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Original article

Comparative efficacy of different insecticides in the treatment of cattle hypodermosis in north-eastern Algeria

Ahmed Benakhla^a, Bertrand Losson^{b*}, Jean-François Lonneux^b,
Chantal Boulard^c, Djamel Benouareth^d

^aInstitut des sciences agronomiques et vétérinaires, Université d'Annaba, Algeria

^bFaculté de médecine vétérinaire, Parasitologie et pathologie des maladies parasitaires,
Bd de Colonster, 20, B 43, Sart Tilman, Université de Liège, 4000 Liège, Belgium

^cPathologie aviaire et parasitologie, Station de Tours, Institut national de la recherche
agronomique, Nouzilly, 37380 Monnaie, France

^dInstitut des sciences naturelles, Université d'Annaba, Algeria

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Abstract – The efficacy of different drugs (microdose of ivermectin, doramectin and moxidectin at the recommended doses and topically applied rotenone) against the first, second and third instar larvae of *Hypoderma bovis* and *H. lineatum* was studied in cattle in the El Tarf area (north-eastern Algeria). Before performing the efficacy trials, the life cycle of the warble fly was studied at the slaughterhouse between July 1993 and June 1994. This survey revealed that both species of warble fly were present in this area: L1 larvae of *H. lineatum* were found around the oesophagus between August and January, whereas L1 larvae of *H. bovis* were observed around the spine from November until March. Warbles were present under the skin from the end of October until May. The nymphal phase took place between February and May. As a result of this study the authors recommend applying chemoprophylaxis (treatment against L1) in September. This work also confirmed the excellent efficacy of avermectins and milbemycins against the three instar larvae of *Hypoderma* sp. under the field conditions prevailing in Algeria. Rotenone has a lower efficacy (95 %) which is limited to the second and third instar larvae. However, this drug appears useful in dairy cattle. © Inra/Elsevier, Paris

cattle / hypodermosis / chemoprophylaxis / chemotherapy / Algeria

Résumé – Efficacité comparative de différents insecticides dans le traitement de l'hypodermose bovine dans le nord-est de l'Algérie. L'efficacité de différents insecticides (ivermectine en microdoses, doramectine et moxidectine à la dose recommandée et roténone en appli-

* Correspondence and reprints

Tel: (32) 4 366 40 90; fax: (32) 4 366 40 97; e-mail: blossom@stat.fmv.ulg.ac.be

cation locale) vis-à-vis des larves de premier, deuxième et troisième stade d'*Hypoderma bovis* et *H. lineatum* a été étudiée chez les bovins dans la région d'El Tarf (Nord-Est algérien). Avant le lancement des essais, la chronobiologie de la mouche du varron a été étudiée lors de visites à l'abattoir d'El Tarf (Nord-Est de l'Algérie) effectuées de juillet 1993 à juin 1994. Cette étude préliminaire a révélé que les deux espèces d'hypodermes étaient présentes dans cette région. Les larves L1 de *H. lineatum* se sont retrouvées autour de l'œsophage d'août à janvier tandis que celles de *H. bovis* ont été observées aux environs de la moëlle épinière de novembre à mars. Les varrons, quant à eux, s'observent sous la peau du dos de la fin du mois d'octobre au mois de mai. La phase nymphale s'est déroulée de février à mai. À partir de ces données, les auteurs recommandent d'appliquer la chimioprophylaxie (traitement contre les L1) en septembre. Le présent travail confirme, sous les conditions de terrain qui prévalent en Algérie, l'excellente activité des avermectines et milbémécines sur les trois stades larvaires d'*Hypoderma*. La rotenone apparaît comme étant moins efficace (95 %) et son activité est limitée aux deuxième et troisième stades larvaires. Elle apparaît néanmoins comme utile chez le bétail laitier. © Inra/Elsevier, Paris

bovins / hypodermose / chimioprophylaxie / chimiothérapie / Algérie

1. INTRODUCTION

Cattle hypodermosis is a widely distributed parasitic infection in the Northern hemisphere. It is present in most countries between latitude 25 and 60°N [22, 23]. In Algeria, a high prevalence (83 % cattle infested) is observed [6, 8, 9]. In this country, both *Hypoderma lineatum* and *H. bovis* (Oestridae) infest cattle.

The two species of warble fly have an annual life cycle. The adult flies are active during the hot sunny days of summer. After mating, the female fly deposits 500–1 000 eggs onto the hair of its cattle host. At hatching, the first instar larvae emerge from the eggs, penetrate actively through the skin of their host and migrate for several months through the host's connective tissue to reach the subcutaneous tissue of the back. There, they molt into second and finally third instar larvae.

The migration patterns of the two species are different; *H. lineatum* first instar larvae follow the intermuscular connective structures and spend some time in the perioesophageal tissue, whereas *H. bovis* larvae follow the thoracic nerves towards the spine and spend some time in the peridural tissue [11].

Hypodermosis has an economic impact on the cattle and leather industry because

it interferes with the zootechnical performances of the animals and decreases the quality of the skin [2, 5, 12]. Controlling hypodermosis in Algeria is a priority. Many different veterinary drugs are now available on the market. Control measures may aim at the destruction of the migrating L1 larvae ('chemoprophylaxis') or at the subcutaneous L2 and L3 larvae ('chemotherapy'). Different types of treatments must be applied according to the biology and prevalence of the parasites. It is well known that the administration of a drug at the wrong time of the year may induce severe side effects [10, 13, 18]. These side effects are mainly observed in cattle treated at a time when the full grown larvae are around the oesophagus (*H. lineatum*) or the spinal cord (*H. bovis*). The present work aims to compare the prophylactic and curative efficacies of different drugs, namely: ivermectin, doramectin, moxidectin, and rotenone in a highly infested area. Ivermectin (Ivomec™) is an avermectin which at the recommended dose of 200 µg·kg⁻¹ has a high activity against insects including *Hypoderma* sp. [15]. Migrating L1 larvae are extremely sensitive to ivermectin even at a dosage as low as 0.2 µg·kg⁻¹ [16]. In fact, the microdosage of 2 µg·kg⁻¹ is routinely used in some countries dur-

ing eradication or control campaigns [3, 4]. Doramectin (Dectomax™) and Moxidectin (Nemadectin or Cydectin™) are members of the avermectin and milbemycin families, respectively. Both have a high efficacy against *Hypoderma* sp. [17, 21]. Rotenone (Tikizid™) is a plant insecticide with no lasting activity. It is mainly used to kill L2 and L3 larvae which have not been killed by winter prophylactic treatments [7, 14].

2. MATERIALS AND METHODS

2.1. Trial site

All trials were performed in the El Tarf area (north-eastern Algeria). In this area both species of fly are present and the level of infestation is high [6, 9].

2.2. Studies on the biological cycle of *Hypoderma* sp.

One year before the start of the efficacy trials, a survey was carried out in the slaughter house of Aïn Assel (El Tarf) in order to characterize the endoparasitic life cycle of the warble flies. The growth of the L1 larvae of *H. lineatum* and *H. bovis* was studied during their period around the oesophagus and the spinal cord, respectively. The goal of this study was to determine the best time of year for the application of the different therapies.

This survey was carried out from July 1993 until June 1994. Weekly visits to the abattoir were performed and a total of 350 animals examined. The collection of L1, L2 and L3 larvae from the slaughtered animals was performed as previously described [8].

2.3. Comparative therapeutic efficacy of different drugs

2.3.1. Animals

For practical reasons the trials had to be carried out on animals maintained under different types of management.

For the evaluation of the prophylactic efficacy (against L1) of the different drugs, 523 animals of a local breed in 54 herds with a mean size of 10 were ear tagged. Sixty percent of the animals were 1–2 years old; over 50 % were heifers, the remaining animals were lactating cows. The animals were maintained under an extensive system in a hilly area of 30 000 ha.

For the evaluation of the therapeutic efficacy (against L2-L3), 325 Black pie and Red pie animals were maintained under more intensive conditions on three different farms. They spent most of their time in pasture (March to November). All were ear tagged. These three herds included 190 Red pie heifers and 135 Black pie heifers. These cattle had been imported into the El Tarf area in order to increase dairy production.

2.3.2. Treatments

2.3.2.1. Chemoprophylactic efficacy

The different chemoprophylactic treatments were given on 26, 27 and 28 September 1994 as follows: the 523 animals were divided into three experimental groups (Groups I, II, III) of 127, 132 and 134 animals, respectively, and a control group of 130 individuals (Group IV) (table 1). One group of animals was given $2\mu\text{g}\cdot\text{kg}^{-1}$ of ivermectin pour-on (Merck Sharp and Dohme, Rahway, USA) applied on the tail basis, whereas two groups of animals received subcutaneously $200\mu\text{g}\cdot\text{kg}^{-1}$ of moxidectin (American Home Product, Princeton, USA) or doramectin (Pfizer, Groton, USA), respectively.

The efficacy of each treatment was assessed by performing warble counts monthly between 25 November 1994 and 25 February 1995. In the control group, warble counts were performed until the end of May 1995, i.e. the end of the emergence period in this area. Extraction of all present warbles was performed on ten randomly selected control animals and the collected larvae were identified as to the species and stage.

2.3.2.2. Chemotherapeutic efficacy

On the three selected farms, the animals were manually examined twice a month from September 1994 onwards. The data collected made it possible to form the different groups and to determine the date of treatment. For each animal the nodules which were present

Table I. Comparative chemoprophylactic efficacy of ivermectin, moxidectin and doramectin in *Hypoderma* infected cattle.

Treatment (dose) ^a	Numbers of animals	Mean numbers of warbles at day				
		0	60	90	120	150
Ivermectin (2 µg·kg ⁻¹)	127	0	0	0	0	0
Moxidectin (200 µg·kg ⁻¹)	132	0	0	0	0	0
Doramectin (200 µg·kg ⁻¹)	134	0	0	0	0	0
Control	130	0	6	14	20	25

^a The different chemoprophylactic treatments were given on 26, 27 and 28 September 1994. The efficacy of each treatment was assessed by performing warble counts monthly between 25 November and 25 February 1995.

were mapped on a grid and numbered. This made it possible to monitor the appearance of new warbles during the experimental period or to note the emergence of the larvae. Five groups of 65 individuals each were formed in such a way that the total number of warbles were similar in all groups (*table II*). Group I animals (1 536 warbles) were given an ivermectin injection at a dose of 200 µg·kg⁻¹, whereas individuals in Groups II (1 490 warbles) and III (1 380 warbles) received a 200 µg·kg⁻¹ injection of doramectin or moxidectin, respectively. In Group IV (1 245 warbles) rotenone (Tikizid®, Siegfried AG, Zofingen, Switzerland), diluted 1 in 10 in water, was applied on each warble with a cotton wool pad. Group V remained as an untreated control and in this group the emergence of warbles was monitored from November 1994 to May 1995. The different treatments were administered on 17 December 1994 and the evaluation of efficacy was performed on 2 January 1995, i.e. 2 weeks after treatment.

2.3.2.3. Expression of results

The efficacy of the different drugs against L1 larvae was expressed as % protection:

$$\left(\frac{I_c - I_t}{I_c} \right) \times 100$$

where I_c is the number of infested controls and I_t is the number of infested treated animal.

The efficacy of the different insecticides against warbles (L2 and L3) is expressed as % efficacy:

$$\left(\frac{W_c - LW_t}{W_c} \right) \times 100$$

where W_c is the mean warble count (controls) and LW_t is the mean count of living warbles after treatment.

The evaluation of chemotherapeutic efficacy is based on the evaluation of the viability of the parasites. This is determined by the visual examination of the nodules and the examination of the extracted larvae under a dissecting microscope.

1) Visual examination of the nodules: two types of nodules were observed following the administration of a chemotherapeutic treatment:

– Nodules that have collapsed. The breathing hole is sometimes plugged or has healed completely; those nodule may contain flacid or dried larvae. The larvae present in this type of nodule are considered as dead. From time to time it is possible to find free larvae in the hair coat. These larvae are always dead.

– Prominent nodules exhibiting a normal appearance; the larvae have visible spiracles in close contact with the breathing hole. In this category, the larvae are extracted from the nodule by the technique of Scholl and Barrett [20] in order to determine the species and parasitic stage.

2) Microscopic examination of the larvae: the viability of the larvae is assessed under a dissecting microscope at $\times 30$ magnification. The larvae are first dried on a filter paper. The viable larvae are motile; some larvae show only slight movement at the level of the stigmata. These larvae are also considered as alive.

2.4. Statistics

For each treated and control group, the animals with a positive warble count at any time were grouped. The differences between each treatment group and the control group in the proportion of animals having a positive warble count at any time of the study were compared using Fischer exact t test (two tail).

For each group, the number of living warbles on the back of each animal on day 0 were totalled. A total dead warble count was also recorded on day 0.

Between the control and each treatment group, the treatment differences in the proportion of dead and living warbles on day 14 were compared using the χ^2 approximation (with Yates correction).

3. RESULTS

3.1. Data on the biological cycle

The results obtained from the survey carried out on 350 animals slaughtered at the abattoir of Aïn Assel (El Tarf) between July 1993 and June 1994 revealed that:

– *Hypoderma* larvae are found in cattle from August until May.

– First instar larvae (L1) of *H. lineatum* are found around the oesophagus between August and January. The infestation intensity, (mean number of L1 per oesophagus), was 2 in August and 8 in

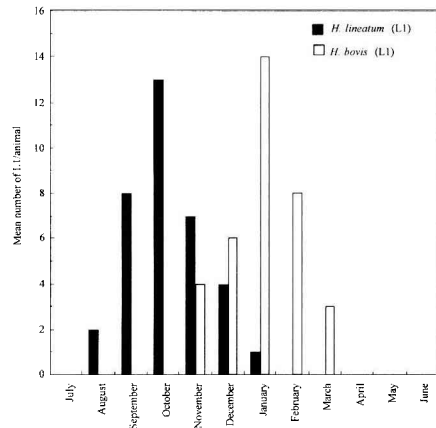


Figure 1. Evolution of mean numbers of L1 per animal.

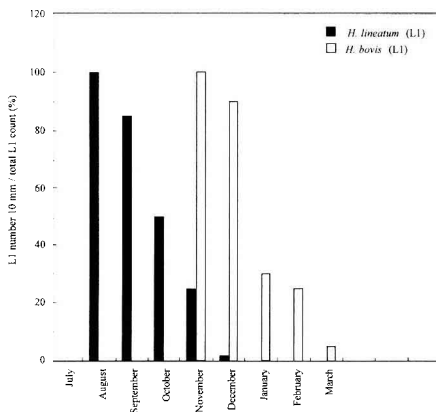


Figure 2. Evolution of the % L1 < 10 mm.

September. A peak number of 13 was reached in October with a gradual decrease to zero in February (figure 1). In addition, the size of the L1 larvae during their passage around the oesophagus varied between 4 and 18 mm. Until the end of September over 80 % of *H. lineatum* larvae were under 10 mm (figure 2).

– L1 of *H. bovis* were found in the spinal cord from November until March (figure 1). The mean level of infestation is maximum in January (14 larvae) (figure 1).

The L1 larvae of *H. bovis* could reach a length of 16 mm. Until December, 90 % of those larvae were below 10 mm (figure 2).

– The L2 larvae of *H. lineatum* were observed for the first time in their subcutaneous location in October; then their numbers decreased to zero in February. The L3 of the same species were observed from November until March (figure 3).

– The L2 of *H. bovis* were found from December until March and the L3 from December until May (figure 4).

– From February onwards many nodules were found to be empty.

3.2. Assessment of the efficacy of the different drugs

3.2.1. Chemoprophylactic activity

Warble counts performed between 60 and 150 days post-treatment were negative in all treated groups indicating 100 % protection, whereas in the control group all the animals were warbled (2–119 parasites) $P < 0.0001$ (table I). The manual

extraction of 360 larvae from ten animals was performed between November 1994 and May 1995 and revealed that 60 % of the larvae were *H. lineatum* and 40 % were *H. bovis*.

3.2.2. Chemotherapeutic activity

In all treated groups, the number of warbles containing living larvae decreased within a few days post-treatment. In the groups given ivermectin, doramectin or moxidectin, all larvae were dead by day 14 (100 % efficacy) and no new warbles were observed. This is in contrast with most of the animals of group IV (receiving rotenone) which did exhibit new nodules. In this group, efficacy reached 95 %.

Two observations were made in the group treated with rotenone: first, rotenone appeared to irritate most of the animals for 10–15 min after application and second, 90 % of the larvae that survived the application of rotenone were mature L3.

In the control animals most of the larvae were alive. The natural mortality reached only 0.5 %. The differences between the percentages of dead larvae

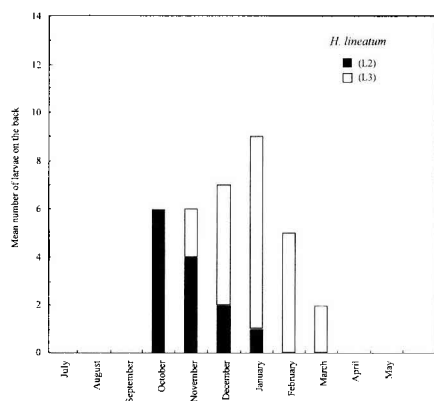


Figure 3. Evolution of mean numbers of *H. lineatum* larvae on the back.

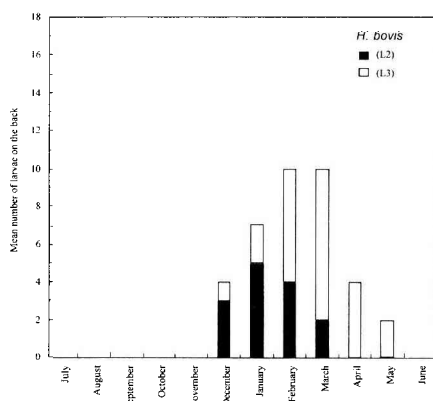


Figure 4. Evolution of mean numbers of *H. bovis* larvae on the back.

between treated and control groups were highly significant ($P < 0.0001$). It is noteworthy that 1–5 new warbles appeared on the back of some control animals between day 0 and day 14 (table II).

4. DISCUSSION

The survey performed between July 1993 and June 1994 at the Aïn Assel slaughterhouse allowed us to define the life cycle of the warble fly in this area of Algeria. This life cycle can be summarized as follows: L1 larvae of *H. lineatum* are found around the oesophagus from August until January and their length varies between 4 and 18 mm, whereas L1 larvae of *H. bovis* are observed around the spinal cord from November until March with a size of between 3 and 16 mm. The warbles are present under the skin from the end of October until the end of May. Finally, the emergence of L3 larvae occurs

from November until March for *H. lineatum* and from December until the end of May for *H. bovis*.

From these results, it is possible to determine the period of activity of the adult flies. Zumpt [23] indicates that the mean time of the larvae under the skin is 2–3 months and the nymphal stage is 17–70 d, whereas the life span of the adults is 3–7 d. Consequently, the period of activity of the adult flies in the El Tarf area is from April until August. A similar study was performed at the slaughterhouse of Constantine [8] and revealed a slightly different timeline. This is probably due to the climatic differences between the two areas (distance of 250 km).

These data are necessary to define the best period for the application of chemoprophylaxis. Chemoprophylaxis must be applied at the start of the endoparasitic phase when the L1 larvae are small and their enzymatic contents are reduced. In

Table II. Comparative chemotherapeutic efficacy of ivermectin, doramectin, moxidectin and rotenone in *Hypoderma* infected cattle.

Treatment ^a (dose)	Warble counts on day 0 Total (mean)	Warble counts on day 14	
		Number of dead larvae (% dead larvae)	New nodules (mean/animal)
Ivermectin (0.2 mg·kg ⁻¹)	1536 (24)	1536 (100)	0
Doramectin (0.2 mg·kg ⁻¹)	1490 (23)	1490 (100)	0
Moxidectin (0.2 mg·kg ⁻¹)	1380 (21)	1380 (100)	0
Rotenone (1/10)	1245 (19)	1169 (95)	67 (1.03)
Control	1325 (20)	7 (0.5)	72 (1.10)

^a Each treatment group comprised 65 animals. The different treatments were administered on 17 December 1994 and efficacy was evaluated 2 weeks after treatment.

fact, the growth of the larvae is accompanied by a progressive accumulation of antigens and toxins in the parasite gut and Boulard et al. [13] demonstrated that the side effects associated with the administration of active drugs in infested animals are due to hypersensitivity reactions which are positively correlated with the size of the larvae.

Ideally, in the El Tarf area, chemoprophylaxis should be administered in August when 100 % of larvae are under 10 mm. However, in August a few flies are still active and consequently it seems better to treat in September (80 % of larvae < 10 mm). If the animals have to be treated at the beginning of the subcutaneous phase treatment is recommended when the number and size of *H. bovis* larvae are low, i.e. in December (90 % of *H. bovis* L1 larvae < 10 mm). However, even at this period of the year it is not possible to avoid the appearance of side effects due to the destruction of larvae.

The efficacy of the microdose of ivermectin and the full dose of doramectin and moxidectin against the first instar larvae of *Hypoderma* sp. was 100 %; this is in agreement with previously published data [19]. The use of microdoses of ivermectin is particularly interesting because of its low cost and the absence of significant level of residues in milk [1].

The efficacy against second and third instar larvae of *Hypoderma* sp. of the full dose of ivermectin, doramectin and moxidectin was 100 %, whereas the efficacy of rotenone was 95 %. The appearance of new nodules on the back of the animals treated with rotenone confirms the strictly local activity of this molecule. These results are in agreement with the data of other teams working under different geographical and management conditions [19]. In addition, the observations made during the present work suggest that rotenone is more efficient on L2 and immature rather than mature L3. This

could be due to the thicker cuticle of the latter.

In conclusion, this study has defined the life cycle of the warble fly infestation in north-eastern Algeria and the best period for the application of chemoprophylaxis. It also confirmed the high efficacy of avermectins and milbemycins against first, second and third instar larvae of *Hypoderma* sp. under field conditions. The activity of rotenone is lower and limited to the subcutaneous phase of the life cycle. Its use should be limited to dairy cattle.

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