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***Invivo* and *invitro* effect of selected commercial acaricide formulations on acaricide resistant ticks in Uganda**

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Abstract

To address the emerging challenge of multi acaricide resistant ticks in Uganda, new acaricide molecules needed to be identified and tested against the Ugandan resistant tick strains. Three novel acaricide products were identified which included eprinomectin (5mg eprinomectin/mL), co-formulated product (cypermethrin (5g/l), chlorpyrifos (7g/l) and citronella) and another co-formulated product containing Cyamizol (17.5%) and cypermethrin (2.5%). The comparison products included a product containing 1% Flumethrin, co-formulated product (50g/L Cypermethrin and 500g/L chlorpyrifos). *Invitro* efficacy of the trial and comparison products was assessed in the laboratory using the FAO recommended larval packet test. A randomized field trial was conducted for a period of six months in two different regions of Uganda to assess the *invivo* efficacy of the selected products. Of the novel products, the laboratory and field evaluation findings showed that eprinomectin was highly effective (efficacy $\geq 90\%$) against all tick species on the randomly selected farms.

Keywords: Acaricide resistance, eprinomectin, field trials, *invivo*, *invitro*, ticks, Uganda

Introduction

In Uganda, ticks and tick-borne diseases (TBDs) remain the most significant constraint to development and improvement of the livestock industry. On dairy and beef farms, the control of ticks and TBDs are responsible for over 90% of disease control budgets and over 60% of the cost of total inputs (Ocaido *et al.*, 2009) [6]. The tick species of major economic and veterinary significance in Uganda include *Rhipicephalus appendiculatus*, *Rhipicephalus decoloratus* and *Amblyoma variegatum*. These cause major diseases: East Coast fever (*R. appendiculatus*), babesiosis (*B. decoloratus*), anaplasmosis (*B. decoloratus*) and cowdriosis (*A. variegatum*). Production losses are attributed to cost of inputs (mainly acaricides) and significantly high mortality rates with up to 30% mortality of the calf crop. The control of ticks is thus a major concern of livestock producers in Uganda. Recent reports also indicate emergence of multi-acaricide resistant tick populations in central and western regions of Uganda where there is excessive acaricide application (Vudriko *et al.*, 2006) [14]. To address the emerging challenge of multi acaricide resistant ticks in Uganda, new acaricide molecules or molecules which were not commonly used in Uganda needed to be identified and tested against the Ugandan resistant tick strains. This could help in identifying the best acaricide candidate for possible use in national tick resistance cleansing programs. Thus, efficacy studies were necessary to determine the effective acaricides which can be deployed for management of resistant ticks in Uganda in addition to controlling flies.

Therefore, the objectives of this study were as follows; to determine the efficacy of the products on multi acaricide resistant ticks of veterinary importance and determine the effective residual time of each product following application on animals. Furthermore, compare the efficacy of the trial products and document any adverse reactions and/or side effects attributable to each product in animals and the people who apply the products. *In vitro*

efficacy of each of the products against multi-acaricide resistant ticks was also determined.

Methods

Trial sites

For each product, trials were conducted under field conditions in areas with heavy tick and nuisance/biting fly challenges. Kyankwanzi, Kiruhura, Kaberamaido, Kyenjojo, Apac, Amolatar and Moyo Districts. Each product had two trial sites in two regions.

Trial animals/herds

Animals/herds were enrolled based on the following criteria:

- Keeping indigenous cattle (Ankole or shorthorn Zebu)
- Heavily infested with major ticks (*R. appendiculatus*, *Amblyoma* and *Boophilus*) and nuisance flies

Trial design

This was a randomized field trial where randomization was done at herd level. Selected herds in each of the trial sites were randomly allocated to receive the trial product or a comparable control product. All the animals in the selected herds received the same randomly allocated product. Each of the three trial products were tested in two herds per trial region. Treatments (dipping, pour on or spraying) strictly followed manufacturer recommended concentrations/dilutions and intervals. Another herd in the neighborhood was selected as the comparable control where a comparable product was applied. The comparable control was any herd in the vicinity applying another product other than the trial products. Each trial site had at least 50 adult animals.

The trial and comparison products

The first trial product contained Eprinomectin (5mg eprinomectin/mL) and was compared with a pour-on product containing 1% Flumethrin. The second trial product was a co-formulated product containing cypermethrin (5g/l), chlorpyrifos (7g/l) and citronella and was compared with a co-formulated product containing Cypermethrin, 50g/L and chlorpyrifos, 500g/L. The third trial product was also a co-formulated product containing Cyamizol (17.5%) and cypermethrin (2.5%) and was compared with a mono-formulation of Amitraz, 12.5%.

Baseline data

During baseline data collection, tick samples were collected for laboratory bioassays (acaricide efficacy testing). Assessment of tick burden in trial and control herds was also conducted for three consecutive days prior to treatments (spraying/dipping). Tick burden/challenge was computed as the total half-body tick counts per animal. Only adult (different levels of engorgement) ticks were counted *in situ*. The different species were noted by inspecting the known predilection sites for particular species. Tick samples were collected for *in vitro* acaricide efficacy testing. *In vitro* efficacy testing employed a standard FAO larval packet tests. For *in vitro* assays, molecules with 90% efficacy were regarded effective while resistance or ineffectiveness was assumed at efficacies less than 90%.

Monitoring of treatments

Tick re-infestation after dipping or spraying was monitored through half-body tick counts performed every two days after application.

Reporting and monitoring of adverse effects

The provision for recording any adverse effects attributable to the trial products in relation to humans and animals was assessed and recorded for all trial herds. All animals were closely observed for at least one hour every after application. Any unusual reactions attributable to product application was noted. The site investigator and the District Veterinary Officer (DVO) received and helped analyze any case of adverse effects or accidents. The investigator reported and managed such incidents in a timely manner.

Trial duration

The acaricide field trials were conducted for a period of six months and hence data was collected through wet and dry seasons.

Statistical analysis

Descriptive statistics were generated using frequency procedures of Epi Info®. Graphs showing the percentage efficacy of both trial and control products over the 6 month period were generated using Microsoft Excel spreadsheet version 13. To compare data between the trial and control products, a paired t-test was performed at 95% confidence level; thus, p-values ≤ 0.05 will be considered significant. To compare the efficacy of the different trial products, repeated measures ANOVA was performed in STATA software, version 14 at 95% confidence level; thus, p-values ≤ 0.05 will be considered significant.

Informed consent and institutional review

The institutional review board (IRB) of the School of Veterinary Medicine and Animal Resources (SVAR), Makerere University approved the trial protocol documents before implementation. The protocol was later approved by Uganda National Council of Science and Technology. Prior to recruitment into the trial, the farmers were educated on the details of the trial protocol and all the possible risks including the adverse or toxicity effects associated with the trial chemicals. Only farmers who willfully consented to the trial were recruited. The trial team underwent rigorous training on trial protocol implementation including all the supporting standard operating procedures. In addition, each of the investigators underwent training in good clinical practice (GCP).

Results

Tick populations used for *in vitro* acaricide susceptibility testing

Engorged ticks suitable for testing were collected from Kyankwanzi (5 farms), Kiruhura (10), Kaberamaido (6), Kyenjojo (4), Apac (1), Amolatar (2) and Moyo (1). A total of 29 tick populations were tested, belonging to three tick species; *Boophilus decoloratus* (14), *Rhipicephalus appendiculatus* (11) and *Amblyomma variegatum* (4).

In vitro Susceptibility of *B. decoloratus* ticks to trial acaricides

For the novel products, samples from all (100%) herds were resistant to the cyamizole-chlorpyrifos (DOD) products. Samples from most (70%) herds were resistant to the potentiated cypermethrin-chlorpyrifos co-formulation (AAO) too, thus only 30% of farmers could opt for the product (Figure 1). However, boophilus samples from up to 96% of the herds were susceptible to eprinomectin (COC/COD).

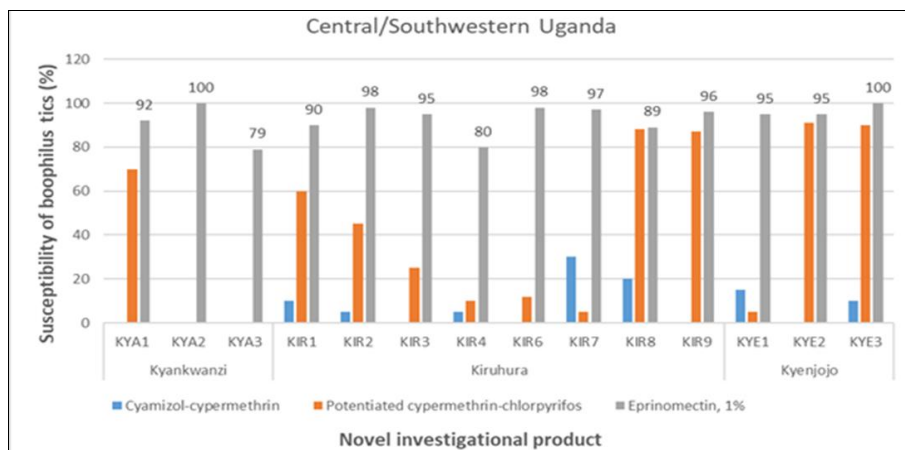


Fig 1: *In vitro* susceptibility of *B. decoloratus* ticks to the novel products

In vitro Susceptibility of *B. decoloratus* ticks to comparison acaricides

Figures 1 and 2 show *in vitro* susceptibility of *B. decoloratus* ticks to the registered and novel acaricide molecules, respectively. Tick samples from up to 57% of the herds in

central and southwestern Uganda were resistant against all registered commercial products (flumethrin, cypermethrin, cypermethrin-chlorpyrifos co-formulation and amitraz) in Uganda (Figure 2). About 43% of the herds could choose between amitraz or cypermethrin-chlorpyrifos co-formulation.

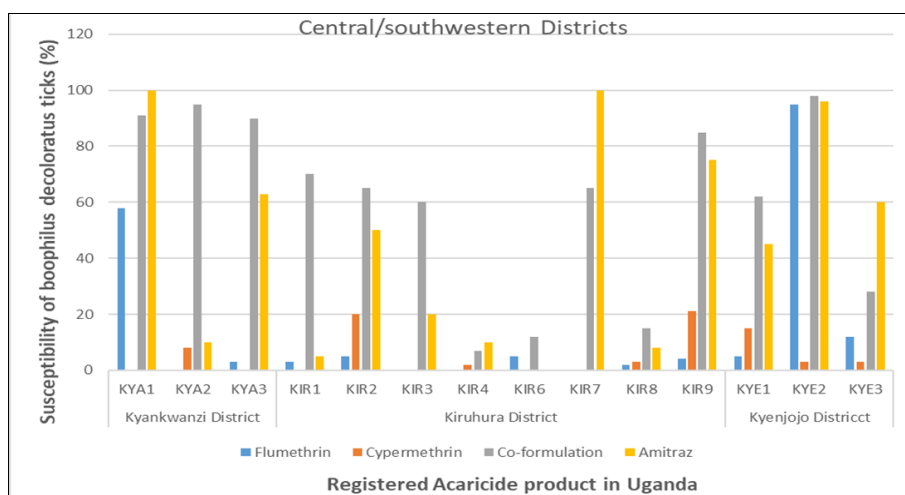


Fig. 2: *In vitro* susceptibility of *B. decoloratus* ticks to registered acaricides

In vitro Susceptibility of *Rhipicephalus appendiculatus* ticks to trial acaricides

In vitro susceptibility of *R. appendiculatus* to the novel cyamizol-cypermethrin co-formulation (DOD), was below 90% in all (100%) farms from central and southwestern Uganda (Figure 3). The product (DOD) was effective in 15% of *R. appendiculatus* samples from the north. The novel

potentiated cypermethrin-chlorpyrifos (AAO) product was efficacious (susceptibility $\geq 90\%$) against *R. appendiculatus* samples from 60 and 83% of herds from southwestern and northern districts, respectively. Eprinomectin (COC/COD) was however effective on at least 80% of farms from both northern and southwestern districts

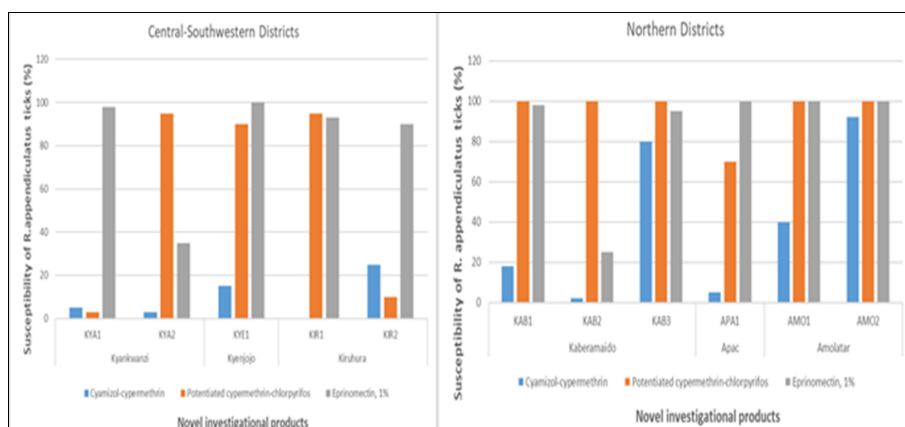


Fig 3: *In vitro* susceptibility of *R. appendiculatus* ticks to novel acaricide products

***In vitro* Susceptibility of *Rhipicephalus appendiculatus* ticks to comparison acaricides**

Figures 3 and 4 show *in vitro* susceptibility of *R. appendiculatus* ticks to registered and novel acaricide products, respectively. Samples from up to 40% of herds in central and southwestern Uganda, were resistant to all

registered acaricide products (flumethrin, cypermethrin, amitraz and cypermethrin-chlorpyrifos co-formulation). In these districts, 60% of the farms could use amitraz or cypermethrin-chlorpyrifos co-formulation (Figure 4). However, in the northern districts, at least 02 (two) or all the registered products were effective on all farms.

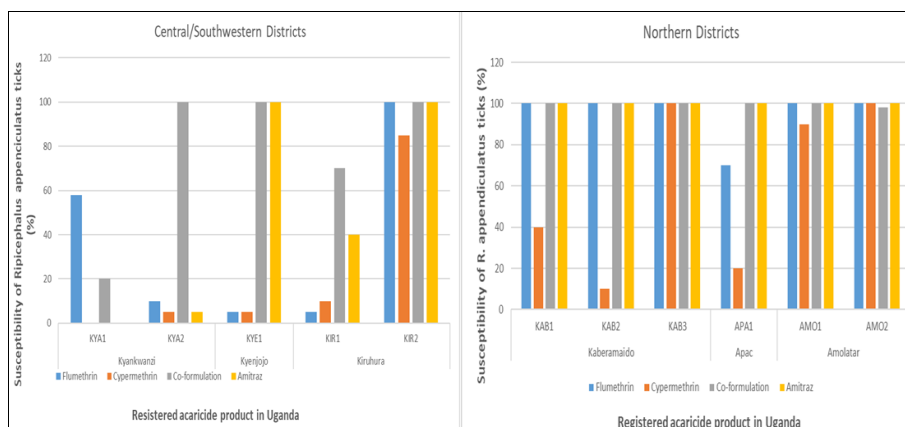


Fig 4: *In vitro* susceptibility of *R. appendiculatus* ticks to registered acaricides

***Invivo* Effect of the study Acaricide Formulations on Acaricide Resistant Ticks**

The effect of eprinomectin on acaricide resistant ticks under field conditions

Mean half-body tick counts and on-farm efficacy of eprinomectin (1%) and flumethrin (1%) products are presented in Figures 5 and 6. Pour-on products eprinomectin (1%) and flumethrin (1%) were effective (efficacy $\geq 90\%$) on

all participating farms in Amolatar District (North). However, for eprinomectin, reattachment of *R. appendiculatus* ticks occurred within 5-10 days after product application. On farms in Kyankwanzi District (central/south), Eprinomectin ($\geq 90\%$) was generally more efficacious than flumethrin ($\leq 90\%$). Like in Amolatar, herds on eprinomectin treatment were highly re-infested by *R. appendiculatus* ticks within just 5-10 days of product application.

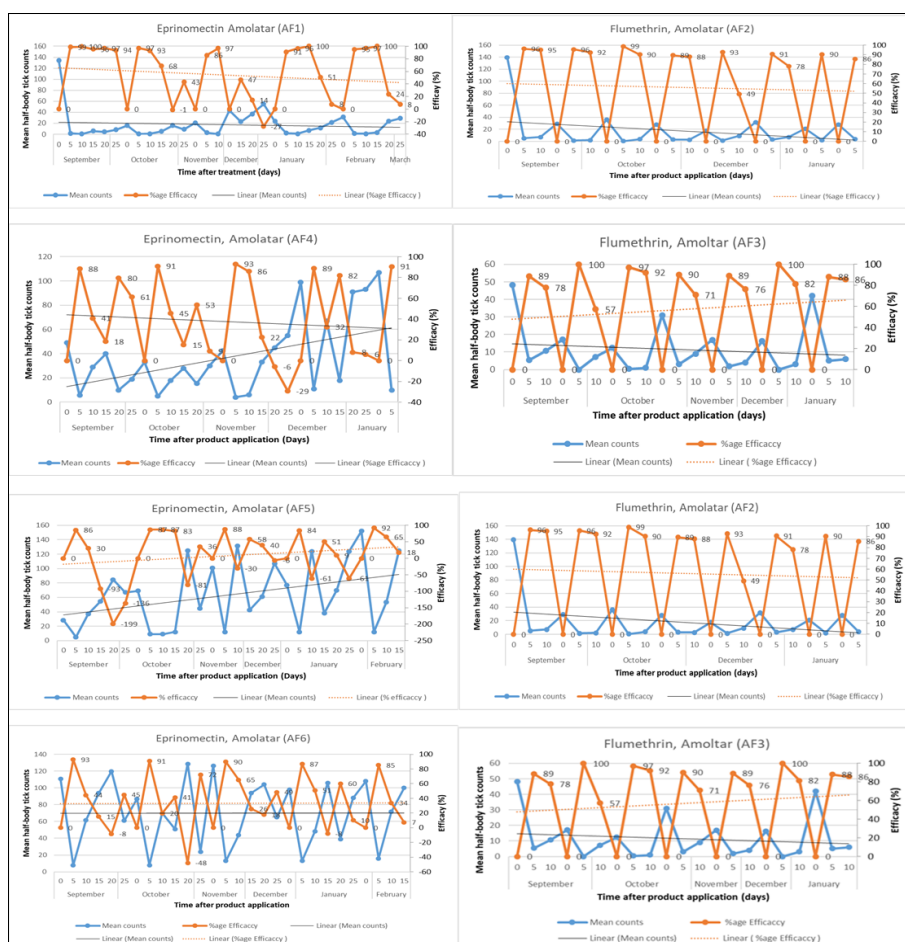


Fig 5: Field efficacy of trial product (Eprinomectin) and comparison product (Flumethrin) in Amolatar District

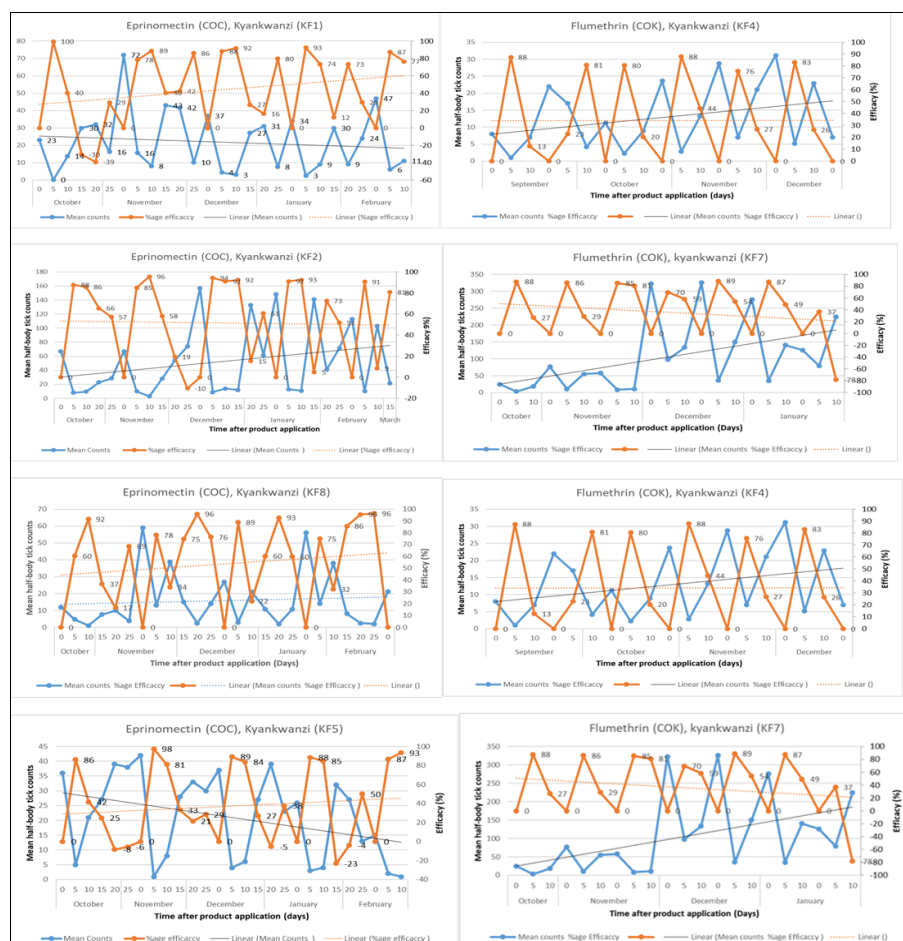


Fig 6: Field efficacy of trial product (Eprinomectin) and comparison product (Flumethrin) in Kyankwanzi District

The effect of potentiated co-formulation of cypermethrin and chlorpyrifos on acaricide resistant ticks under field conditions

Mean tick counts and efficacy of the novel potentiated cypermethrin-chlorpyrifos product (AAO) as compared to the registered cypermethrin-chlorpyrifos product (AIA) are presented in Figures 7 and 8. In the southern districts (Gomba,

Kiruhura and Nakaseke), both products generally had poor efficacy ($\leq 90\%$) in all experimental herds. In northern Uganda (Aswa), the products (AAO & AIA) were generally effective (field efficacy $\geq 90\%$) in Aswa, but poor (field efficacy $\leq 90\%$) on farms in Maruzi. In all cases where the products were effective, efficacy ($\geq 90\%$) lasted not beyond 10 days.

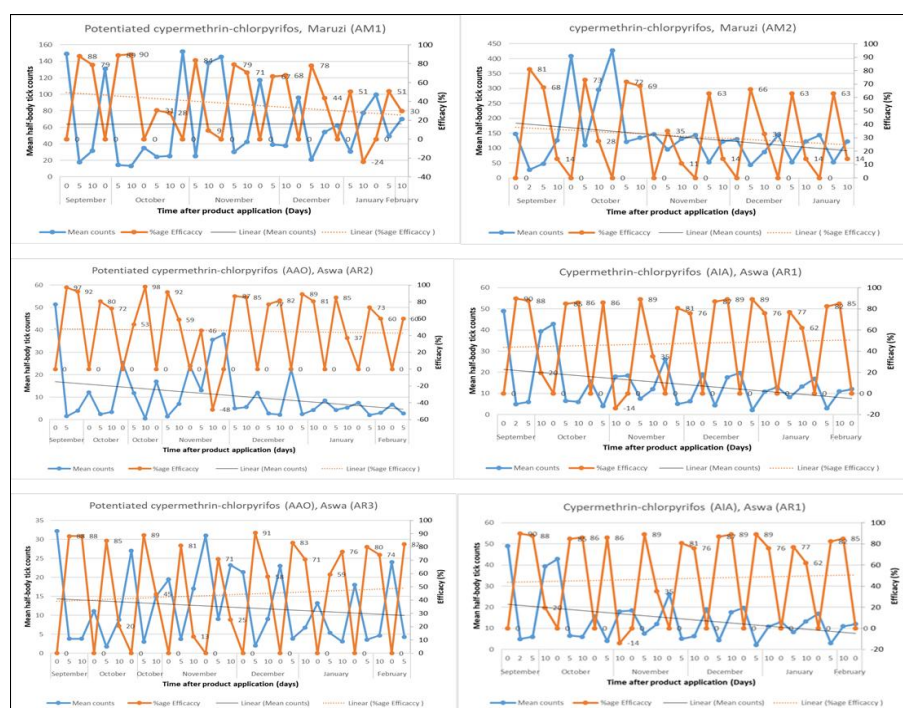


Fig 7: Efficacy potentiated cypermethrin-chlorpyrifos (AAO) and registered cypermethrin-chlorpyrifos (AIA) products in Maruzi and Aswa

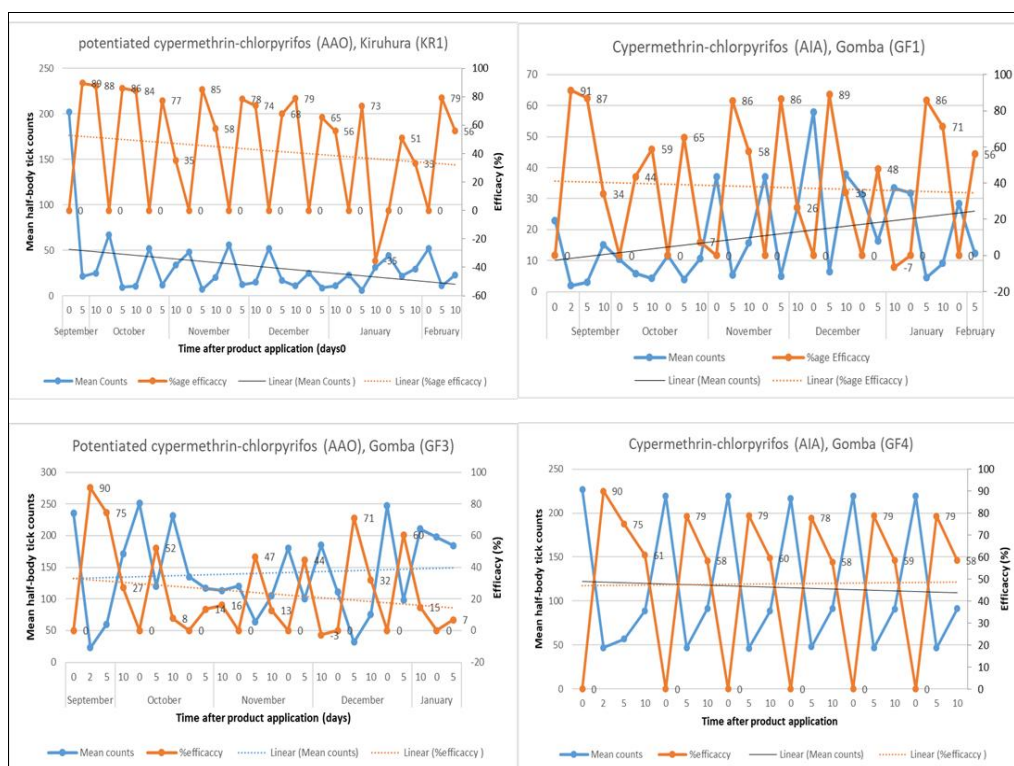


Fig 8: Efficacy of potentiated cypermethrin-chlorpyrifos (AAO) and registered cypermethrin-chlorpyrifos (AIA) products in Kiruhura and Gomba

The effect of potentiated co-formulation of cyamizol and cypermethin on acaricide resistant ticks under field conditions

In Kaberamaido (North), the co-formulated cyamizol-cypermethin product (DOD) registered efficacy below 90%

on all participating farms throughout the study while amitraz, 12.5% (D2D) was generally effective (efficacy $\geq 90\%$) on most farms, at least within 5 days of application. An almost similar picture was observed on herds in Kyenjojo (Figures 9 and 10).

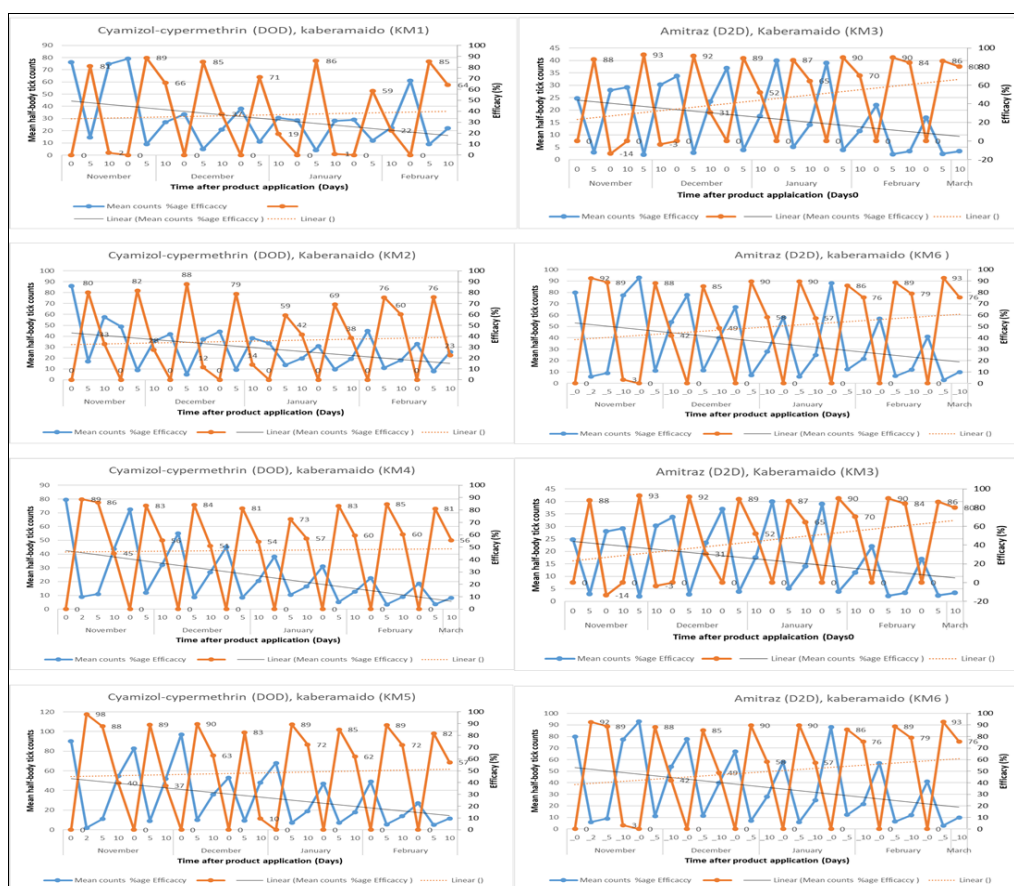


Fig 9: Efficacy of Cyamizol-cypermethrin (DOD) and Amitraz products (D2D) in Kaberamaido District

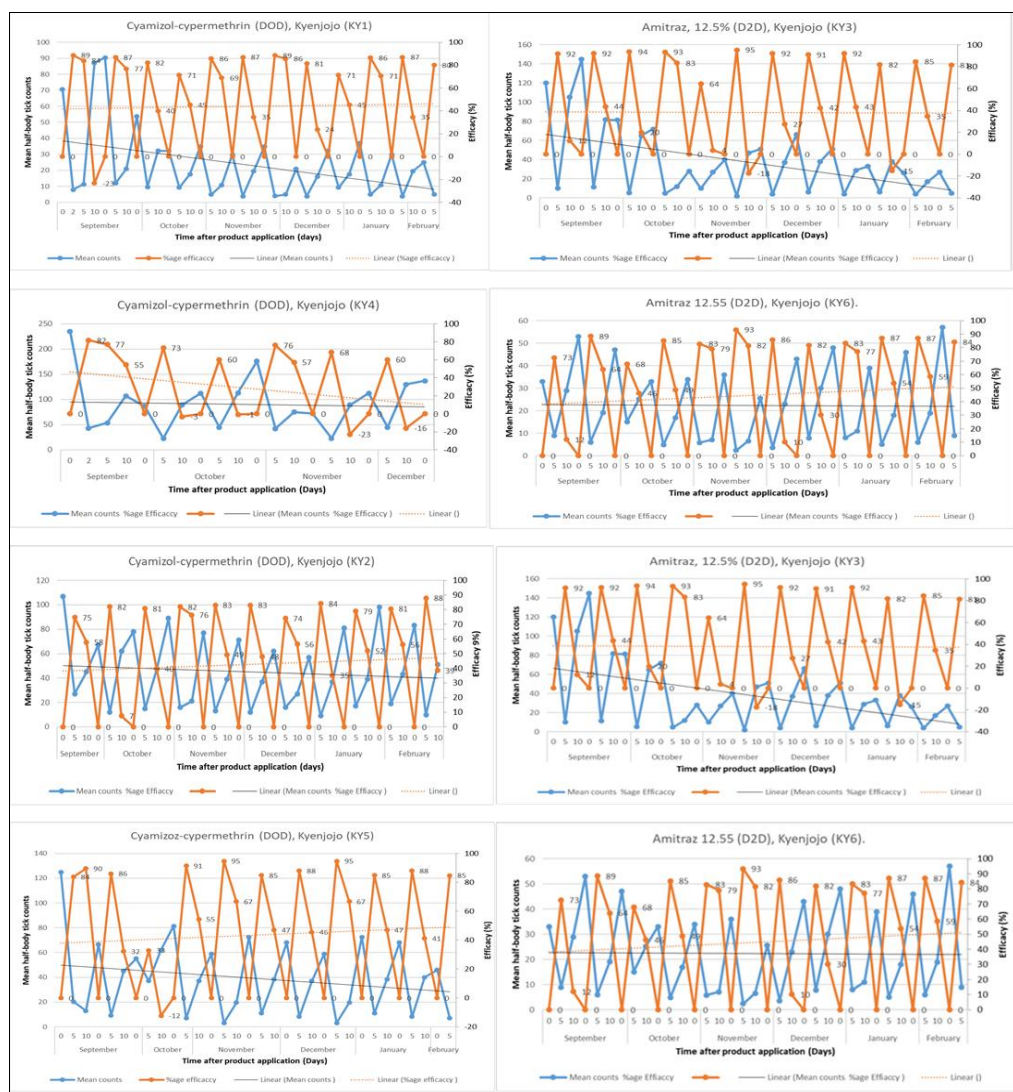


Fig 10: Efficacy of Cyamizol-cypermethrin (DOD) and Amitraz (D2D) products in Kyenjojo

Reported Adverse events

The following adverse events were recorded with the various products:

Scold wounds in the back parts behind the ears (Dorsal pinna) were reported in two out of eighty cattle on Eprinomectin treatment in Amolatar District. All farmers complained of massive brown ear tick re-invasion 5-10 days after Eprinomectin product application. All farmers that used Cyamizol-cypermethrin co-formulation product complained of reduced farm milk production during the first 2-4 days following the products application, in addition to calf drowsiness on the first 2 days of product application. Animal deaths recorded during the experimental period attributable to tick-borne diseases: one (01) sentinel animal, potentiated cypermethrin chlorpyrifos product in Kiruhura District (KR1), three heifer calves on Cyamizol-cypermethrin co-formulation product on farms in Kyenjojo District (KY4). The spraying herdsmen complained of irritation after using Cyamizol-cypermethrin co-formulation in Kyenjojo District (KY4 and KY5).

Discussion

Laboratory efficacy evaluation revealed that *Boophilus decoloratus* tick populations that are resistant to all classes of registered acaricides are prevalent in herds, especially in central and south-western Uganda. Earlier surveys reported similar finding (Vudriko *et al.*, 2016) [14]. Being 'single-host'

ticks, boophilus species get the most exposure to acaricidal products applied onto their hosts. If such products are used for a prolonged time or are applied too frequently, naturally resistant populations of the most exposed parasites are quickly selected, hence development of resistance (Bruce and Marshall, 1999) [1]. *Boophilus* is thus, an indicator species of resistance resulting from insensible acaricide use practices. Again, the need for concerted extension and improved regulation at consumer level cannot be over-emphasized.

The study observed that 57% of *B. decoloratus* tick samples from central and southwestern Uganda were resistant to all registered acaricide molecules. Also 40% of *R. appendiculatus* samples from the same herds were resistant to all registered molecules. Multiple resistance against common acaricides in Uganda was reported by Vudriko *et al.*, (2016) [14]. This may explain why many herd owners have resorted to using unlicensed products, especially organophosphate or acelamectin-based agro-chemicals, besides reduced frequencies of product application, use of higher concentrations or smuggling products from neighboring countries. Resistance to cypermethrin and flumethrin (pyrethroids) was first reported in 2012 (Vudriko *et al.*, 2016) [14]. Its persistence to date could be attributed to continued use of pyrethroids in co-formulated products otherwise resistance should have decayed since pyrethroid products are currently not in use in the southwestern districts. Multiple resistance could be controlled by adopting a policy on pesticide import

regulation, where the country only allows limited classes of pesticides into the country at a given time (3-5 years), after which, they are changed. For acaricides, since there are mainly three classes (amidines, organophosphates and pyrethroids), it is advisable that the molecules are used following an import-controlled rotation policy. In other words, only one acaricide class is prescribed by MAAIF for use throughout the country or a region at a time while its effectiveness is monitored. Co-formulations are however not commendable, since they distort and limit the options for an import-controlled national products rotation policy.

Of the novel products, the laboratory and field evaluation findings showed that eprinomectin was highly effective (efficacy $\geq 90\%$) against all tick species on the randomly selected farms. Whereas engorged *Boophilus* ticks were not observed on treated animals till next application (30 days), massive re-infestation with *R. appendiculatus* occurred within 5-10 days after treatment on the experimental farms. This probably is why eprinomectin products as acaricides are indicated for routine control of *Boophilus* ticks only (Snoop *et al.*, 1996) ^[10]. In the current situation where, multiple resistance to common acaricides by mainly *Boophilus* has been reported, targeted use of eprinomectin to knock out resistant populations from affected herds is realistic. It is prudent that the use of eprinomectin in a herd should follow laboratory confirmation of *Boophilus* resistance. The eprinomectin treatment must be supplemented with application of an acaricide product that is effective against *R. appendiculatus*, which unlike *Boophilus*, re-infest eprinomectin treated cattle within 5-10 days of treatment.

The product AAO (cypermethrin-5g/L, chlorpyrifos -7g/L, piperonyl -5g/L, citronella 0.5g/l) and its control AIA (Cypermethrin, 50g/L-chlorpyrifos, 500g/L) were highly effective on farms in the north. In Maruzi however, efficacies were poorer, and this was attributed to the fact that herds got immediately re-infested from communal grazing grounds that are heavily tick infested. This re-affirms the emphasis on the need to encourage farmers to enclose their farms and limit communal grazing.

The AAO and AAI efficacies on farms in Gomba, Kiruhura and Nakaseke did not reach the 90% susceptibility cut-off. In the lab too, only 80% of tick samples from these districts were susceptible to the products. This supports earlier report by Vudriko *et al.*, (2016) ^[14] who reported a 40% resistance to cypermethrin-chlorpyrifos co-formulations in tick from south-western Uganda. Apparently, potentiation of the co-formulation with citronella or piperonyl may not augment its performance since original individual constituent components (cypermethrin and chlorpyrifos) are already independently resisted. Therefore, the novel product (AAO) is not a suitable alternative to already registered co-formulated products in the market, especially if the aim is to eradicate resistance.

The cyamizol-cypermethrin (DOD) product was less effective than amitraz-12.5% (D2D) *in vitro* and on farms. This co-formulation is not in use in Uganda much as cyamizol belongs to the amidine class of acaricides that are widely used in Uganda. Cypermethrin resistance in south-western Uganda is reported at about 90% (Vudriko *et al.*, 2016) ^[14]. What is difficult to explain is why, amitraz, 12.5% performed better than the co-formulation: could it be that there is antagonism between the constituent molecules? By implication, cyamizol-cypermethrin co-formulation is not a suitable alternative to products in the market with respect to the fight against tick resistance in Uganda.

Of the evaluated novel products, only eprinomectin demonstrated sufficient efficacy against *Boophilus* ticks of known resistance to common acaricides. Performance of the other novel/investigational products were more or less similar to related registered products. They are thus, not suitable alternatives to registered acaricide molecules in regard to control of tick resistance in Uganda.

Conclusion

Of the evaluated novel products, only eprinomectin demonstrated sufficient efficacy against *Boophilus* ticks of known resistance to common acaricides. Thus, novel products (potentiated cypermethrin-chlorpyrifos co-formulation-AAO or the cyamizol-cypermethrin coformulation-DOD) are not suitable alternatives to registered acaricide molecules in regard to control of tick resistance in Uganda. Against *R. appendiculatus*, eprinomectin is effective only for 10-15days i.e. *R. appendiculatus* larvae/nymph massively reattach prior to the next recommended application time (30 days).

Recommendations

To address multiple resistance, it is advisable that molecules are used following an import-controlled rotation policy i.e. one acaricide class is prescribed by MAAIF for use throughout the country or a region at a time while its effectiveness is monitored. Whenever there are reported incidence of failure or resistance, the molecule is recalled and another effective one prescribed for the country or region. If import-controlled molecule rotation policy is adopted, the use of co-formulation products should be discouraged since they limit the options for molecule rotation in addition to delaying decay of resistance against withdrawn products. Registered amidine and organophosphate formulations, or pyrethroids may still be applied for routine tick control in Uganda except on farms with confirmed multi-acaricide *Boophilus* resistance.

On farms with confirmed multi-acaricide *Boophilus* resistance, *eprinomectin* may be applied. It's advisable that the use of eprinomectin should be state regulated with emphasis on prescription and supervision by a competent veterinary authority. Herds on eprinomectin for *B. decoloratus* resistance control should continue with routine application of a registered common product that is effective against *R. appendiculatus* ticks which usually reattach 10-15 days of eprinomectin application

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Conflict of Interest

Not available

Financial Support

Not available

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