Acaricide resistance in the cattle tick, *Amblyomma variegatum*, in the coastal savanna zone of Ghana

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SUMMARY

Field strains of the cattle tick, *Amblyomma variegatum*, collected from 10 herds scattered in the western part of the coastal savanna zone of Ghana, were tested for resistance to acaricides, according to the FAO Acaricide Test kit. Discriminating doses were obtained from tests with a reference susceptible strain of *Boophilus microplus*. The LC_{50} values, where possible, were derived from fitted lines for graphs of mortality rates versus acaricide concentration for the various herds. Tick larvae from all the herds were completely susceptible to cypermethrin (0.025, 0.05, 0.10, 0.20, 0.40, and 0.80 %) and flumethrin (0.003, 0.006, 0.012, 0.015, 0.03, 0.06, 0.12, 0.25, and 0.50 %). However, eight herds had significant proportions of tick larvae showing resistance to diazinon at 0.2 per cent concentration, while three herds showed resistance to 0.2 per cent dieldrin. At the discriminating dose of 0. 2 per cent coumaphos, none of the ticks from the herds tested showed resistance. The results indicate possible resistance of field strains of *A. variegatum* to organophosphates, toxaphene, and/or lindane. The implications of this finding are important, if tick control measures should be effective in the coastal savanna zone of Ghana.

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RÉSUMÉ

TURKSON, P. K. & BOTCHEY, M.: *La résistance de la tique bovine* (Amblyomma variegatum) à *l'acaricide dans la zone de savane côtière du Ghana.* Quatre races de la tique bovine *Amblyomma variegatum* du champs, enlevées de 10 troupeaux dispersés à l'ouest de la zone de la savane côtière du Ghana, étaient mises à l'essai pour la résistance à l'acaricide, selon le Kit d'Essai d'acaricide de la FAO. Des doses discriminantes étaient obtenues des essais utilisant une race susceptible de *Boophilus microplus* comme une référence. Les valeurs LC_{50} étaient dérivées, dans la mesure du possible, de lignes ajustées pour les graphiques de taux de mortalité par opposition à la concentration d'acaricide pour les divers troupeaux. Les larves de tique de tous les troupeaux étaient complètement susceptibles à cypermethrin (0.025, 0.05, 0.10, 0.20,0.40 et 0.80 %) et flumethrin (0.003, 0.006, 0.012, 0.015, 0.03, 0.06. 0.12, 0.25. et 0.50 %). Cependant, 8 troupeaux avaient des proportions considérables de larves de tique montrant une résistance à diazinon à la concentration de 0.2 pour cent alors que 3 troupeaux montraient une résistance à 0.2 pour cent de dieldrin. Avec les doses discriminantes de 0.2 pour cent de coumaphos, aucune de tiques des troupeaux mis à l'essai ne montrait une résistance. Les résultats indiquent une résistance possible de races du champ d'*Amblyomma variegatum* aux organophosphates, toxaphene et/ou lindane. Les conséquences de ces résultats sont importantes, si les mesures de la lutte contre la tique devraient être efficaces dans la zone de la savane côtière du Ghana.

Introduction

Ticks are important in livestock production in Ghana either as vectors or reservoirs of diseases or as pests. In heavy infestations, ticks are responsible for economic losses resulting from loss of production of meat and milk as well as decreased value of hides (FAO, 1984). The predominant species of cattle ticks in Ghana is

Amblyomma variegatum (Morrow, Koney & Heron, 1996; Bell-Sakyi *et al.*, 1996). In one study, *A. variegatum* strains formed about 70 per cent of ticks from Friesian, Ndama, and Sanga cattle (Morrow *et al.*, 1996). In Ghana, *Amblyomma* ticks transmit heartwater and two normally non-pathogenic species of bovine theileria, *Theileria mutans* and *T. velifera,* and are also associated with dermatophilosis, a bacterial skin disease of cattle (Bell-Sakyi *et al.*, 1996).

The major tick control method in Ghana is the use of acaricides to reduce infestations. Resistance to acaricides may occur wherever acaricides have been used against ticks (FAO, 1984). In a survey, Awumbila (1996) reported that farmers and herdsmen in Ghana used 16 out of 24 different types of acaricides. Acaricides have been introduced on the Ghanaian market purely on marketing basis, without considering whether ticks were resistant or still susceptible to those already on the market. There is scanty or no published information on monitoring of acaricide resistance in Ghana (Mitchell, 1996).

This study, therefore, aimed at using a standardized kit of five indicator acaricides to investigate the extent of acaricide resistance in the cattle tick, *A. variegatum,* in the coastal savanna zone of Ghana.

Materials and methods

Area

The survey was done on two institutional farms, and eight cattle herds were chosen purposively by the willingness of the owners to co-operate. These herds were located within the coastal savanna zone of Ghana, which is a humid ecozone with a thicket and grassland type of vegetation.

Cattle herds

The institutional farms were the University of Cape Coast farm (UCC) in Cape Coast (with 13 Ndama cattle) and the Agricultural Research Station of the University of Ghana (ARS) in Accra (with 50 mixed breed cattle). Routine disease control measures were in place on these farms. The other cattle herds in the survey (Herds 1 to 8; average size of 176 cattle; minimum 70, maximum 450) were scattered around in the Winneba District (now Awutu-Effutu-Senya District), which lies between longitudes 0° 30' and 0° 45' W and latitudes 5°15' and 5° 40' N in Ghana. This district has one of the highest concentrations of cattle in the coastal savanna zone. The common breeds were mixed or cross breeds, West African Short Horns, Sanga, and few Zebus and Ndamas. These animals were managed under a system of sedentary pastoralism. Diseases and pests in herds in this area were controlled on an *ad hoc* basis (Turkson, 1993).

Tick collection

Engorged females (20-30), and 2-5 males were collected by hand from the perineum, dewlap, ears, scrotum, groin, and udders of cattle with visible ticks in each herd between June 1993 and May 1994. They were transported in plastic containers with gauze tops to the laboratory, where taxonomic keys were used to identify each tick (Shah-Fisher & Say, 1989). Engorged *A. variegatum* females were placed in universal bottles with gauze covers and incubated at 27 °C and a relative humidity of 80 to 90 per cent. Eggs were laid between 10 and 16 days. These were collected and incubated further under the same conditions. Hatching took 37 to 41 days. For resistance testing, larvae of 14 to 21 days of age were used.

Acaricide resistance testing

The Acaricide Test kit, supplied by the World Acaricide Resistance Research Center in Berlin, Germany, was used. This test is a modification of the larval packet test (FAO, 1984). About 100 tick larvae were placed into packets of Whatman filter paper impregnated with an oil solution of an acaricide at specific concentrations. The acaricides used were diazinon (0.05, 0.1, 0.2, 0.4, 0.8%), dieldrin (0.1, 0.2, 0.4, 0.8, 1.6 %), coumaphos (0.025, 0.05, 0.1, 0.2, 0.4, 0.8, 1.6 %) cypermethrin (0.025, 0.05, 0.1, 0.2, 0.4, 8 %), and flumethrin (0.003, 0.006, 0.012, 0.015, 0.03, 0.06,

0.125, 0.25, 0.5 %). These acaricides were used as indicators of resistance in the various acaricide groups. Dieldrin was used as an indicator of toxaphene and lindane resistance, while diazinon was a general indicator of organophosphorus resistance. Flumethrin and cypermethrin were indicators of resistance against synthetic pyrethroids.

The packets were incubated at 27 °C and a relative humidity of 80 to 90 per cent for 24 h, after which the numbers dead or alive were counted for mortality rate calculations. Dosage-mortality responses were plotted on log-probit paper, and straight lines fitted by eye, from which the lethal concentration killing 50 per cent of the larvae (LC_{50} values) for each acaricide and herd were determined. No LC_{50} values were derived where all or most larvae were killed. Each test included filter paper impregnated with the oil solvent alone as control.

TABLE 1

List of Acaricides in Use or Previously Used in the Herds

Herd	*In use*	*Previously used*
UCC	Steladone©	Gammatox©
ARS	Bayticol©	Gammatox©/Delnav©
1	Gammatox©	Ectomin©/Supamix©
2	Gammatox©/Supamix ©	-
3	Actellic©	Supamix©
4	Steladone©/Ectopor©	-
5	Gammatox©	-
6	None over past 2 years	-
7	Steladone©	Gammatox©
8	Delnav©	-

Steladone© = chlorfenvinphos, an organophosphate.
Gammatox© = lindane or gamma benzene hexachloride, an organochlorine.
Delnav© = dioxanthion, an organophosphate.
Supamix© = mixture of chlorfenvinphos and dioxanthion.
Ectomin©/Ectopor© = cypermethrin, a pyrethroid.
Actellic© = pyrimiphosmethyl, an organophosphate (a crop pesticide).
Bayticol© = flumethrin, a pyrethroid.

For baseline data, a known reference acaricide-susceptible tick, the Yeerongpily strain of *Boophilus microplus* was tested for susceptibility to the specified acaricides under the laboratory conditions. This strain, an example of a one-host tick, is used as an internationally recognized standard of resistance or of susceptibility by the World Acaricide Resistance Reference Center in Berlin, with the justification that *Boophilus* ticks are good indicators of future acaricide resistance in all ticks in an area, since acaricide resistance develops faster in one-host ticks.

Ninety-nine percent confidence intervals for proportions were calculated as per Fleiss (1981) to test for significance in the difference in proportions between the suspected resistant strains and the reference susceptible strain.

Results

Table 1 lists the acaricides in use and previously used in the herds. In a survey of major pharmaceutical companies in Accra, other acaricides (apart from those in Table 1) available in the market for use in livestock included Asuntol © (coumaphos), Ivomec© (ivermectin, an avermectin), Sebacil© (an organophosphate), and " Spot-on© " (deltamethrin, a pyrethroid).

Table 2 shows the larval mortality rates for the different herds for diazinon, dieldrin, and coumaphos. There were 100 per cent mortality rates for tick larvae from all the cattle herds tested with the range of concentrations of cypermethrin and flumethrin (except for Herd 2 and Herd 6 where no flumethrin tests were done).

Table 3 shows the LC_{50} values for the various herds for diazinon, dieldrin, and coumaphos. The discriminating doses established for the acaricides was set at twice the lowest dose at which the reference susceptible strain showed complete susceptibility, and were diazinon 0.10 %, dieldrin 0.20 %, coumaphos 0.20 %, cypermethrin 0.10 %, and flumethrin 0.03 %.

Significant differences (P=0.01) were seen in the proportions of ticks from UCC, ARS, and Herds 1 to 8 that survived diazinon 0.10 %, and in

TABLE 2

Mortality Rates for Amblyomma variegatum *Larvae from 10 Cattle Herds in the Coastal Savanna Zone of Ghana*

	Herd									
Acaricide	*UCC*	*ARS*	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*
Diazinon 0.05%	44	44	0	0	3	0	33	0	0	0
Diazinon 0.10%	80	74	0	7	4	7	36	21	16	12
Diazinon 0.20%	99	98	54	35	18	26	84	75	75	37
Diazinon 0.40%	100	100	86	92	75	85	100	88	88	99
Diazinon 0.80%	100	100	100	100	100	100	100	100	100	100
Dieldrin 0.10%	44	82	100	92	76	34	100	100	100	100
Dieldrin 0.20%	92	94	100	98	100	93	100	100	100	100
Dieldrin 0.40%	100	99	100	100	100	100	100	100	100	100
Dieldrin 0.80%	100	98	100	100	100	100	100	100	100	100
Dieldrin 1.60%	100	100	100	100	100	100	100	100	100	100
Coumaphos 0.025%	nt	nt	nt	nt	nt	nt	10	6	0	0
Coumaphos 0.05%	nt	nt	nt	nt	nt	nt	45	66	79	48
Coumaphos 0.10%	100	100	100	100	100	100	95	97	100	100
Coumaphos 0.20%	100	100	100	100	100	100	nt	nt	nt	nt
Coumaphos 0.40%	100	100	100	100	100	100	nt	nt	nt	nt
Coumaphos 0.80%	100	100	100	100	100	100	nt	nt	nt	nt
Coumaphos 1.60%	100	100	100	100	100	100	nt	nt	nt	nt

nt = not tested

TABLE 3

Values for Amblyomma variegatum *Strains from 10 Herds in the Coastal Savanna Zone of Ghana*

Herd	*Diazinon %*	*Dieldrin %*	*Coumaphos %*
UCC	0.06	0.11	nd
ARS	0.07	nd	nd
1	0.28	nd	nd
2	0.22	nd	nd
3	nd	nd	nd
4	0.18	0.12	nd
5	0.11	nd	0.04
6	0.15	nd	0.04
7	0.16	nd	0.04
8	0.17	nd	0.04

nd = not determined

the proportions from UCC, ARS and Herd 4 that survived dieldrin 0.2 %, when compared to the reference susceptible strain. Interestingly, six herds (Herds 1, 2, 3, 4, 6, and 7) had significant proportions of ticks surviving diazinon 0.4 %. This was four times the discriminating dose.

Discussion

The significant proportion of ticks surviving diazinon 0.1% and dieldrin 0.2 % indicates that resistant ticks were present in all 10 herds, for diazinon, and in three herds, for dieldrin. In the test kit, diazinon is used as a general indicator of organophosphate resistance, while dieldrin is an indicator of toxaphene and lindane resistance. In essence, the findings indicate widespread resistance of field strains of *A. variegatum*, in the herds studied, to organophosphates and to a limited extent, resistance to toxaphene and/or lindane.

Table 1 indicates that lindane (as Gammatox) was or had been in use in six of the 10 herds in the study. Awumbila & Bokuma (1994) reported that a survey of

pesticides used on farm animals in Ghana in 1989 included organophosphates, synthetic pyrethroids, carbamates, and organochlorines; the most widely distributed and used pesticide is lindane, an organochlorine.

The mode of application of the acaricides varied. In one herd and the two institutional farms, a backpack sprayer was used in spraying the animals. In the other herds, two methods were common. Diluted acaricide was either squirted from a squeeze bottle on to the infested sites after milking, or was used to soak a piece of cloth and rubbed on the infested areas. Awumbila (1996) reported similar methods.

Awumbila & Bokuma (1994) stated that none of the herdsmen interviewed in their survey complained about resistance to acaricide, explaining that with 24 different acaricides in the country, resistance to any one compound was likely to develop slowly. However, this study shows some evidence of resistance of ticks to organophosphates, and to a limited extent, to lindane/toxaphene. It is possible that the herdsmen failed to recognize this problem because of the different types used, the frequency, and the erratic mode of applying the acaricides. It is also possible that the problem was not recognized because acaricide resistance in Ghana was just developing.

The mode and frequency of acaricide application is of concern. Luguru, Musisi & Chizyuka (1985) indicated that poor management of acaricides, use of low concentrations, inadequate application, and frequent changes from one acaricide to another accelerated selection, while increasing the proportion of ticks resistant to acaricides. As Awumbila (1996) noted, over 90 per cent of herdsmen interviewed hand-dressed their animals with diluted acaricide prepared by adding unknown amount of acaricide to unknown quantity of water. This, often, led to low strength since herdsmen tried saving money by stretching the amount of acaricide used. Herdsmen need to be taught how to use acaricides – dilution, application, and disposal – through extension materials in local languages and demonstrations by the Veterinary Services Department and companies importing acaricides.

Another concern is the large variation in types of acaricides available on the market with no information on their efficacies under local conditions and their effect on the environment. There is an urgent need to screen these acaricides and retain a few found effective as backup when others fail.

In conclusion, the significance of these findings is that this study has, for the first time, shown the existence of acaricide resistance in the cattle tick, *A. variegatum,* in the coastal savanna zone of Ghana. This calls for more work to cover other areas of Ghana and to monitor resistance in other tick species.

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