Efficacy of VERYL® in the treatment of cattle naturally infected with gastrointestinal nematodes in Kenya.

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Introduction

- Animal African trypanosomosis (AAT) and helminthosis are among the economically most important diseases affecting cattle in sub-Saharan Africa (SSA).
- Losses attributed to these diseases include lowered productivity, mortalities and decreased reproduction rates. AAT alone is estimated to cost African livestock farmers from 2 to 5 billion USD per year (Kristjanson *et al*., 1999).
- Mixed infections and pathologic effects on the animal attributed to both conditions have been well-documented (Kaufmann *et al*., 1992; Van Wyk, 2014).
- AAT and infections by gastro-intestinal nematodes occur mainly during the rainy season. Moreover, it has been demonstrated that the severity of trypanosome infections is worsened in animals co-infected with certain nematode species like *Haemonchus* sp.

Introduction (2)

- The cumulative effect of certain strongyle nematodes at high level and *Trypanosoma* species co-infections have been shown to cause the most significant decrease in packed cell volume % (PCV) on African Short-horn Zebu calves when compared with other co-infections.
- The cumulative impact of these two pathogens can potentially result in anaemia severe enough to cause mortality (Van Wyk, 2014).
- Chemotherapy is the most preferred method of controlling AAT and helminthosis
- The IVP called VERYL® was developed for the control of both AAT and helminthosis by combining two anti-parasitic agents comprising of diminazene aceturate and levamisole chloride for the intramuscular administration in cattle.

Description of IVP product

Manufacturer	**Ceva Santé Animale, 10 av. de La Ballastière, 33500 Libourne, France**
Code	C753
Active ingredients	A97290 and A71575
Pharmaceutical dose form	Powder for injection
Conditioning	4.9g sachets
Storage conditions	To be stored away from light, in dry place below 30°C Shelf life: use immediately after opening Reconstituted solution to be used within 24hrs
Dose	3.5mg A97290/kg bwt and 5mg A71575/kg bwt (10ml/100kg bwt after reconstitution)
Route of administration	Deep im injection, maximum of 15mls per site
Reconstitution	Add 26mlsof sterile water (coded 2526 and supplied by sponsor) to each 4.9g sachet to make 30ml
Withdrawal period	Meat and affals: 42 days

Description of CP product

Manufacturer	**Ceva Santé Animale, 10 av. de La Ballastière, 33500 Libourne, France**
Code	$R_1$5262
Active ingredients	A97290
Pharmaceutical dose form	Powder for injection
Conditioning	2.36g sachets
Storage conditions	To be stored away from light, in dry place below 30°C Shelf life: use immediately after opening Reconstituted solution to be used within 24hrs
Dose	3.5mg A97290/kg bwt (5.8ml/100kg bwt after reconstitution)
Route of administration	Deep im injection, maximum of 15mls per site
Reconstitution of CP	Add 15mls of sterile water (coded 2526 and supplied by sponsor) to each 2.36g sachet to make a final solution of 17.3ml
Withdrawal period	Meat and affals: 21 days

Justification for a combination product

- Broadening of the spectrum of activity covering the main blood and gastro-intestinal parasites of cattle in Sub-Saharan Africa.
- The simplified treatment and drug management: single intramuscular application for the control of the main parasitic infestations occurring during the wet season

Study objective

- To demonstrate the efficacy and safety of C753 at the dose of 1 ml/10 kg bwt by intramuscular route in treatment of natural nematodes infection (the anthemintic component).
  - The results of this study was to be used in obtaining marketing authorization for the product in the East Africa countries and elsewhere

Materials and methods

- Type of study

A semi-cross over, randomised, blinded, experimental design, comparing the efficacy of C753 *versus* a negative control product against gastrointestinal nematodes

Animals

- Cattle (bought from Kajiado County and transported to VRI, Muguga) were used in the study as they are the target species for the product
- The trial used 38 cattle aged 6-12 months, naturally infected with gastrointestinal nematodes.
- These were randomly allocated to two groups, a treated group that received IVP intramuscularly at 1mls/10 kg bwt and a control which received the CP intramuscularly at 3.5mg/kg bwt. Faecal egg counts (Day 0, 7, 14 and 21), coprocultures, packed cell volume and local tolerance at injection sites were monitored.

Field selection of animals

Field selection criteria

- age: 6-12 months
- Weight: 50-200kg
- faecal egg count ≥ 350 epg
- Negative parasitaemia for trypanosomes

Inclusion criteria

- Feacal egg count of ≥ 350 epg on D -1
- negative parasitaemia for trypanosomes
- good general health
- no trypanocide or anthelmintic treatment within 15 days or 4 weeks prior to inclusion, respectively

Exclusion criteria

- Animals not fulfilling the inclusion criteria and study animal requirements (age, bodyweight).
- Animal known to have received
  - any trypanocide treatment within 15 days prior to inclusion
  - any anthelminthic treatment within 4 weeks prior to inclusion
- Animals with positive parasitaemia for trypanosome (buffy coat technique) the day before treatment.
- Animals with poor general health (according to physical examination performed on treatment day and PCV the day before treatment: PCV < 10%).

Post-inclusion removal criteria

- Animals presenting the following after inclusion were to be excluded from the study during follow up:
  - Decision of the Investigator in the interest of the animal welfare, any animal observed with unacceptable suffering at the discretion of the Investigator, to be euthanized, or treated to alleviate distress.
  - Severe concomitant disorder or disease that may interfere with the evaluation of response to the tested treatment.
  - Major protocol deviation, such as administration of a forbidden treatment.
  - Decision of the Sponsor to stop the study.

Animal welfare and ethical approval

- Approval by VSRI IACUC
- Daily monitoring of animals by clinician
- Post-inclusion removal of animals in distress
- Barn house conditions (water, feeding, lighting, ventilation, cleaning and hygiene)

Randomization and allocation to pens

- According to a computer-made randomisation list
- Randomisation was stratified on basis of faecal egg count and body weight on D-7.
- Once an animal fulfilled the inclusion criteria (on D-1), it was allocated to pens (one treatment group per pen) by Sponsor.
- The "Allocation to treatment and pen" form was then furnished to the Drug Dispenser and animals were allocated to pens according to the plan on D0.

BLINDING METHOD

- Blinding was achieved by the involvement of two categories of Investigators:
  - Clinician, responsible for the monitoring of the animals before and after treatment
  - Drug Dispenser, responsible for the treatment administration/accountability.
  - Laboratory staff involved in the study remained blinded (coproscopy, coproculture, packed cell volume (PCV) and parasitaemia assessment).
- Blinding of the study could be broken by the Drug Dispenser on animal welfare

Treatments

Group	n	Treatment	Dose
1	19	Veryl	1ml/10kg bwt
2	19	Control (Veriben)	3.5mmg/kg bwt)

Parameters monitored

- Faecal egg counts (Days 0, 7, 14, 21)
- Coproculture
- PCV
- Local tolerance at injection site
- FECR on Day 7, 14 and 21
- Live weight, Day 0, 7, 14 and 21
- Physical examination with local tolerance assessment

Results

FEC and FECR from Day 0 to Day 21

Parameter	statistics	Treatment group			
		VERYL® (N=19)	CP (N=19)	Percent efficacy	p
FEC on Treatment day (Day 0)	GM	560.5	566.7		-
	AM(SD)	660.3(431.2)	650(324)		-
	[Min; Max]	[200; 2050]	[200; 2050]	[6.0; 12.0]	
	% (n) of infected animals	100% (29)	100% (19)		
FEC on Day 7	GM	0.3	587	99.95%	<0.0001
	AM(SD)	3.4(12.9)	863.9(949.9)	99.6%	
	[Min; Max]	[0; 50]	[50; 4400]		
	% (n) of infected animals	6.9% (2)	100% (18; 1 missing)		
FEC on Day 14	GM	0.2	386	99.95%	<0.0001
	AM(SD)	3.6(18.9)	441.2(213.8)	99.19%	
	[Min; Max]	[0; 100]	[100; 850]		
	% (n) of infected animals	3.6% (1)	100% (17; 2 missing)		
FEC on Day 21	GM	0.6	583.8	99.90%	<0.0001
	AM(SD)	8.9(27.4)	838.2(1038.3)	98.94%	
	[Min; Max]	[0; 100]	[100; 4700]		

Evolution of larvae (GM) for the different helminth species

Group	Species	Larvae D0	Larvae D7	Larvae D14	Larvae D21
VERYL®	Haemonchus	353.6	0.7	0.2	0.5
	Cooperia	25.2	0.2	0	0
	Ostertagia	1	0	0	0
	Trichostrongylus	66.9	0.4	0	0.1
	Oesophagostomum	104.1	0	0.1	0.4
Control	Haemonchus	144.5	659.6	655.4	607.5
	Cooperia	7.6	24.2	27.2	6.4
	Ostertagia	0.1	1	0.6	0.9
	Trichostrongylus	23.9	85.6	36.9	71.3
	Oesophagostomum	54.8	208	183.1	130.3

Body weight and daily body weight gain

- Means of body weight were comparable between groups on D-1.
- Body weight 21 days after treatment was lower in the control group compared to the VERYL® group.
- This suggested a positive impact of VERYL® treatment on zootechnical performances

Drug safety

- The injection site was examined on Day 0 before treatment and daily up to 7 days post treatment. No reaction at the injection site was observed during the study either in the VERYL® or in the control group.
- Following administration of products (VERYL® and CP), 7 animals exhibited the following signs: tremors, salivating, lying down, aggression, urinating and ataxia. Three animals belonged to the VERYL® group and 4 were in the control group.
- All reactions to treatment were transitory and resolved within 30 minutes and without any sequel.

Conclusions

- VERYL® was highly effective against nematodes (overall species) when compared to CP at each time point (7, 14 and 21 days post treatment). Indeed, efficacy was above 98% and faecal egg counts were significantly lower in the VERYL® group compared to the control group ($p<0.0001$).
- Therefor the study demonstrated the efficacy and safety of VERYL® administered once by intramuscular injection at the dose of 1 ml/10 kg body weight and recommended the drug for the treatment of natural nematode infections in cattle.

THANK YOU