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Original papers

Evidence of *Fasciola* spp. resistance to albendazole, triclabendazole and bromofenofos in water buffaloes (*Bubalus bubalis*)

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ABSTRACT. Fasciolosis caused by *Fasciola* spp. is considered the most important helminth infection of ruminants in tropical countries. Anthelmintic resistance has become a global concern. This study compared the efficacy of the commonly used anthelmintics, determined the toxicity level and any indication of resistance. Thirty two water buffaloes naturally-infected with *Fasciola* spp. were used to determine the efficacy of triclabendazole (TBZ), albendazole (ABZ), and bromofenofos (BRO) using Fecal Egg Count Reduction Test (FECRT). To test the toxicity of the drugs given, serum glutamic-pyruvic transaminase (SGPT) was evaluated before and within one week after treatment. One dose administration of ABZ registered an efficacy of 79.17%, 73.33% for TBZ and 70.83% for BRO. Efficacy in two dose-treatment group was 83.33% for both BRO and ABZ, and 90.00% for TBZ. Two dose-treatment was effective for TBZ (90%), ineffective for BRO and ABZ. SGPT levels were not significantly different between pre-treatment and post-treatment across all treatments. Giving one or two doses of anthelmintics, at one month interval, does not increase the efficacy of the three drugs tested. The study also implies that anthelmintic resistance may have developed in the animals.

Key words: *Fasciola* spp., albendazole, bromofenofos, triclabendazole, anthelmintic resistance

Introduction

The total water buffalo population in the Philippines as of 2014 was 2.86M with more than 99.5% raised by smallhold farms. The number of commercial farms went up by 5.92% from last year's inventory while the backyard sector dropped by 1.03% [1]. This scenario suggests that there is a need to improve the production management of smallhold-raised farms that represent the large segment of the industry.

One of the problems that beset the carabao farms is the occurrence of fasciolosis. The disease is caused by *Fasciola* spp. which is considered the most important helminth infection of ruminants in tropical countries [2]. It is the leading cause of morbidity and mortality in ruminants in the Philippines and classified as a disease of farm concern by the Bureau of Animal Industry.

Fasciolosis has been considered as the most destructive parasitic disease of farm animals in the Philippines. In a review reported by Copeman and Copland [3], the prevalence of fasciolosis in buffaloes in various parts of the country ranged from 37–69%.

Surveys in some Asian countries have shown that amongst domestic animals, buffaloes suffer more frequently from fasciolosis [4]. This may be attributed to the high risk of infection to these animals in view of their wallowing habit that increases their chance of access to the infective stage of the worm [2]. Immature *Fasciola* spp. parasitizes the liver while adults damage the bile ducts [5]. The worms compete with host nutrients leading to slow and poor development, liver condemnation and poor carcass [6].

The most common method of worm control in ruminants is the use of chemical anthelmintics. However, evidence of resistance to various anthelmintics has led to treatment failures [7]. Anthelmintic resistance has become a global concern with the recent report of problems in the efficacy of a number of anthelmintic preparations.

Local outbreaks of fasciolosis have recently been reported in some towns in Nueva Ecija (Abes, personal communication, 2013). This was alarming because there is a deworming program for fasciolosis in the affected areas. Assessment of the efficacy of available anthelmintics will provide a basis on planning a sustainable control program against fasciolosis. Options may be developed in order to preserve the efficacy of the drug by judicious use and integration with other biological worm control methods.

The study determined the efficacy of albendazole, triclabendazole, and bromofenofos against *Fasciola* spp. of naturally-infected water buffaloes in one-dose and two-dose treatments. Indication of resistance to the drugs was based on the efficacy. The level of toxicity to these drugs following treatment was also measured.

Materials and Methods

Identification of experimental animals. Animals owned by farmer cooperatives in the province of Nueva Ecija, Philippines were used in the study. These animals were utilized to determine the efficacy of three types of fasciolicides as reports of high morbidities and mortalities have been accounted in this area (Abes, personal communication, 2013).

A total of 32 buffaloes naturally-infected with *Fasciola* spp. regardless of sex, non-pregnant with ages ≥8 months were used in the study. Each of the two treatment sets: one dose-treatment (Set 1) and two dose-treatment (Set 2), and the control group consisted of four animals for each experimental set. The average individual pre-treatment fecal egg count (FEC) using standard sedimentation technique was taken two weeks before administration of anthelmintics. At day 0, all 32 animals were ranked according to their FEC such that heavy, moderate, and low burdens of fasciolosis were equally distributed in the groups for each set. The animals were treated with triclabendazole (TBZ), albendazole (ABZ), bromofenofos (BRO) in their respective designated groups according to the manufacturer's recommended dose.

Fecal collection and fecalysis. About 5g of feces were collected directly from the rectum of each animal and properly labeled into zipped plastic bags. Collected samples were transported to the laboratory for analysis. Individual FEC was done using the standard Sedimentation Technique [8].

Fecal Egg Count Reduction Test (FECRT). Data for the average pre-treatment FEC was determined at 7 and 0 day post-treatment. Post-treatment analysis of fecal egg counts were done on the 14th day after treatment. Efficacy rate was based on the pre- and post-treatment FEC using this formula [9]:

$$\text{FEC (EPG)} = \frac{\text{number of egg} \times \text{amount of fecal suspension}}{\text{amount examined} \times \text{amount of feces used}} \times 100$$

where: EPG = egg per gram

The efficacy of the anthelmintic compound was determined based on reduction of egg excretion at 14 days post-treatment using the formula below [10]:

$$\text{Efficacy} = \frac{\text{pre-treatment FEC} - \text{post-treatment FEC}}{\text{Pre-treatment FEC}} \times 100$$

Liver Enzyme Assay. The effect of anthelmintics on the liver function was determined by the Serum glutamic-pyruvic transaminase (SGPT) level determination. Initial SGPT level were determined on day 0, prior to drug administration, and succeeding test were done on 1st week post-treatment. A 10ml blood sample was collected from the jugular vein of the animal using vacutainer tubes. Samples were kept on ice until SGPT analysis.

Statistical analysis. Analysis of Variance (ANOVA) was used for the overall comparison of the different treatments at 95% confidence interval and *P* value of<0.05.

Results and Discussion

Efficacy of TBZ, ABZ, and BRO against *Fasciola* spp. infection using FECRT

The efficacy of the three drugs used namely TBZ, ABZ, and BRO that were given one dose-

Table 1. Efficacy of different anthelmintics against *Fasciola* spp. infection in buffaloes with one-dose treatment

Drug	Dose	Faecal egg count (eggs per gram)		Efficacy (%)
		Pre-treatment	Post-treatment	
Albendazole	0.07 ml/kg	675	150	79.17[a]
Triclabendazole	0.12 ml/kg	450	150	73.33[a]
Bromofenofos	0.05 g/kg	325	100	70.83[a]
Control		450	600	00.00[b]

Means of the same letter are not significantly different at $P<0.05$

treatment is shown in Table 1. No significant differences were found in the efficacy of ABZ (79.17%), TBZ (73.33%), and BRO (70.83%). Nevertheless, the three drugs are all significantly different from the control in one dose treatment. The drugs used in the study have claims of efficacy to different developmental stages of *Fasciola* spp. TBZ is effective against early immature, immature, and mature stages [11,12]. ABZ, a broad spectrum dewormer, is effective against adult stages of the parasite [13]. BRO on the other hand is known to be effective against mature and immature fluke stages [11]. The present study did not establish the specific stage at which the drugs used were effective as the only basis for the calculation of efficacy is the FECRT. Nevertheless, this method is considered reliable in field trials of drug efficacy. Resistance or failure of treatment with the tested drug was defined as efficacy of less than 90% based on the standards set by the World Association for the Advancement of Veterinary Parasitologists (WAAVP) [14]. Others have also used 90% reduction in FECRT as the threshold for resistance based on a set country standard. For example, Brockwell et al. [15] followed 90% reduction as cut off based on the Australian flukicide efficacy trials.

Guidelines and standard tests to determine drug resistance in *F. hepatica* is currently not available [16]. However, previous studies in experimentally-infected cattle [17] and in sheep [18] show that the FEC have suitable characteristics for resistance tests.

One dose treatment with TBZ was found to be ineffective with efficacy of 72.22%. This is contrary to the reports of efficacy of TBZ against liver flukes in the country [19]. There are factors that may be associated with the inefficacy of the drug. It is worth mentioning that TBZ's claim of efficacy specifically refers to *Fasciola hepatica*. This may be considered a limiting factor in the drug efficacy considering that the preponderant species in the Philippines is *Fasciola gigantica* [7]. Differences in reaction to a drug may occur at the species level, which may be the case in the present study. However, the eggs counted in the present study were not identified to the species level. It is possible that the worm load of the animals were predominated by *Fasciola gigantica* which may not have high susceptibility to TBZ.

The present findings suggest the development of resistance of *Fasciola* sp. against the three drugs used. According to the WAAVP standards, a highly effective anthelmintic should have an efficacy of >98% [14]. An efficacy of less than 90% is considered an indication of resistance development. Several studies have established development of resistance of *Fasciola* spp. against different types of anthelmintic. A recent study on *Fasciola hepatica* sheep isolate showed resistance against ABZ while being susceptible to TBZ [20]. On the contrary, resistance of liver flukes to TBZ was observed in a clinical trial in a cattle population [21].

An earlier study demonstrated resistance of liver flukes to TBZ in experimentally-infected sheep with very low efficacy of 10%. In buffaloes, resistance of liver flukes to flukecides have also been reported [22].

The mean pre-treatment and post-treatment FEC after one-dose administration of ABZ, TBZ, and BRO are shown in Table 1. Except for the control, there was a decreasing trend from pre-treatment to post-treatment FEC in all of the three treatments. Data shows a mean pre-treatment FEC of 675 EPG in ABZ, 450 EPG in TBZ, 325 EPG in BRO, and 450 EPG in the control animals. Both ABZ and TBZ groups registered a FEC of 150 EPG 14 days after treatment. Post-treatment FEC of 100 EPG and 600 EPGwere recorded for BRO and Control groups, respectively.

Differences in the pre-treatment and post-

Table 2. Efficacy and faecal egg counts of different anthelmintics against *Fasciola* spp. infection in buffaloes with two-dose treatment

Drug	Dose	Faecal egg count (eggs per gram)		Efficacy (%)
		Pre-treatment	Post-treatment	
Albendazole	0.07 ml/kg	725	100	83.33[a]
Thiabendazole	0.12 ml/kg	475	50	90.00[a]
Bromofenofos	0.05 g/kg	250	50	83.33[a]
Control		375	300	10.83[b]

Means of the same letter are not significantly different at $P<0.05$

treatment FEC of the different treatment groups can be possibly attributed to the fact that these animals were owned by different farmers and were located in different areas. It is likely that they have differences in the level of infection and different amount of eggs excreted in the feces. Statistical analysis reveals no significant difference $P=0.05$ between the FEC after using ABZ, TBZ, and BRO in treatment animals.

Efficacy of TBZ, ABZ, and BRO against *Fasciola* spp. infection using FECRT in two dose-treatments

The efficacies of ABZ, TBZ, and BRO in buffaloes after two dose-treatments with interval of one month in-between treatments are shown in Table 2. ABZ had an efficacy of 83.33% which was similar to those treated with BRO. The efficacy of TBZ (90%) was higher compared to the two other treatments. However, there was no significant difference ($P<0.05$) between TBZ and the two other treatments.

Administration of ABZ and BRO against liver flukes in buffaloes two times at one month interval was effective with efficacy rates of 83% for both drugs. The efficacy of TBZ (90%) indicates that the drug was effective if given at two-dose treatment regimen. TBZ is claimed to be effective against early immature until the adult stage. On the other hand, BRO and ABZ are known to be effective against adult and immature stages. It is possible that the immature stages were killed at first dose of TBZ and hence reduced the number of egg-laying adults. It seems likely as well that immature flukes were not affected by ABZ and BRO allowing them to mature and lay eggs.

There is an indication based on the present findings that resistance to ABZ and BRO may be present. ABZ, a broad spectrum dewormer, is

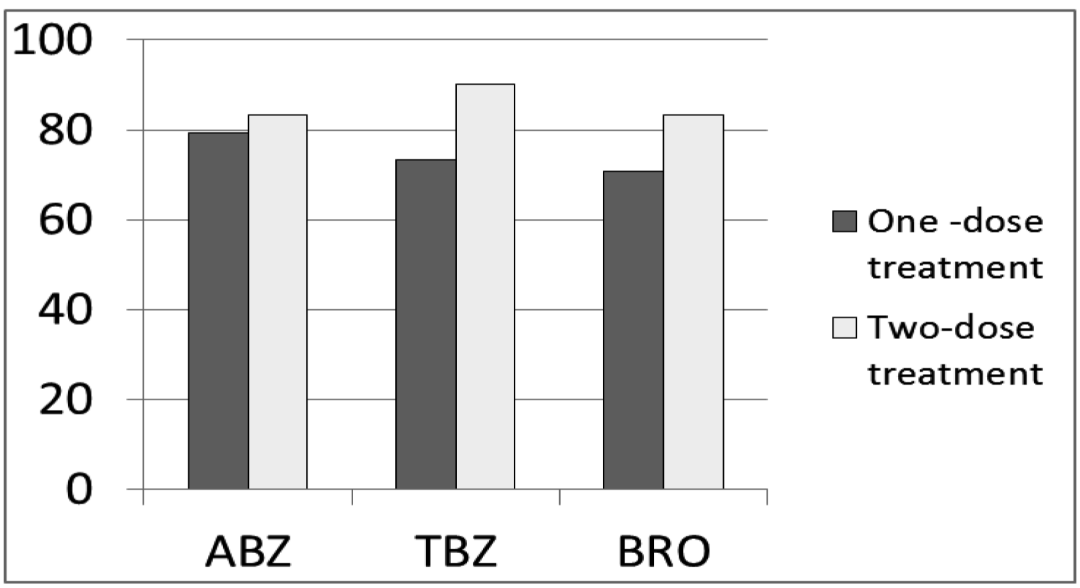

Fig. 1. Efficacy (%) of albendazole (ABZ), triclabendazole (TBZ), and bromofenofos (BRO) against *Fasciola* spp. in buffaloes

Table 3. Comparison of SGPT values in triclabendazole (TBZ), albendazole (ABZ), and bromofenofos (BRO) treated buffaloes and Control (CON) in one-dose treatment

Anthelmintic	Mean SGPT Level (U/L)	
	Pre Tx	Post Tx
ABZ	54.83[a]	50.43[a]
TBZ	47.80[b]	49.77[b]
BRO	53.13[c]	41.90[c]
CON	46.87[d]	61.33[d]

Means with the same letter within rows are not significantly different at $P<0.05$

commonly used in ruminants. Indication of ABZ resistance in nematodes of buffaloes has been reported in the Philippines [19]. Frequent use of the same anthelmintic has been proven to be one of the factors that contribute to resistance development. Abes (personal communication, 2013) had disclosed that ABZ had been in use for more than five years in most cooperator farms. Hence, the probability that selection pressure for resistance development has increased throughout the years. TBZ may be considered effective at 90% but since the drug is expected to be highly effective (98%) with single dose as a commercial drug, the current efficacy shows a tendency for the drug to develop resistance.

The mean pre-treatment and post-treatment FEC after two-dose administration of ABZ, TBZ, and BRO with interval of one month are shown in Table 2. The data shows a mean pre-treatment FEC of 725 EPG in ABZ, 475 EPG in TBZ, 250 EPG in BRO, and 375 EPG in the control animals. Both TBZ and BRO groups registered a FEC of 50 EPG 14 days after treatment, while a post-treatment FEC of 100 EPG and 300 EPG respectively were recorded for ABZ and the control groups.

On the other hand, BRO group had an initial FEC average of 250 EPG which was reduced to 50 EPG 14 days after treatment. Meanwhile, the control group had an initial FEC of 375 EPG which slightly reduced to 300 EPG after 14 days. The difference in the FEC of the different treatment groups from the pre-treatment to the 14th day post-treatment can be possibly attributed to the different farm locations of the farms. Reduction of FEC in the untreated control group can be attributed to the possible natural death and expulsion of worms that reach their life span. Statistical analysis reveals no significant difference between the FEC after using ABZ, TBZ, and BRO in treatment animals P=0.05.

Comparison of efficacy of TBZ, ABZ, and BRO against *Fasciola* spp infection using FECRT in one-dose treatment and two dose-treatment

Comparison on the efficacy of the three drugs given at one dose and two dose- treatment at one month interval is shown in Fig. 1. Analysis of the data shows that the efficacy of ABZ is comparable between one dose and two-dose treatments with all three anthelmintics used. There was no significant difference between the two treatment sets for ABZ at P =0.80. Similarly, one-dose and two-dose treatments with TBZ (P=0.41) and BRO (P=0.62) were not significantly different. These findings indicate that administration of one or two-dose treatments of any of the three drugs used have comparable levels of efficacy. Results also suggest that giving a subsequent dose of any of the three anthelmintics after a month will not increase their efficacy. Some farmers have disclosed that they tend to give a follow-up dose of anthelmintics if they observe that the animal's weight based on visual estimation do not improve after one treatment dose. This study proves that a follow-up treatment at one month interval will not improve the efficacy of the drug. Thus, it only shows that it is impractical to give a second dose of the respective anthelmintics as it will be an added cost without additional benefit.

Table 4. Comparison of SGPT values in triclabendazole (TBZ), albendazole (ABZ), bromofenofos (BRO) and CON (Control)

	SGPT (U/L) 1st Treatment		SGPT (U/L) 2nd Treatment	
	Pre	Post	Pre	Post
ABZ	53.13[a]	41.90[a]	55.00[a]	49.90[a]
TBZ	50.10[b]	51.90[b]	48.77[b]	55.67[b]
BRO	41.00[c]	42.67[c]	48.43[c]	44.67[c]
CON	85.10[d]	49.23[d]	56.77[d]	57.00[d]

Means with the same letter within rows are not significantly different at $P<0.05$

SGPT Levels of buffaloes before and after treatment with TBZ, ABZ, and BRO in one dose-treatment and two-dose treatment

The mean values of SGPT on each treatment are shown on Table 3. ABZ had an average SGPT of 54.83 U/L prior to treatment which was reduced slightly to 50.43 U/L after treatment. Conversely, SGPT level before treatment with TBZ was 47.80 U/L which had a subtle increase after treatment (49.77 U/L). The group treated with BRO had mean of 53.13 U/L SGPT before treatment which was reduced to 41.90 U/L after treatment. The SGPT of the control group was 46.87 U/L before treatment which rose to 61.33 U/L after treatment. These differences in SGPT levels before and after treatment in all treatments including the control are not significantly different at P<0.05.

The normal SGPT value in cattle and buffalo may vary from 6.9–35 U/L depending on associated condition. Except for the post-treatment SGPT levels in the control group, the SGPT levels obtained in the current study is within the normal range, assuming that the standards set for cattle are the same with buffaloes. However, there is no significant increase or decrease in the level of the SPGT values in all the animals treated with different anthelmintics, hence it can be concluded that there was no significant effect on the liver. References show that increase in the SGPT values are related to toxicity of the drug to the liver, primarily because an increase in the enzymes means the liver overworked for the release of these enzymes in the blood, hence the detected high values [23]. However, other physiological processes such as increase exercise, pregnancy, and even muscle damage can increase SGPT values. An extreme fluctuation however is indicative of liver disease.

The mean SGPT on one dose- and two dose-treatment with TBZ, ABZ, and BRO, as well as the untreated control are shown in Table 4. With one dose- treatment, ABZ showed an average SGPT of 53.13 U/L before treatment which was decreased to 41.90 U/L after treatment. This observation was inconsistent with TBZ wherein SGPT level before treatment was 50.10 U/L which increased slightly to 51.90 U/L after treatment. The group treated with BRO has a mean of 41.00 U/L SGPT before treatment which increased very slightly to 42.67 U/L SGPT after treatment. However, the control group has a mean of 85.10 U/L SGPT before treatment which plummeted to 49.23 U/L after treatment. With two dose-treatments, ABZ had a mean of 55.00 U/L SGPT before treatment and was reduced to 49.90 U/L SGPT after treatment. TBZ had a mean of 48.77 U/L SGPT before treatment and has increased to 55.67 U/L SGPT after treatment. Animals treated with BRO had a mean of 48.43 U/L SGPT before treatment which was lowered to 44.67 U/L SGPT after treatment. Control group however has a mean of 56.77 U/L SGPT before treatment and 57.00 U/L after treatment. These differences in SGPT levels before and after treatment in all treatments including the control are not significantly different at P<0.05. Non-significant reduction in liver function tests after anthelmintic treatment against *Fasciola* spp. is inconsistent with the findings of Pal and Dasgupta [24]. They demonstrated that aspartate amino-transferase (AST), alanine aminotranferase (ALT) and alkaline phosphatase (ALP) decreased significantly after treatment with TBZ. Others have shown increase in SGPT following treatment [25]. High SGPT in control animals before treatment may be attributed to other unknown physiological difference in these animals. In the same manner, SGPT is normally present in large concentrations in the liver due to worm infestation.

Conclusions

There is an indication of resistance of *Fasciola* spp. in Philippine water buffaloes to albendazole, triclabendazole, and bromofenofos based on the low efficacy. One or two-dose treatment with the three anthelmintics has no effect on efficacy of the drugs. None of the drugs tested are potentially toxic based on the SGPT levels after treatment.

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References

[1] BAS 2014. Bureau of Agricultural Statistics. Department of Agriculture. http://www.bas.gov.ph/

[2] Sharma R.L., Godara R., Thilagar M.B. 2011.

Epizootiology, pathogenesis and immunoprophylactic trends to control tropical bubaline fasciolosis: an overview. *Journal of Parasitic Diseases* 35: 1-9.

[3] Copeman D.B., Copland R.S. 2008. Importance and potential impact of liver fluke in cattle and buffalo. http://aciar.gov.au/files/node/9010/MN133%20part%202.pdf.

[4] Maqbool A., Hayat C.S., Akhtar T., Hashmi H.A. 2002. Epidemiology of fasciolosis in buffaloes under different management conditions. *Veterinarski Arhiv* 72: 221-228.

[5] Soulsby E.J.W. 1982. Helminths, Arthropods, and Protozoa of Domesticated Animals.7th ed. Bailliere Tindall.

[6] Molina E.C., Gonzaga E.A., Lumbayo L.A. 2005. Prevalence of infection with *Fasciola gigantica* and its relationship to carcass and liver weights, fluke and egg counts in slaughter cattle and buffaloes in South Mindanao, Philippines. *Tropical Animal Health and Production* 37: 215-221.

[7] Moll L., Gaasenbeek C.P.H., Vellema P., Borgsteede F.H.M. 2000. Resistance of *Fasciola hepatica* against triclabendazole in cattle and sheep in the Netherlands. *Veterinary Parasitology* 91: 153-158.

[8] Coles E.H., Ewing S.A. 1980. Veterinary Clinical Pathology. W.B. Saunders, Philadelphia, USA.

[9] Coles G.C., Bauer C., Borgsteede F.H.M., Geerts S., Klei T.R., Taylor M.A., Waller P.J. 1992. World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.) methods for the detection of anthelmintic resistance in nematodes of veterinary importance. *Veterinary Parasitology* 44: 35-44.

[10] Young K.E., Jensen J.M., Craig T.M. 2000. Evaluation of anthelmintic activity in captive wild ruminants by fecal egg reduction tests and a larval development assay. *Journal of Zoo and Wildlife Medicine* 31: 348-352.

[11] Boray J.C., Crowfoot P.D., Strong M.B., Allison J.R., Schellenbaum M., Von Orelli M., Sarasin G. 1983. Treatment of immature and mature *Fasciola hepatica* infections in sheep with triclabendazole. *Veterinary Record* 113: 315-317.

[12] Estuningsih S.E., Stevenson P., Beriajaya, Knox M.R. 1990. Triclabendazole in the treatment of *Fasciola gigantica* infection in swamp buffalo (*Bubalus bubalis*). *Australian Veterinary Journal* 67: 234-235.

[13] Junquera P. 2013. Parasites of Dogs, Cats & Livestock: Biology & Control. http://www.http://parasitipedia. net/index.php?option=com_content&view = article&id=2730 &Itemid=2971.

[14] Wood I.B., Amaral N.K., Bairden K., Duncan J.L., Kassai T., Malone J.B., Pankavich J.A., Reinecke R.K., Slocombe O., Taylor S.M., Vercruysse J. 1995. World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.) second edition of guidelines for evaluating the efficacy of anthelmintics in ruminants (bovine, ovine, caprine). *Veterinary Parasitology* 58: 181-213.

[15] Brockwell Y.M., Elliott T.P., Anderson G.R., Stanton R., Spithill T.W., Sangster N.C. 2014. Confirmation of *Fasciola hepatica* resistant to triclabendazole in naturally infected Australian beef and dairy cattle. *International Journal of Parasitology: Drugs and Drug Resistance* 4: 48-54.

[16] Coles G.C., Jackson F., Pomroy W.E., Prichard R.K., Von Samson-Himmelstjerna G., Silvestre A., Taylor M.A., Vercruysse J. 2006. The detection of anthelmintic resistance in nematodes of veterinary importance. *Veterinary Parasitology* 136: 167-185.

[17] Brockwell Y.M., Spithill T.W., Anderson G.R., Grillo V., Sangster N.C. 2013. Comparative kinetics of serological and copro-antigen ELISA and faecal eggcount in cattle experimentally infected with *Fasciola hepatica* and following treatment with triclabendazole. *Veterinary Parasitology* 196: 417-426.

[18] Flanagan A.M., Edgar H.W.J., Gordon A., Hanna R.E.B., Brennan G.P., Fairweather I. 2011. Comparison of two assays, a faecal egg count reduction test (FECRT) and a coproantigen reduction test (CRT), for the diagnosis of resistance to triclabendazole in *Fasciola hepatica* in sheep. *Veterinary Parasitology* 176: 170-176.

[19] Reyes G.M. 2006. Field Trial on the Efficacy of Albendazole and Ivermectin on naturally-infected Buffaloes against *Fasciola* spp. Undergraduate thesis. College of Veterinary Science and Medicine, Central Luzon State University, Science City of Muńoz, Nueva Ecija, Philippines.

[20] Sanabria R., Ceballos L., Moreno L., Romero L., Lanusse C., Alvarez L. 2013. Identification of a field isolate of *Fasciola hepatica* resistant to albendazole and susceptible to triclabendazole. *Veterinary Parasitology* 193: 105-110.

[21] Ortiz P., Scarcella S., Cerna C., Rosales C., Cabrera M., Guzmán M., Lamenza P., Solana H. 2013. Resistance of *Fasciola hepatica* against triclabendazole in cattlein Cajamarca (Peru): a clinical trial and an in vivo efficacy test in sheep. *Veterinary Parasitology* 195: 118-121.

[22] Overend D.J., Bowen F.L. 1995. Resistance of *Fasciola hepatica* to triclabendazole. *Australian Veterinary Journal* 72: 275-276.

[23] Ozer J., Ratner M., Shaw M., Bailey W., Schomaker S. 2008. The current state of serum biomarkers of hepatotoxicity. *Toxicology* 245: 194-205.

[24] Pal S., Dasgupta C. 2006. Haemato-biochemical profiles of buffalo in anthelmintics treatment against *Fasciola gigantica* infection. *Buffalo Bulletin* 25: 40.

[25] Bharti P., Prasad K.D. 2001. Biochemical profiles of cattle and buffalo infected with *Paramphistomum* spp. and *Fasciola gigantica*. *Journal of Veterinary Parasitology* 15: 149-151.

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