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Comparative Efficacy and Safety Study of a Newly Developed Moxidectin Containing Formulation (Drench) in Sheep

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Abstract:

In the present study, several clinical field trials were conducted to evaluate the efficacy and safety of moxidectin oral drench for sheep. Trial I was designed to evaluate the efficacy and toxicity 0.2 % moxidectin (Rivermec(R)) oral drench , whereas in trial II, a comparative efficacy between Rivermec(R) LV and Cydectin LV containing 0.2% moxidectin oral drench formulation was evaluated in the Winter season (August 2013). Trial III was a comparable efficacy of Rivermec (R) LV drench for sheep and Cydectin LV (reference product) containing 0.2% moxidectin oral drench formulation was evaluated in summer season (December 2013). Stability study of Rivermec (R) LV Sheep Drench was conducted at both real time and accelerated conditions. The efficacy was measured on the basis of the reduction of the faecal egg counts by modified McMaster method. On the basis of egg reduction, irrespective of season, the efficacy of the treatment was almost 95-100% effective in trials (trial I, II & III) for both Rivermec (R) LV and Cydectin LV. Treatment of sheep with 0.2, 0.4, 1.0 mg/kg BW (1X, 2X and 5X levels) of Rivermec (R) LV Drench for Sheep did not cause clinical signs of toxicity in any of the test animals. Faecal samples were also analysed to identify the larval species. *Tricostrongyle* was predominant followed by *Ostetragia, Oesophagostomum* and *Haemonchus.* Recovery study in simulated Body Fluid (SBF) suggested that both candidates could behave similar after administration. Biometrical analysis of study data suggested that no significant differences (at $p<0.05$) were observed between the Rivermec LV and Cydectin® LV treated groups on Days 14 and 28 in trials 2 and 3.

Key Words: Rivermec (R) LV, Moxidectin, Cydectin LV, Sheep nematode, Efficacy, Safety

1. INTRODUCTION:

Gastrointestinal nematodes are the most common parasite in sheep production areas [1] and the use of strategic anthelmintic treatments has become a routine management activity of flocks. These types of parasite control procedures provide beneficial effects on the animals' weight gain, milk and wool yield [2]. Great efforts have been made in the pharmacological field searching for products with high efficacy, reduced therapeutic doses, a broad spectrum of activity and better tolerance than those currently used. However, the continuous use of a particular chemical product generally results in the appearance of resistant strains [3] which require the use of an alternative anthelmintic with a different mode of action to keep the parasite population under control.

Moxidectin (American Cyanamid Company, USA) is a new broad spectrum parasiticide belongs to the milbemycin group of compounds. It has been produced by chemical modification of nemadectin, the principal component of the LL28249 antibiotic complex [4], a natural fermentation product of the micro-organism *Streptomyces cyaneogriseus noncyanogenus.* Nemadectin has been shown to have potent activity against a range of parasites of domestic animals [5], and more recently the efficacy of moxidectin against various nematodes in ruminant livestock has been assessed in several studies [6-10].

It is well known that same drug can produce significantly different results based on the formulation. Similar liquid preparation may produce different results due to carrier effect. The efficacy, safety and withholding period for meat/egg/milk consumption of an antiparasitic compound is related not only to the dose rate but also to its formulation and route of administration [11]. So, it is always recommended to evaluate the safety and efficacy of a newly formulated drug in the target species to assess the commercial viability.

The purpose of this study was to evaluate the efficacy and safety of moxidectin oral drench (Rivermec (R) LV Drench , a new formulation developed by Vetafarm Pty Ltd, Australia) against naturally infected nematodes and establish comparable efficacy of Rivermec (R) LV drench for sheep with the reference product Cydectin® LV (Virbac, Australia). This study also includes an in vitro recovery study of moxidectin in two test compound from simulated body fluid to predict the fate of the moxidectin in newly developed product after administration. A comprehensive biometric analysis was also conducted to validate the obtained study data.

2. MATERIALS AND METHODS:

2.1 Study farms:

The study was conducted in Wagga Wagga, Riverina, NSW, Australia, in two contiguous regions, Study location for trial I & III was Southdown View, Estella Road, North Wagga Wagga, NSW. PIC: ND 553372 (marked as B in the map) and location for second trial was Maxwelton, The Gap Via Wagga Wagga, NSW 2650. PIC: NB 554410 (marked as A in the map). The climate of wagga is dry in summers and cool to cold in winters. The maximum temperatures in summer are hot and dry averaging between 29^0C and 32^0C. Annual mean rainfall is 570 mm. Relative humidity however remains low in the summer months with

a 3pm average of about 30%. The winters are cool to cold with overnight minimums averaging 3^0C and daily maximums climbing to only 12^0 C to 14^0 C on average. Relative humidity is much higher in winter with a 3 pm average of over 60% and a 9 pm average just below 90%. Two commercial sheep farms were used for the study which were small in size with an average area of approximately 50 ha characterized by small pasture areas mainly cultivated with cereal crops. No cattle co-grazed with the sheep and the number of sheep ranged from 150 (Farm 1) to 2500 (Farm 2). The breeds of sheep were mixed in the study farms.

2.2. Flock parasitological status:

Faecal examinations were performed on sheep from the two farms before the beginning of the study (August 2013 on Farms 1 and December 2013 on Farms 2) and sheep with natural mixed parasite infections were selected for the study.

2.3 Experimental design:

The field trials were performed using commercially obtained young sheep of mixed sex. All the animals were selected from a naturally infected sheep flocks on the basis of the positive faecal egg counts. Each sheep was individually identified by numbered ear tags. Vetafarm has performed three field trials to establish the Efficacy and Safety of Rivermec (R) LV Sheep Drench. Field trial one was a dose Confirmation Study, and toxicity trial using Rivermec (R) LV sheep drench alone. In the dose confirmation study the animals were randomly allotted into two groups (1 and 2) of 20 animals each. The animals in group 1 remain as untreated control. The animals in group 2 were drenched orally with 0.2 % oral liquid moxidectin at a dose of 0.2 mg/kg body weight (b.w.). In the toxicity trial study the animals were randomly allotted into two groups (3 and 4) of 25 animals each. The animals in group 3 were drenched orally at a dose of 0.4 mg/kg (2X) body weight (b.w.). The animals in group 4 were drenched orally with 0.2 % oral liquid moxidectin at a dose of 1 mg/kg (5X) body weight (b.w.) Field Trial Two was a comparative efficacy study of Rivermec (R) LV Sheep Drench and Cydectin LV drench for sheep (APVMA Number 46517) in winter (August 2013). In this study the animals were randomly allotted into three groups (A, B and C) of 30 animals each. The animals in group A remain as untreated control. The animals in group B & C were drenched orally with 0.2 % moxidectin oral liquid (Rivermec (R) and Cydectin respectively) moxidectin at a dose of 0.2 mg/kg body weight (b.w.). Field trial three was a comparative efficacy study of Rivermec (R) LV sheep drench and Cydectin LV drench for Sheep in summer (December 2013). In this study, the animals were randomly allocated into three groups (A, B and C) of 20 animals in control group and 25 animals in each treatment group. The animals in group A remain as untreated control. The animals in group B & C were drenched orally with 0.2 % oral liquid (Rivermec (R) and Cydectin respectively) where moxidectin at a dose of 0.2 mg/kg body weight (b.w.)

No other treatments were given throughout the trial periods. After treatments, all of the sheep were observed daily for any sign of adverse reactions. The general condition was evaluated visually, with special reference to changes in behaviour of treated animals by a registered veterinarian.

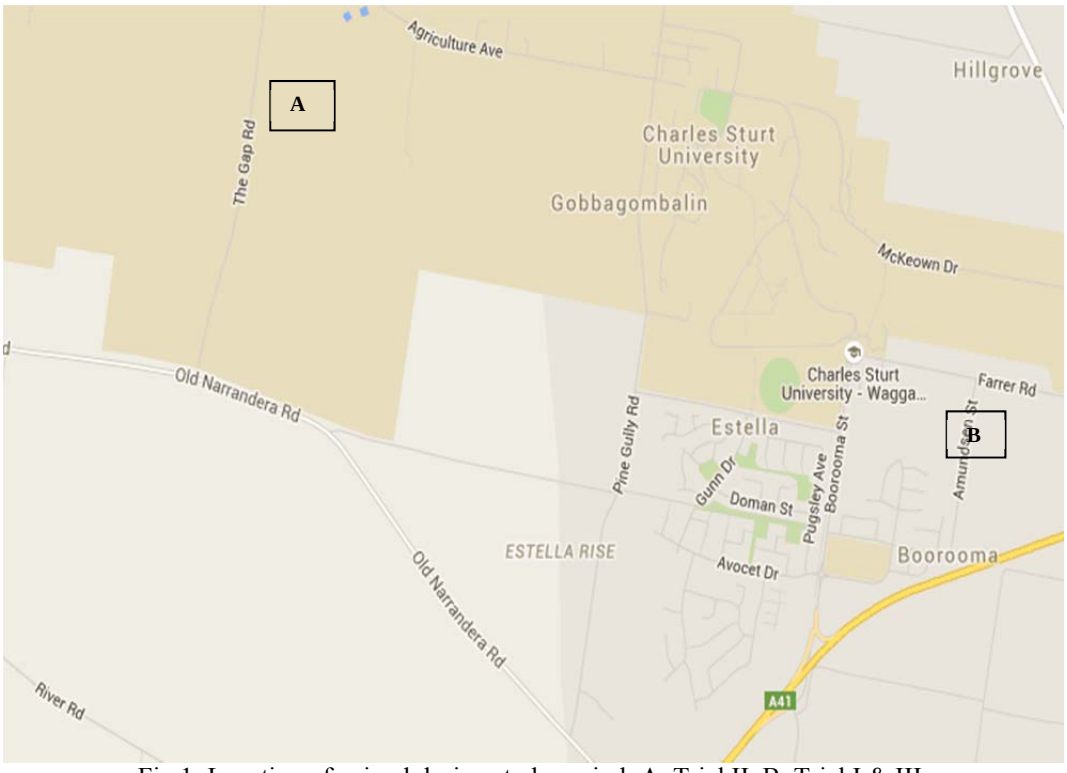

Fig 1: Location of animal during study period: A. Trial II, B. Trial I & III.

2.4. Procedures:

Faecal samples were analysed by Modified McMaster method. The total number of eggs present in faeces was determined and the number of eggs present expressed in terms of eggs per gram (epg) of faeces following the World Association for the Advancement of Veterinary Parasitology (WAAVP) guidelines [12]. Larval species were identified by the School of Veterinary Science, Charles Sturt University, Wagga Wagga, Australia.

2.5. Calculations and statistical analysis:

The anthelmintic efficacy of Rivermec (R) LV and Cydectin(R) LV Sheep Drench was assessed by applying the Faecal Egg Count Reduction test (FECRT) as described by the World Association for the Advancement of Veterinary Parasitology (WAAVP) [13]; Treatment efficacies were then calculated (for both arithmetic and geometric means) using Abbott's Formula:

Efficacy (%) = (Untreated Control Mean$_{time\ t}$ – Treated Mean$_{time\ t}$)/ (Untreated Control Mean$_{time\ t}$) x 100

Anthelmintic treatment is considered to be effective if the percentage reduction in arithmetic mean faecal egg count is above 95%.

The percent efficacy results were analysed for significance by a comprehensive biometrical analysis method to validate the obtained study results. Individual animal Faecal Egg Counts (FECs) from 3 Faecal Egg Count Reduction Tests (FECRTs) were recorded in Microsoft EXCEL format. Data were arranged by treatment, sample ID and time point. Group arithmetic mean FECs and FEC standard deviations were calculated for each treatment group and time point using built in EXCEL formulae. Data were also log transformed:

y = LOG (x + 1)

and geometric means calculated using the formula:

Geometric Mean = 10^ (average (x_a:x_z))-1.

Group mean FECs and calculated treatment efficacies are presented in **Tables 1, 4** and **6**.

Data were then examined to determine the most suitable statistical methodology. Levene's Tests (to test homogeneity of variances) were performed (using Statistix 10.0, Analytical Software 2013) to assess the suitability of parametric One-Way Analysis of Variance (ANOVA) on either untransformed or log transformed data. In all trials while parametric ANOVA was suitable for Day 0, the assumption of homogeneity of variances could not be met using untransformed data for Days 14 and 28. In all cases, this assumption appeared valid using log-transformed data, hence all statistical analyses were performed using log transformed data.

Data were compared using parametric ANOVA and the model

FEC ~ Treatment and TIBCO Spotfire S+ 8.2, TIBCO Software Inc 2010.

Statistical comparisons of treatment group FEC data (including observed p-values, point estimates of the difference in mean FEC where appropriate and 95% Confidence Intervals for the difference in FECs) are presented in **Tables 2, 5** and **7**. Means were compared using Tukey's Multiple Comparison Test.

Validity of ANOVA was checked during analyses via examination of residual plots; in a number of instances (Trial 2, Days 14 and 28 and Trial 3, Day 14) residual plots suggested parametric ANOVA assumptions were not being fully met. In these instances, data were also analysed using the equivalent non-parametric tests (Kruskal-Wallis ANOVA and Dunn's All-Pairwise Comparison Test). In all 3 instances, similar results were observed using both parametric and non-parametric ANOVA.

2.6 Stability Study:

Stability study of 3 batches of Rivermec (R) LV oral drench was conducted at room temperature (30^0C) and accelerated (40^0C) conditions in order to assess the commercial viability of the newly formulated product.

2.7 Preparation of simulated Body Fluid (SBF):

Simulated Body Fluid which contains similar ionic concentration of human blood plasma was prepared as follows:

The Simulated Body Fluid was prepared using the reagents listed in the **Table 10.** These reagents were added to 700 mL of water in the order given in the table one by one after each reagent was completely dissolved. The pH was adjusted to 7.4 with 1 M HCl and final volume adjusted to 1L with water. In most of the studies, a volume ranges from 15 mL to 200 mL of SBF was used to evaluate the samples. In this study, 200 mL of SBF solution was used to recover Moxidectin.

3. RESULTS:

3.1. Trial I:

After treatment, on day 14 the arithmetic mean of EPG in group B decreased sharply. The efficacy based on faecal egg counts reduction was 97.842% on day 14 and corresponding geometric means was 99.90%. The efficacy of moxidectin against various species of nematodes based on faecal egg counts was 98.571 % and corresponding geometric mean was 99.91% on the following sampling dates (day 28) (Table 1). Highly significant differences ($P<0.05$) in group mean FECs were observed between the untreated control group and the Rivermec LV treated group on day 14 and 28 (Table 2).

Table 1: Rivermec LV trial 1- group mean FECs (epg), standard deviations and treatment efficacies

Group	Treatment	Day 0	Day 14	Day 28
Arithmetic Means				
1	Untreated Controls	670.0	695.0	700.0
	St Deviation	180.9	176.1	133.8
2	Rivermec LV	640.0	15.0	10.0
	St Deviation	193.0	48.9	30.8
Treatment Efficacy		---	**97.84%**	**98.57%**
		Geometric Means		
1	Untreated Controls	648.3	673.0	688.3
2	Rivermec LV	612.8	0.6	0.6
Treatment Efficacy		---	**99.90%**	**99.91%**

Table 2: Rivermec LV trial 1- statistical comparison of group mean FECs.

Parameter	Value/Conclusion
Day 0	
F-statistic	0.39
p-value	0.535
Conclusion	No significant difference between groups at p<0.05
Day 14	
F-statistic	300.2
p-value	0
Conclusion	Significant difference at p<0.05
Point estimate of difference in FEC (Controls - Rivermec LV)	406.4
Lower 95% Confidence Interval for difference	203.2
Upper 95% Confidence Interval for difference	830.8
Day 28	
F-statistic	359.4
p-value	0
Conclusion	Significant difference at p<0.05
Point estimate of difference in FEC (Controls - Rivermec LV)	435.5
Lower 95% Confidence Interval for difference	228.1
Upper 95% Confidence Interval for difference	830.8

The daily weight gain of sheep in the treatment group (175.000±15.058) was significantly different (P < 0.05) from the control group (139.286±14.555) during the trial 1 (Table 3).

Table 3: Effect of Rivermec (R) LV treatment on mean live weight gain (g/day)

Untreated control group	Treatment group
139.286±14.555	175.000±15.058

No clinically detectable side effects to the treatment with recommended dose of 0.2 % Rivermec (R) LV oral drench were observed and a progressive recovery of treated animals was observed throughout the trial.

The second part of trial 1 was to assess the toxicity of 0.2 % Rivermec (R) LV oral drench at 2X and 5X of recommended dose. All sheep grew similarly throughout the study period. The animals were pastured so food consumption could not be measured. During the trial period, there was an outbreak of Pink Eye (*Morexella spp* infection) in the sheep. This is common and is easily differentiated from toxicity by close examination of the eye. Pink Eye infections show ocular discharge, conjunctivitis and/or keratoconjunctivitis. Pink eye is commonly unilateral and pupil size is not affected. The infection will respond rapidly to antibiotic therapy (Cloxacillin eye ointment) and the animal dose not exhibit any other sign of illness.

Additionally, toward the end of the trial period, several animals showed ocular discharge and conjunctivitis due to Barley Grass Seeds embedded in the conjunctiva.

Lameness related to minor injury was observed in 3 animals of Toxicity study animals. These animals were yarded and examined to reveal one with a swollen left fore carpometacarpal joint and the other a gash above the coronet on the left rear foot. Rapid breathing was occasionally noted among the animals. Due to the sheep being in open pastures, it was not possible to isolate the animal without yarding and catching – which increases respiratory rate. Increased respiration was not considered significant if the animal showing symptoms could not be located on the following day. While sheep with increased respiratory rate or lameness were significant, there was no clinical relevance, and therefore these observations were not attributed to the treatment effect. In accordance with the study protocol, the observation schedule was terminated seven days after the third treatment based on the absence of any signs of toxicity up to that point in the study.

3.2 Trial II:

Trial II was conducted to establish/assess a comparable efficacy between Rivermec (R) LV and Cydectin(R) LV oral drench at the same dose, environmental conditions and worm species in the winter season. All of the animals selected for the trial showed positive faecal egg counts. After treatment, on day 14 the arithmetic mean of EPG in group Rivermec (R) LV & Cydectin(R) LV decreased sharply. The efficacy based on faecal egg counts reduction was 98.020 % for group Rivermec (R) LV and 98.564 for group Cydectin(R) LV on day 14 and their corresponding geometric mean was 99.87% and 99.91% respectively. The efficacy of moxidectin against various species of nematodes based on faecal egg counts was 98.978 % for Rivermec (R) LV group and 99.015 for Cydectin(R) LV group on the following sampling dates (day 28) and the corresponding geometric mean was 99.94% and 99.94% respectively (**Table 4**). There was no significant difference (P<0.05) in percent of efficacy between two test compounds (**Table 5**).

Table 4: Rivermec LV trial 2- group mean FECs (epg), standard deviations and treatment efficacies.

Group	Treatment	Day 0	Day 14	Day 28
	Arithmetic Means			
1	Untreated Controls	626.7	696.6	725.0
	St Deviation	222.7	199.1	171.3
2	Rivermec LV	680.0	13.8	7.4
	St Deviation	207.4	35.1	26.7
	Treatment Efficacy		**98.02%**	**99.98%**
3	Cydectin® LV	686.7	10.0	7.1
	St Deviation	192.5	30.5	26.2
	Treatment Efficacy		**98.56%**	**99.01%**
	Geometric Means			
1	Untreated Controls	591.7	668.4	704.0
2	Rivermec LV	649.7	0.9	0.4
	Treatment Efficacy	---	**99.87%**	**99.94%**
3	Cydectin® LV	660.3	0.6	0.4
	Treatment Efficacy	---	**99.91%**	**99.94%**

Table 5: Rivermec LV trial 2- statistical comparison of group mean FECs.

Parameter	Value/Conclusion		
	Day 0		
F-statistic	1.06		
p-value	0.351		
Conclusion	No significant difference between groups at p<0.05		
	Day 14		
F-statistic	220		
p-value	0		
Conclusion	Significant differences at p<0.05		
	Controls-Rivermec LV	**Controls-Cydectin® LV**	**Rivermec LV-Cydectin® LV**
Point estimate of difference in FEC	353.8	425.6	
Lower 95% Confidence Interval for difference	161.2	194.0	No significant difference at p<0.05
Upper 95% Confidence Interval for difference	775.2	911.0	
K-W non-parametric ANOVA	Significant difference at p<0.05	Significant difference at p<0.05	No significant difference at p<0.05
	Day 28		
F-statistic	356		
p-value	0.0		
Conclusion	Significant difference at p<0.05		
	Controls-Rivermec LV	**Controls-Cydectin® LV**	**Rivermec LV-Cydectin® LV**
Point estimate of difference in FEC	500.2	511.9	
Lower 95% Confidence Interval for difference	262.0	268.2	No significant difference at p<0.05
Upper 95% Confidence Interval for difference	954.0	954.0	
K-W non-parametric ANOVA	Significant difference at p<0.05	Significant difference at p<0.05	No significant difference at p<0.05

Faecal samples were analysed to identify the larval species. *Tricostrongyle* was predominant followed by *Ostetragia*, *Oesophagostomum* and *Haemonchus*. These results showed that Rivermec (R) LV and Cydectin LV showed a similar efficacy profile. No significant difference was found (P < 0.05) between the two test candidates. No animal was died or removed throughout the trial period. No clinically detectable side effects to the treatment with recommended dose of 0.2 % Rivermec (R) LV drench for sheep were observed and a progressive recovery of treated animals was observed throughout the trial.

3.3 Trial III:

Trial III was conducted to establish/assess a comparable efficacy between Rivermec (R) LV and Cydectin(R) LV oral drench at the same dose, environmental conditions and worm species in Summer season. All the animals selected for the trial showed positive faecal egg counts. After treatment, on day 14 the arithmetic mean of EPG in group Rivermec & Cydectin decreased sharply. The efficacy based on faecal egg counts reduction was 99.416 % for group Rivermec and 98.971 for group Cydectin on day 14 and their corresponding geometric mean were 99.97% and 99.94% respectively. The efficacy of moxidectin treatment against various species of nematodes based on faecal egg counts was 98.699 % for group Rivermec and 97.960 for group Cydectin on the following sampling dates (day 28) and their corresponding geometric mean was 99.89% and 99.81 % respectively (Table 6). No significant difference was noticed between two test compounds at P< 0.05 (Table 7).

Table 6: Rivermec LV trial 3- group mean FECs (epg), standard deviations and treatment efficacies.

Group	Treatment	Day 0	Day 14	Day 28
	Arithmetic Means			
1	Untreated Controls	620.0	777.8	668.4
	St Deviation	365.1	288.1	321.5
2	Rivermec LV	612.0	4.5	8.7
	St Deviation	435.2	21.3	28.8
	Treatment Efficacy	**---**	**99.42%**	**98.70%**
3	Cydectin® LV	692.0	8.0	13.6
	St Deviation	406.1	27.7	35.1
	Treatment Efficacy		**98.97%**	**97.96%**
	Geometric Means			
1	Untreated Controls	528.1	729.5	459.0
2	Rivermec LV	416.0	0.2	0.5
	Treatment Efficacy	**---**	**99.97%**	**99.89%**
3	Cydectin® LV	592.0	0.4	0.9
	Treatment Efficacy	**---**	**99.94%**	**99.81%**

These results showed that Rivermec (R) LV and Cydectin LV showed a similar efficacy profile irrespective of seasonal conditions. No significant difference was found (P < 0.05) between these two test candidates. No animal was died or removed throughout the trial period. No clinically detectable side effects to the treatment with recommended dose of 0.2 % Rivermec(R) LV oral drench were observed and a progressive recovery of treated animals was observed throughout the trial.

Table 7: Rivermec LV trial 3- statistical comparison of group mean FECs

Parameter	Value/Conclusion		
	Day 0		
F-statistic	0.89		
p-value	0.415		
Conclusion	No significant difference between groups at p<0.05		
	Day 14		
F-statistic	259		
p-value	0		
Conclusion	Significant differences at p<0.05		
	Controls-Rivermec LV	**Controls-Cydectin® LV**	**Rivermec LV-Cydectin® LV**
Point estimate of difference in FEC	587.8	500.2	
Lower 95% Confidence Interval for difference	274.4	238.9	No significant difference at p<0.05
Upper 95% Confidence Interval for difference	1257.9	1070.5	
K-W non-parametric ANOVA	Significant difference at p<0.05	Significant difference at p<0.05	No significant difference at p<0.05
	Day 28		
F-statistic	94		
p-value	0.0		
Conclusion	Significant difference at p<0.05		
	Controls-Rivermec LV	**Controls-Cydectin® LV**	**Rivermec LV-Cydectin® LV**
Point estimate of difference in FEC (Controls - Rivermec LV)	308.0	244.5	
Lower 95% Confidence Interval for difference	99.0	78.4	No significant difference at p<0.05
Upper 95% Confidence Interval for difference	932.3	757.6	

3.5 Identification of Larvae:

Faecal samples were tested to identify the larval species. *Tricostrongyle* was predominant followed by *Ostetragia, Oesophagostomum, Cooperia* and *Haemonchus* (**Table 8**). Thus, it is evident that the formulation is effective against the strains most frequently affect the sheep industry.

3.6 Stability Study:

The stability of Rivermec [(R)] LV Oral drench was evaluated over three months at two different temperatures. There was no physical change in the solution; moreover, moxidectin degradation in these three batches was not significant, suggesting that the drug is stable in the formulation (**Table 9**) but further study is required to establish the stability of moxidectin in this formulation for a longer period of time.

3.7 Recovery from SBF:

It was assumed that moxidectin could be recovered from simulated body fluid for both formulations in similar amounts which would confirmthat a change of inert excipient does not affect the level of moxidectin in body fluid and hence its levels in tissue and fat. Thus, we have conducted a comparable recovery study of moxidectin from Simulated Body Fluid between Cydectin LV (Reference product) and Rivermec [(R)] LV. The simulated body fluid composition is given in table 10. The hypothesis was to demonstrate that despite a variation in excipients both formulations behave in a reasonably similar fashion in Simulated Body Fluid. It is worthy to mention that correlations between *in vitro* and *in vivo* data (IVIVC) are often used during pharmaceuticals development in order to reduce development time and optimize the formulation. A good correlation is a tool for predicting *in vivo* results based on *in vitro* data. IVIVC allows dosage form optimization with the fewest possible trials, fixes dissolution acceptance criteria, and can be used as a surrogate for further bioequivalence studies.

Table 8: Identification of Larvae

Larva species	Trial 2 (%)	Trial3 (%)
Tricostrogylus	71	47
Ostertagia	27	48
Cooperia	-	2
Oesophgostatomum	1	3
Haemonchus Contortus	1	-

Table 9: Stability results

Time and Storage Condition	4068530823 A (Moxidectin, mg/mL)	4068530823 B (Moxidectin, mg/mL)	4068530823 C (Moxidectin, mg/mL)
Initial	2.109 mg/mL	2.113 mg/mL	2.112 mg/mL
3 months at 30 ^{0}C	2.051 mg/mL	2.030 mg/mL	2.020 mg/mL
3 months at 40 ^{0}C	2.018 mg/mL	2.002 mg/mL	1.998 mg/mL
6 months at 30 ^{0}C	2.057 mg/mL	2.027 mg/mL	2.042 mg/mL
6 months at 40 ^{0}C	1.997 mg/mL	1.965 mg/mL	2.019 mg/mL

Table 10: Reagents for preparation of Simulated Body Fluid [14]

Reagents	Amount per L of SBF
Sodium Chloride	8.035 g
Sodium Bicarbonate	0.355 g
Potassium Chloride	0.225 g
Potassium Phosphate dibasic trihydrate	0.231 g
Magnesium Chloride Hexahydrate	0.311 g
1 M HCl acid	39 mL
Calcium Chloride	0.292 g
Sodium Sulfate	0.072 g
tris(hydroxymethyl) aminomethane	6.118 g

Table 11 shows % of moxidectin recovered from Simulated Body Fluid for both Cydectin LV and Rivermec (R) LV and both formulations showed similar % of recovery from SBF. *In vitro* Simulated Body Fluid (SBF) test is a good predictor of possible *in vivo* bioactivity **[14]**. It could be concluded that despite variation in formulation excipients, it did not have any effect on moxidectin concentration in SBF and hence could be assumed that absorption, distribution, elimination and tissue residue will be similar for both test products.

Table 11: % of Moxidectin recovered from Simulated Body Fluid for both Cydectin LV (Reference product) and Rivermec (R) LV formulations.

Product	% Recovery
Cydectin LV (n=3)	98.105 ± 0.180
Rivermec (R) LV (n=3)	97.272 ± 0.523

4. DISCUSSION:

This study shows that the newly developed formulation Rivermec (R) LV drench and commercially available Cydectin(R) LV 0.2% oral moxidectin solutions at a dose rate of 0.2 mg moxidectin/kg b.w. are safe and highly effective (97-100%) against naturally acquired internal parasitic **(***Tricostrogylus*, *Ostertagia***,** *Haemonchus Contortus***,** *Oesophgostatomum***,** *Nematodirus***)** infection in sheep. The high efficacy observed for these parasites even on day 25 of post treatment indicates a residual effect of the drug [18]. Both formulations had similar efficacy and showed that the action of moxidectin was rapid against nematodes becasue after 12 days of treatment, the efficacy was greater than 97%. These results of the current trials are in congruent with those from other reports using the similar formulations at the same dose rate [15-17].. Rivermec (R) LV and Cydectin(R) LV also showed similar efficacy profile irrespective of seasonal differences suggesting the newly developed formulation is effective in different climatic conditions. The daily weight gain of sheep in the treatment group was significantly different from the control group during the trial 1. The similar trend of weight gain was observed by Skys and Juma (1984), Uriarte et al. (1993) where the growth rate in treatment group was significantly higher than control group. The toxicology study results are an indication of high level of safety margin even at 5 times of recommended dose. Larval species identification results showed that *Tricostrongyle* was predominant followed by *Ostetragia*, *Oesophagostomum*, *Cooperia* and *Haemonchus*. It is important to mention that during faecal egg count by McMaster method, there was prevalence of *Nematodirus* in a good number of samples but during larval species identification, no *Nematodirus* were found. This is may be due to random sampling of faecal sample. Similar recovery percentage of moxidectin from simulated body fluid medium suggests the possibility of similar behaviour in vivo. A comprehensive biometric analysis of efficacy study results suggests that obtained stability study is scientifically valid and the results of newly formulated Rivermec(R) LV drench showed that the formulation is stable for a period of six months at both room temperature (30^0C) and accelerated conditions(40^0C).

5. CONCLUSION:

The comparable high treatment efficacy against various gastrointestinal nematodes, high safety margin and stability results indicated that the newly formulated Rivermec (R) LV drench for sheep could be a potential candidate for common gastrointestinal nematodes in sheep industry.

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