The extent of acaricide resistance in 1-, 2- and 3-host ticks on communally grazed cattle in the eastern region of the Eastern Cape Province, South Africa

Z Ntondini^a, E M S P van Dalen^{b*} and I G Horak^c

ABSTRACT

In order to determine the extent of acaricide resistance in the eastern region of the Eastern Cape Province 1-, 2- and 3-host ticks were collected from cattle at 59 dip-tanks over a period of 2 years. These ticks were tested for resistance against 3 compounds, namely amitraz, cypermethrin and chlorfenvinphos. The Shaw Larval Immersion Test detected emerging resistance to amitraz in *Rhipicephalus* (*Boophilus*) *microplus* at 2 dip-tanks and resistance at a 3rd. It also revealed resistance in this tick to cypermethrin at 1 dip-tank and emerging resistance to chlorfenvinphos at 8 dip-tanks and resistance at 2. *Rhipicephalus evertsi evertsi* was susceptible to amitraz and cypermethrin at all dip-tanks, but showed emerging resistance to chlorfenvinphos at 7 dip-tanks and resistance at 4. *Rhipicephalus appendiculatus* was susceptible to amitraz and chlorfenvinphos at all dip-tanks and demonstrated emerging resistance to cypermethrin at 1. With the exception of *R*. (*B*.) *microplus*, in which emerging resistance to amitraz was detected at 1 dip-tank by the Reproductive Estimate Test, all 3 tick species at all dip-tanks at which sufficient numbers of ticks had been collected were susceptible to the 3 acaracides in both the Egg Laying Test and the Reproductive Estimate Test. The localities at which acaricide resistance was recorded were mapped.

Key words: acaricide resistance, amitraz, chlorfenvinphos, cypermethrin, Eastern Cape Province, ixodid ticks, *Rhipicephalus appendiculatus, Rhipicephalus (Boophilus) microplus, Rhipicephalus evertsi evertsi.*

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INTRODUCTION

Ixodid ticks are without doubt the most important external parasites of cattle in large regions of Africa. The principal species that infest cattle in South Africa are *Amblyomma hebraeum*, a vector of *Ehrlichia ruminantium*, the cause of heartwater, *Rhipicephalus* (*Boophilus*) spp., which transmit *Babesia bigemina* and *Babesia bovis*, the cause of babesiosis, and *Rhipicephalus appendiculatus*, which transmits *Theileria parva*, the cause of East Coast Fever¹⁰. A 4th tick, *Rhipicephalus evertsi evertsi*, is also a common parasite of cattle¹².

Dipping cattle for the control of ticks and coincidentally tick-borne diseases has been practised in southern Africa for close on a century⁷. Considering the length of this period, it is not surprising that selection for resistance to acaricides has occurred in some tick species. Various studies have been conducted in South Africa and particularly in the Eastern Cape Province to determine the extent of this resistance^{2-5,8,9}.

The northeastern region of the Eastern Cape Province comprises the territory formerly known as the Transkei. This used to be a self-governing state in which dipping was a function monitored by the state and dipping compounds were supplied free of charge to stock farmers. All cattle owners were, however, required to pay a livestock levy, which was used to subsidise the purchase of the dipping compounds (S Gwababa, Department of Agriculture, Mthatha, pers. comm., 2006). In 1987 a new regional government came into power, and with it the livestock levy was stopped. The purchase of dipping compounds now became the responsibility of the state, which bought them on tender. During this time new compounds

came onto the market. These included organophosphates and synthetic pyrethroids as well as combinations of chemicals. At one stage 3 groups of compounds, namely amidines, organophosphates and synthetic pyrethroids, as well as combinations of organophosphates and pyrethroids, were used in various parts of the region, with each being used nearly exclusively in a particular area. During summer, weekly dipping of cattle in the coastal regions and fortnightly dipping further inland were enforced, while during winter the dipping interval along the coast was extended to 28 days, with a total suspension of dipping inland.

The purchase of dipping compounds on tender could promote the selection for acaricide resistance, because the dipping compound selected by the state authorities, namely amitraz (Triatix 125, Coopers Veterinary Products), has now been used nearly exclusively for a number of years within the region. Selection for resistance would be particularly intense in the 1-host species Rhipicephalus (Boophilus) decoloratus and Rhipicephalus (Boophilus) microplus, both of which spend approximately 3 consecutive weeks on their hosts to complete their life cycles¹, of which more than 1 can be completed annually. Consequently, if each of these ticks completes 2 life cycles annually at a locality at which weekly dipping is practised, they and their offspring could theoretically be exposed to the same compound on 6 occasions during the course of a year. Recent studies on commercial farms in the Eastern Cape Province indicated that there is considerable resistance in these ticks to the 3 most commonly used groups of chemicals^{8,9}. The present paper records the results of a survey conducted on ticks on communally grazed cattle to determine the extent of acaricide resistance in the eastern region of this province.

MATERIALS AND METHODS

Sampling localities

A list of all the cattle dip-tanks in the eastern region of the Eastern Cape Province was obtained and 1057 of these,

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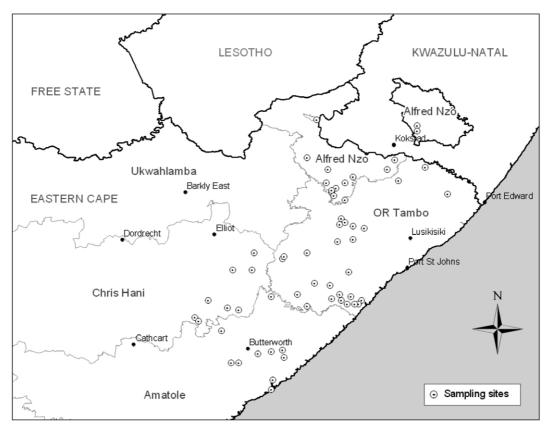


Fig. 1: Dip-tanks at which ticks were collected for an acaricide resistance survey in the eastern region of the Eastern Cape Province, South Africa.

at each of which more than 200 animals were dipped, were identified. Using tables of random numbers 55 of these dip-tanks were selected for the survey. Ticks for acaricide resistance testing were to be collected from cattle at 50 of the dip-tanks during the summers of 2004 and 2005. However, because insufficient numbers of ticks were collected at some dip-tanks during 2004, additional collections were made during 2005 and eventually collections were made at a total of 59 dip-tanks (Fig. 1). Cattle at 8 of the dip-tanks were sampled both in 2004 and 2005. At the same time a survey on the geographic distribution of ticks in this region was in progress and ticks were collected not only from cattle, but also from goats and dogs at the abovementioned 59 dip-tanks, and at an additional 14 tanks, as part of the $latter\ study^{11,12}.$

Sampling procedure

To ensure that the animals had not recently been dipped in an acaricide, arrangements were made with cattle owners to bring their animals to the dip-tanks on non-dipping days. If, however, dipping days were used for tick collection, ticks were collected before dipping commenced. Year-old cattle were selected and collections of adult engorged female ticks were made according to the predilection sites of the various species. At each of the dip-tanks an attempt was

made to collect engorged female *Rhipicephalus* (*Boophilus*) spp., *R. e. evertsi* and *R. appendiculatus*. These ticks were chosen as they represent 1-, 2- and 3-host species. Both *R.* (*B.*) *microplus* and *R.* (*B.*) *decoloratus* were to be collected, either individually or together, depending on the localities at which they were found. *Amblyomma hebraeum* was not considered as a 3-host target species as its distribution is restricted to the coastal regions of the survey area.

The sampling period of January to May was chosen because of the greater probability of collecting adult engorged female ticks during the warmer months of the year. The ticks were stored in separate plastic bottles with perforated lids according to the body site from which they had been collected. The bottles contained crumpled soft tissue paper to restrict movement and supply protection, and were placed, together with ice packs, in cooler boxes to prevent or delay oviposition before the ticks reached the laboratory.

The cooler boxes were dispatched to an acaricide resistance-testing facility at the University of the Free State, Bloemfontein. On arrival the specific identities of the engorged female ticks that had been collected were confirmed, and the number required for the tests selected. Although it was originally intended to use the indigenous tick *R.* (*B.*) decoloratus as the

1-host target species, it soon became apparent that it had to a large extent been displaced by the introduced 1-host tick *R*. (*B*.) *microplus*. Consequently the latter tick was used as the 1-host species.

Acaricide-susceptible reference strains

A susceptible strain of *R.* (*B.*) *microplus* was collected from cattle at Sabié, Moamba District, Maputo Province in Mozambique during April 2004 and imported into South Africa under a veterinary permit. No acaricides had been applied to cattle at the Sabié dip-tank during the previous 3 years. Unengorged male and female R. e. evertsi and R. appendiculatus were collected from a chemically immobilised Burchell's zebra in the northeastern region of the Hluhluwe-iMfolozi Nature Reserve in northern Kwazulu Natal Province, South Africa. The 3 susceptible reference species were transferred to an animal facility near Bloemfontein where they were cycled on cattle. These ticks were first tested for susceptibility to the 3 acaricides and were thereafter used as susceptible reference strains for comparison with the same 3 tick species collected from cattle in the Eastern Cape Province.

Acaricides

The 3 acaricides selected were amitraz, cypermethrin (Curatik, Bayer, Veterinary Health Division) and chlorfenvinphos (Disnis N.F., Bayer, Veterinary Health

Table 1: Shaw Larval Immersion tests conducted on 3 tick species in the eastern region of the Eastern Cape Province, South Africa.

Tick species and acaricide	No. of localities at which tests were done	Number of localities at which there was:		
		Susceptibility	Emerging resistance	Resistance
Rhipicephalus (Boophilus) microplus	5			
Amitraz	45	42	2	1
Cypermethrin	44	43	0	1
Chlorfenvinphos	36	26	8	2
Rhipicephalus evertsi evertsi				
Amitraz	49	49	0	0
Cypermethrin	43	43	0	0
Chlorfenvinphos	34	23	7	4
Rhipicephalus appendiculatus				
Amitraz	51	51	0	0
Cypermethrin	43	42	1	0
Chlorfenvinphos	35	35	0	0

Division). Amitraz, representing a compound with an amidine as its active component, was chosen because of its long uninterrupted use in the eastern region of the Eastern Cape Province. Cypermethrin was selected because it represents a 4th generation synthetic pyre-throid and is thus one of the more recently developed acaricides. Chlorfenvinphos, representing a compound with an organophoshate as its active component, was chosen because of its widespread and prolonged use as an acaricide throughout South Africa.

Acaricide resistance tests

During the National Acaricide Resistance Survey that had been conducted country-wide previously, the Shaw Larval Immersion Test (SLIT) was used as the standard test procedure ^{14,15}. Consequently this test was assigned the 1st priority in the current survey in order to generate data that could be compared with those obtained in the National Survey. The other tests performed were the Egg Laying Test and the Reproductive Estimate Test.

i. The Shaw Larval Immersion Test (SLIT)

Engorged female ticks of the 3 species were incubated at 27 °C and >75 % RH until they had oviposited and the eggs had hatched. The resultant larvae, between 16 and 21 days after hatching, were used in the SLIT, and were tested against concentrations of amitraz between 0.000006% and 0.1% of the active ingredient, cypermethrin between 0.00002 % and 0.2 % of the active ingredient, and chlorphenvinphos between 0.00013 % and 0.1 % of the active ingredient. Corrected mortality was used in a log-probit programme to determine the LC₅₀ and LC₉₉, and finally the Factor of Resistance was calculated by comparison with the LC₅₀ values obtained for the susceptible reference strains. Depending on the availability of larvae, they were tested against all 3 compounds.

ii. The Egg Laying Test (ELT)

Engorged female ticks of approximately uniform size and without any visible abnormality were selected from the samples and separated into 4 groups, each containing 10 ticks. Three of the groups were used as treatment groups, and the 4th served as an untreated control group. Each group was immersed for 10 min in the test acaricide at the concentration recommended for its use in the field, namely amitraz at 0.025 %, cypermethrin at 0.015 %, and chlorfen vinphos at 0.05 %. The ticks were then air-dried at room temperature for an hour, separated into groups of 5 ticks, weighed per group, pasted onto double-sided adhesive tape strips on glass test panels and incubated at 27 °C and >75 % RH. The control group of ticks was immersed in distilled water for 10 min and thereafter incubated separately from the treated groups to avoid acaricide contamination. After 7 days the ticks were examined to ascertain the numbers that had survived and were re-examined after 21 days when the eggs laid by each treatment group were weighed.

Twenty-nine of the 59 dip-tanks at which ticks were collected from cattle yielded sufficient ticks for the ELT. Resistance was calculated using the formula:

$$\frac{\text{No. of treated ticks laying eggs}}{\text{No. of untreated ticks laying eggs}} \times 100.$$

A value of above 80 % indicated resistance, and between 50 and 80 % emerging resistance. Anything below 50 % indicated susceptibility.

iii. The Reproductive Estimate Test (RET)

The same initial procedures as for the ELT were followed, and thereafter the eggs that had been weighed were trans-

ferred to labelled flasks and incubated at the same temperature and relative humidity as used in the ELT. The eggs were examined for hatching after 42 days and using the formula below to estimate the number of larvae that had hatched, the Reproductive Estimate (RE) of the engorged females was calculated:

$$\mathrm{RE} = \frac{m_1 \times n \times h}{m_2 \times s \times h/4}$$

where m_1 = mass of eggs per treatment group (mg); m_2 = mass of engorged female ticks per treatment group (mg); n = number of ticks per group; h = hatchability of the eggs (scale of 0 to 4: 0 = none hatching to 4 = 75–100 % hatching); and s = number of ticks surviving after 7 days of incubation

The %RE for female ticks was then calculated using the formula:

$$\%$$
RE = $\frac{\text{RE of ticks treated with acaricide}}{\text{RE of untreated ticks}} \times 100.$

An RE value above $80\,\%$ indicated resistance, and between $50\,\text{and}\,80\,\%$ emerging resistance, anything below $50\,\%$ indicated susceptibility.

Questionnaire survey

At each of the 72 dip-tanks at which ticks were collected for the survey on their geographic distribution, including the 59 dip-tanks used for the acaricide resistance survey, an owner's perception questionnaire was completed.

RESULTS

The results of the SLIT test indicated that there was emerging resistance to amitraz in *R*. (*B*.) microplus at 2 dip-tanks and resistance at a 3rd (Table 1, Fig. 2). This tick was also resistant to cypermethrin at 1 dip tank, while emerging resistance to chlorfenvinphos at 8 tanks and resistance at 2 were also recorded. The 2-host tick *R. e. evertsi* showed emerging resistance to chlorfenvinphos at 7 localities and resistance

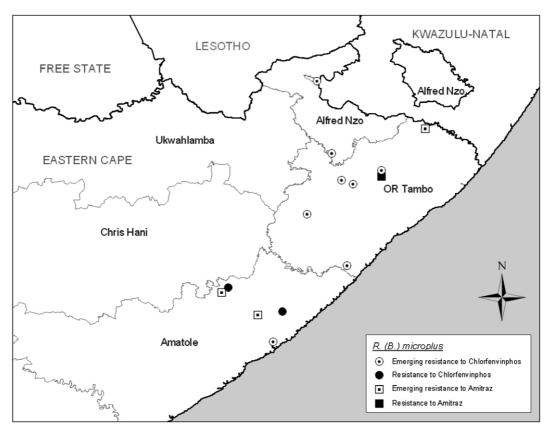


Fig. 2: Dip-tanks at which the 1-host tick *Rhipicephalus* (Boophilus) microplus displayed resistance to acaricides in the eastern region of the Eastern Cape Province, South Africa.

tance at 4 (Fig. 3), while emerging resistance to cypermethrin was recorded in the 3-host species *R. appendiculatus* at a single locality.

The ELT detected no resistance to any of the acaricides in *R*. (*B*.) *microplus* or *R*. *appendiculatus* (Table 2), while the RET indicated emerging resistance to amitraz in *R*. (*B*.) *microplus* at 1 dip-tank. At the Tutura dip tank, at which cattle were sampled in 2004 and 2005, the RET indicated that *R*. (*B*.) *microplus* was susceptible to amitraz in 2004 and showed emerging resistance in 2005.

Because too few female *R. e. evertsi* had been collected, ELTs and RETs with amitraz could only be performed on ticks collected at 2 dip-tanks and with cypermethrin on ticks collected at a single dip-tank. No tests with chlorfenvinphos could be conducted. No resistance could be detected in *R. e. evertsi* at the 3 dip-tanks.

Answers to the questionnaire survey indicated that the cattle owners perceived protection against tick-borne disease as the main advantage to be gained from dipping. All respondents grazed their cattle on communal land throughout the year and all dipped their cattle in summer, during which 88 % of respondents said that they dipped once every 2 weeks. Virtually all respondents (97 %) also dipped during winter at a frequency of once a month (91 %). At 31 of the 72 dip-

tanks at which ticks had been collected for the geographic distribution survey, the owners stated that they were not satisfied with the efficacy of the acaricide.

DISCUSSION

The spread of acaricide resistance can be ascribed to any one, or combination, of at least 3 factors. The 1st is the continuous regular use of compounds belonging to the same chemical group, without concurrent frequent monitoring for emerging resistance. The 2nd is the system of communal grazing practised throughout much of the northeastern region of the Eastern Cape Province, in which cattle belonging to different owners graze unfenced communal land⁵. Animals that harbour acaricide-resistant ticks would disseminate these within the communal grazing area. The 3rd is the purchase of animals from nearby commercial farms on which they had been subjected to intense, regular applications of acaricide and were thus likely to harbour acaricideresistant ticks5. Cattle on one such commercial farm in a region adjacent to the present survey area harboured a strain of R. (B.) decoloratus that was resistant to all 3 chemical groups currently tested⁹.

Although amitraz had been used for several years against ticks on cattle at most, if not all, of the dip-tanks sampled, there were very few at which emerging resistance or resistance was detected. This

result is contradicted by the opinions of stock owners at 31 of the 72 dip-tanks sampled in the geographic distribution survey, who indicated that they were not satisfied with the efficacy of the compound. It thus seems that at these 31 localities either dipping in under-strength dip-wash had occurred, or that the mode of action of amitraz, which is expellant and not immediately lethal against attached ticks¹⁷, resulted in the owners observing ticks on their animals a day after treatment. It could also be that the particular formulation of amitraz used for tick control was one that had very little residual effect. If cattle were dipped at greater than weekly intervals with this formulation during seasons of high tick abundance, farmers might also perceive that it was not effective. If, however, under-strength dipping was the reason for the owners' dissatisfaction, it might also have led to selection for low-intensity resistance. In this event the ticks should ideally be monitored for the emergence of resistance. In contrast to the low prevalence of resistance to amitraz detected in the present survey, a study on a commercial farm in the Eastern Cape Province indicated emerging resistance in R. (B.) decoloratus to amitraz in the SLIT and resistance to this chemical in the ELT and RET9.

According to Roush¹³, selection for resistance is most rapid when there is discrimi-

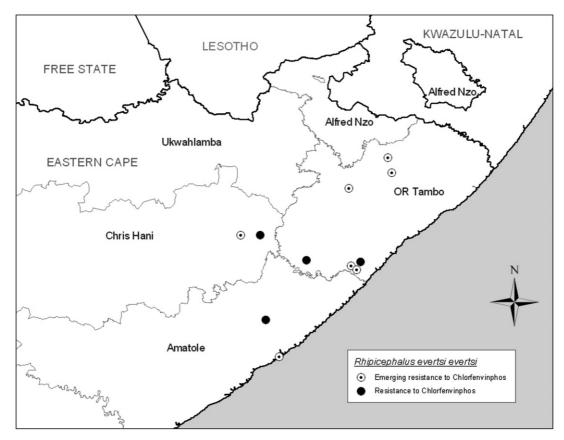


Fig. 3: Dip-tanks at which the 2-host tick *Rhipicephalus evertsi evertsi* displayed resistance to acaricides in the eastern region of the Eastern Cape Province, South Africa.

nation between heterozygotes and susceptible homozygotes within a population, in other words when resistance is effectively dominant. Selection would in general be slower when resistance is effectively recessive and heterozygotes are killed by ordinary field applications of the chemical¹³. The apparent low selection for resistance against amitraz indicated by the laboratory assays in the

present study might thus be due to a recessive factor, with heterozygotic individuals being killed by application of the acaricide in the field.

With the exception of emerging resistance to cypermethrin, observed in the SLIT in 1 of the 43 lots of *R. appendiculatus* tested, no resistance to this compound was evident in any of the tests applied. Cypermethrin would thus be a good

replacement compound should a change in acaricides be contemplated.

In 1982 resistance to organophosphorus-based acaricides was recorded in *R*. (*B*.) *microplus* at at least 11 localities within the current survey area⁵. In the present study this tick was resistant to or displayed emerging resistance to chlorfenvinphos at 10 of the 36 localities at which it had been collected (Table 1).

Table 2: Egg Laying and Reproductive Estimate tests conducted on 2 tick species* in the eastern region of the Eastern Cape Province.

Test, tick species and acaricide	No. of localities at which tests were done	Number of localities at which there was:		
		Susceptibility	Emerging resistance	Resistance
Egg Laying Test				
Rhipicephalus (Boophilus) microplus				
Amitraz	39	39	0	0
Cypermethrin	37	37	0	0
Chlorfenvinphos	32	32	0	0
Rhipicephalus appendiculatus				
Amitraz	31	31	0	0
Cypermethrin	26	26	0	0
Chlorfenvinphos	24	24	0	0
Reproductive Estimate Test				
Rhipicephalus (Boophilus) microplus				
Amitraz	39	38	1	0
Cypermethrin	37	37	0	0
Chlorfenvinphos	32	32	0	0
Rhipicephalus appendiculatus				
Amitraz	31	31	0	0
Cypermethrin	26	26	0	0
Chlorfenvinphos	24	24	0	0

^{*}Too few engorged female R. e. evertsi were collected to carry out these test procedures.

However, it is probable that neither this chemical nor other organophosphatebased dips had been used regularly or at all in the region during the previous 8 years. A similar result was obtained on 2 commercial farms in the Eastern Cape Province and on 1 in North West Province on which chlorfenvinphos had not been used for the past 10 years8. On the Eastern Cape farms, R. (B.) decoloratus was resistant or displayed emerging resistance to chlorfenvinphos, while in North West R. evertsi evertsi was resistant to this compound8. It would thus appear that once resistance to an organophosphate-based acaricide has become established in a population of ticks, the return to susceptibility is either very slow or does not take place^{5,16}. Even when considerable 'dilution' of the original resistance has occurred after several years of non-use of a particular acaricide, it would seem that reselection for resistance is rapid once the compound is again applied⁵.

The answers to the questionnaire that accompanied the survey on the geographic distribution of ticks also highlight the reliance of stock owners on dipping as a means of tick, and hence disease, control. This practice could promote not only acaricide resistance through high-frequency, long-term application, but could also reduce natural herd immunity through lack of adequate tick challenge.

The fact that, because of its scarcity, we had to replace R. (B.) decoloratus as the 1-host tick of choice in the acaricide resistance tests, was supported by the findings of the geographic distribution survey. These indicated that the introduced Asiatic blue tick, R. (B.) microplus, had effectively displaced the indigenous blue tick, R. (B.) decoloratus. R. (B.) microplus was present on cattle at 69 of the 72 dip-tanks sampled¹², while R. (B.) decoloratus was collected from cattle at only 4 of these localities. This displacement has serious implications for the transmission of Babesia bovis in cattle, an organism that is not transmitted by the indigenous R. (B.) decoloratus, but only by the introduced R. (B.) microplus.

The geographic distribution survey also highlighted the role played by goats as hosts of adult ticks that are normally associated with cattle. In fact, in this survey more goats than cattle were infested with *R. appendiculatus* and *R. e. evertsi*, and goats were also found to be good hosts of adult *A. hebraeum* and *R.* (*B.*) *microplus*¹². Therefore, unless goats are included in tick control programmes, the long-term results may be unsatisfactory. From another perspective, however, the non-

inclusion of goats in tick control programmes means that they could serve as refugia for untreated susceptible ticks and thus delay selection for acaricide resistance.

Provided an inexpensive, reliable and rapid acaricide resistance testing service was available, 6-monthly testing of at least 1-host ticks at each locality would be ideal. This would enable the cattle owner or provincial authority to change to a dipping compound belonging to a different group of chemicals soon after emerging resistance to a particular compound or group of compounds was detected at a specific locality. It could also preclude frequent switching between acaricides for no valid reason.

Several veterinarians, animal health technicians and stock owners have expressed concern at the perceived increase in myiasis in cattle in the survey region. This is particularly so for the coastal areas, where *A. hebraeum* is present. Amitraz does not affect blow flies or their larvae. Its use to the exclusion of other compounds that have both acaricidal and insecticidal properties has probably contributed to this situation. Some form of intervention with a chemical with insecticidal properties to contain the spread of myiasis seems advisable.

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