lacrimation. In contrast, temporary coughing, sneezing, and ocular inflammations without dermatitis were observed in birds sprayed with DMT.

4. Discussion

The chewing louse, *C. columbae*, was solely recorded among examined pigeons, in the present study. The same observation had been reported for pigeons from different geographical regions [16,17]. Our data indicated that the prevalence of lice infestation was 85%. Similar high prevalence was recorded in Iran, 79.41% [22]; Turkey, 89.8% [23]; Bangladesh, 100% [24]; Pakistan, 91% [17] and 70.5% and 100% in feral and wild pigeons, respectively [1]. Lower prevalences were recorded for male and female pigeons, 64.4% and 57.6%, respectively, in Iraq [25]. The mean intensity of infestations with *C. columbae*, in the present work, was 550 ± 50 (100–800). Almost similar burden of infestation was recorded form feral and wild pigeons, 438.2 ± 29 (115–906) and 715.8 ± 21 (152–1186), respectively [1]. Lower parasitic burdens were recorded in male and female pigeon's (60 and 45, respectively) [25]. Heavy infested pigeons with *C. columbae*, in this study, showed restlessness accompanied by increased preening activity, an apparent itching, and damaged skin and feathers. As a result, pigeons would feed less than uninfested birds, grow much slower, and eventually lay fewer eggs with low hatchability. Similar symptoms were also recorded [1,5].

In the present study, *in vitro* bioassays indicated that 100% lousicidal efficacy was achieved 1 h post-treatments with 1% CAM and DPH and the LC50 values were 0.25% and 0.28%, respectively. Likewise, high efficacy for the same materials had been reported against the buffalo louse, *Haematopinus tuberculatus*, the LC50 values of CAM and DPH were 2.74% and 1.17%, respectively [13]. Two minutes post-treatments, all larvae of the fowl tick, *Argas persicus*, were killed when exposed to 0.005% DMT and LC50, LC90, LC95, and LC99 values were 0.003%, 0.005%, 0.005%, and 0.006% (33.20, 46.76, 51.53, and 61.82 mg/L), respectively [19]. Furthermore, *in vitro* larval immersion tests determined the efficacy of CAM for the first time, to the best of our knowledge, against the second and third larval stages of *Cephalopina titillator* [14]. Our *in vitro* results also indicated that the LT50 values of CAM and DPH, post-treatments with 0.5% for both materials, were almost 0.8 h (48 min) Similar high speed of efficacy had been reported for CAM [13,14], DPH [13], as well as peracetic acid and DMT [19].

All treated lice, in the present study, were died 14 days post-treatment with CAM and DPH and 21 days post-treatment with DMT. It was observed that most dead mallophages dropped down from the feathers after spraying as soon as they were dry again. Similar result was observed [5]. Poultry ectoparasites have been controlled though chemical control agents,

Table 4 *In vivo* treatments (spraying) of pigeons with CAM, DPH, and DMT.

	PrT	Days PT					Reduction (%)				
	0	1	7	14	21	28	1	7	14	21	28
Gr1: CAM	531.00 ± 15.14	92.20^{c} ± 3.18	0.88^{c} ± 0.28	0.00^{b} ± 0.00	0.00^{b} ± 0.00	0.00^{b} ± 0.00	82.64	99.83	100.00	100.00	100.00
Gr2: DPH	481.64 ± 26.48	98.20^{c} ± 3.92	5.64^{c} ± 0.64	0.00^{b} ± 0.00	0.00^{b} ± 0.00	0.00^{b} ± 0.00	79.61	98.83	100.00	100.00	100.00
Gr3: DMT	546.84 ± 16.16	283.76^{b} ± 3.73	96.64^{b} ± 2.97	9.52^{b} ± 0.48	0.00^{b} ± 0.00	0.00^{b} ± 0.00	48.11	82.33	98.26	100.00	100.00
Gr4: control	503.56 ± 18.45	565.76^{a} ± 13.69	703.6^{a} ± 33.25	802.80^{a} ± 8.41	940.80^{a} ± 7.66	1023.29^{a} ± 4.65	−12.35	−39.73	−59.42	−86.83	−103.34
LSD	0.00	185.56*	91.00*	793.23*	940.80*	1023.92*	0.00	0.00	0.00	0.00	0.00

Mean number of alive lice ± standard error.
One hundred pigeons were randomly divided into four equal groups (25 birds/each).
PrT = mean number of louse/bird (naturally infested) on day 0, just before treatment.
PT = post-treatment.
(Gr1 = the first group, sprayed with 8% camphor oil and few drops of Tween 80.
(Gr2) = the second group, sprayed with 9% *d*-phenothrin(Item®).
(Gr3) = the third group, sprayed with 0.005% (50 mg/L or 1 ml/L) deltamethrin (Butox® 50 EC).
(Gr4) = the fourth group, sprayed with distilled water and few drops of Tween 80.
Data were analyzed by one-way ANOVA.
Means with different letters in the same column are significantly different using Duncan tests at $P \leqslant 0.05$.
* LSD = Least significance difference among means at $P \leqslant 0.05$.

such as organochlorines, organophosphates, pyrethroids, and carbamates [26,27]. Analogous to our *in vivo* results, ivermectin effectively controlled pigeon lice [16,17]. A single treatment with the preparations containing the insecticidal substances, cypermethrin, permethrin, and propoxur, removed completely the infestation with chewing lice, *Menopon gallinae*; *Eomenacanthus stramineus*; *Menacanthus cornutus*, and *Goniocotes gallinae*, 6 h post-treatments [28].

A comparable prolonged effect of DMT against *A. persicus* [19] and the sheep body louse, *Damalinia ovis* [29] was recorded. As sunlight does not break pyrethroids down, they stick to surfaces for weeks, killing any bypassing insect, which explains the lengthy effect of DMT [30] observed in the present study. Despite their efficacy in controlling ectoparasites, conventional insecticides, including synthetic pyrethroids, pollute the environment, undesirably affect non target orgasms including humans [11], adversely affect reproduction directly, leading to egg shell thinning due to its effect on calcium metabolism [31]. Acute effects of DPH and DMT are caused by concentrations > 2500 and > 4640 mg/kg body weight of birds, respectively [32]. It is worthy to mention here that we applied highly diluted concentrations of both pyrethroids, *in vivo*. Besides the previous side effects of conventional insecticides, marked levels of resistance has been developed due to the repeated use and inadequate application methods of lousicides, such as DMT [9] and DPH [10]. Consequently, health-care providers now face a serious lack of new commercial pediculicides. Solutions form the Mother Nature could be safer and helpful than currently used conventional insecticides.

Several botanicals, analogues to our *in vivo* results, efficiently controlled poultry lice infections. Successful control of *Menacanthus stramineus* infestations after dipping of laying hens (3 times) in neem (*Azadirachta indica*), Ruda (*Ruta graveolens*), and Solanacea (*Ardisia solanacea*) reduced lice infestation by 93.6%, 85.2%, and 98.2%, respectively [33]. One hour after dipping the infested birds completely into the 1:33 tap water-diluted MiteStop® solution (based on a neem seed extract), all motile stages (nymphs and adults) of the shaft louse, *M. gallinae*, the elongate feather louse, *Lipeurus caponis*, and *Columbicola* sp. were dead [5]. Pestoban (an herbal product with unknown constituents) was also very effective against lice infestations of poultry [34]. Our result is in harmony with that of Khater et al. [13] as the *in vivo* treatment with essential oils (pour-ons) against the carabao louse revealed 100% pediculicidal activity 0.5 and 120 min post-treatments with CAM (22%) and DPH (9%), respectively, and the number of lice infesting treated buffaloes was significantly (P < 0.05) reduced several days post-treatments. Data of the present study indicated that CAM was more effective than DPH and DMT. Analogues study indicated that essential oils was found to be at least, if not more, effective against human lice than DPH and pyrethrum, two commonly used pediculicides [35].

Essential oils and other botanical preparations induce ovicidal effects as the developmental stages in the nits of *M. gallinae*, *L. caponis*, and *Columbicola* spp. had been killed during the treatment of birds with MiteStop®, either the treatment for controlling biting lice was repeated or not [5]. In the same token, CAM and DPH induced ovicidal effect to the eggs of *H. tuberculatus* [13]. Consequently, it is expected from the previously mentioned work and our data that the applied materials could induce ovicidal effect toward *C. columbae* as natural lice infestations were effectively controlled for 28 days post-treatments.

Botanicals reduce egg hatchability probably due to toxicity of the oil vapors to nits [36]; diffusion of some chemical ingredients into eggs, thus affecting vital processes associated with embryonic development [13]; or blockage the aeropyles of the eggs, thus preventing the embryos of lice from accessing oxygen and from releasing carbon dioxide [37]. Botanicals repel insects as well as killing them [38,39]. CAM and DPH repelled *H. tuberculatus* and nuisance flies infesting buffaloes several days post-treatments [13]. Neem seed extract, MiteStop® *in vitro*, repelled cutoff feathers contaminated with *L. caponis* [5]. Consequently, it is anticipated that CAM and DPH could repel not only lice, but also the other ectoparasites infesting pigeons.

No abnormal health observations or skin irritations were noted on either birds or spraying operators after exposure to CAM and DPH. Similar result was reported [13]. An exception that temporary lacrimation had been observed in few sprayed birds with CAM and DPH. In contrast, coughing, sneezing, and temporary ocular inflammations without dermatitis were observed shortly among pigeons after spraying with DMT. This may be attributed to the obvious kerosene-like odor of DMT. Similar observation for DMT had been reported [19].

In respect to the mode of action of essential oils, they interfere with basic metabolic, biochemical, physiological, and behavioral functions of insects. Essential oils or their related products are mostly nontoxic to non-target organisms, but some of the purified terpenoid ingredients of essential oils are moderately toxic to mammals. Therefore, using crude oils is much safer than using purified ingredients. Many of the commercial products that include essential oils are on the 'Generally Recognized as Safe' (GRAS) list fully approved by the Food and Drug Administration (FDA) and Environmental Protection Agency (EPA) in USA for food and beverage consumption [38–40]. Being a green-plant product, camphor is quite an eco-friendly source of insecticide [12]. As a consequence; EO-based pesticides have become research hot spots because of their environmental safety and efficacy [38–40].

5. Conclusion and recommendations

It is very crucial to inspect pigeons at regular intervals for the occurrence of lice that may harm the health of the pigeon and cause enormous damages and economical losses in egg production and/or meat growth which decrease the economic revenue of their owners. Chemical lousicides should be considered the last resort after sanitation and management methods have been tried considering that sole reliance on lousicides often results in resistance, control failures, and higher pest populations. CAM has potential for the development of new and safe control product for poultry lice which might be used as prophylaxis besides treatment of diagnosed cases as well as air refresher [12]. Treatment is usually effective and is best carried out 2 weeks apart [5,13] to ensure that the life cycle is completely broken and all birds on the property should be treated at the same time. All new birds should be treated on arrival and not mixed with resident birds until deloused.

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