Efficacy of triclabendazole and albendazole against *Fasciola* spp. infection in cattle in Côte d'Ivoire: a randomised blinded trial

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ABSTRACT

Triclabendazole is the anthelminthic of choice for the treatment of fascioliasis, however, it is only registered in a few countries. We investigated the efficacy of a single-dose of triclabendazole (12 mg/kg) or albendazole (15 mg/kg) against *Fasciola* spp. infection in cattle on farms in the northern part of Côte d'Ivoire in a randomized clinical trial. Faecal samples were obtained from 196 cattle, of which 155 (79.1%) were found positive for *Fasciola* spp. by the sedimentation technique. Cattle infected with *Fasciola* spp. were randomly allocated (3:3:1) to receive triclabendazole (n = 66), albendazole (n = 67) or left untreated to serve as control (n = 22). Follow-up faecal samples were collected on days 21, 28, 90 and 188 post-treatment. No adverse events were observed as reported by farmers in any of the treatment groups. The proportion of non-egg shedding cattle, assessed at day 21 (primary outcome), was significantly higher in cattle treated with triclabendazole (95.4%) compared to those receiving albendazole (70.3%; odds ratio [OR] 8.73, 95% confidence interval [CI] 2.43-31.28, p <0.001). The egg reduction rate (ERR) expressed as number of eggs per gram of faeces, a secondary endpoint assessed at day 21 post-treatment, was significantly higher in the triclabendazole arm (arithmetic mean (AM) ERR = 99.8%) than in the albendazole arm (AM ERR = 92.2%), with a difference of 7.6%-points (95% CI: 0.9-14.5%-points, p=0.026). This is the first report of efficacy of triclabendazole against *Fasciola* spp. in naturally infected cattle in Côte d'Ivoire. Our results confirm that triclabendazole is the most effective treatment of fascioliasis and therefore, should be considered for the control of livestock fascioliasis; if resources allow in combination with intermediate host snail control and raising farmers awareness of pasture and livestock management to avoid reinfection.

1. Introduction

The liver flukes *Fasciola gigantica* and *Fasciola hepatica* are responsible for fascioliasis, a chronic, zoonotic disease of considerable veterinary and public health importance (Bennema et al., 2017; Fürst et al., 2012; Mas-Coma et al., 2018). *Fasciola hepatica* is a cosmopolitan species adapted to temperate regions, while *F. gigantica* is found in tropical and subtropical parts of Africa and Asia (Greter et al., 2017; Malatji et al., 2019; Parkinson et al., 2007). Fascioliasis affects mammals, mainly cattle, goats and sheep, but can also infect humans (Fürst et al., 2012;

Gandhi et al., 2019). It causes economic loss in livestock, mainly through reduced fertility and productivity, liver condemnation in abattoirs, stunted growth and premature death (Kaplan, 2001; Suleiman et al., 2015; Yusuf et al., 2016). Fascioliasis is considered one of the most widespread food-borne trematode infections (Fürst et al., 2012).

Since its introduction in the early 1980s in Australia (Boray et al., 1983), triclabendazole – a benzimidazole carbamate – has become one of the most widely used anthelminthic drugs for the treatment and control of fascioliasis in cattle and in humans (Gandhi et al., 2019; Keiser et al., 2005). It has shown over 95% efficacy in reducing the

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excretion of *Fasciola* spp. eggs in cattle and sheep faeces (Keiser and Utzinger, 2004; Rapic et al., 1988; Shokier et al., 2013; Stevenson et al., 2002). Unlike other anthelminthics, which are only efficacious against adult flukes, triclabendazole has a lethal effect against both adult and immature flukes aged 2 weeks and above (Boray et al., 1983; Fairweather, 2005; Smeal and Hall, 1983). The lethal activity against immature flukes is particularly relevant, as the migration of the developing flukes out of the small intestine and into liver is the most damaging stage of the infection (Keiser et al., 2005).

In terms of mechanism of action, triclabendazole and other benzimidazoles (e.g. albendazole and mebendazole) disturb the secretory processes of the fluke. Triclabendazole, in particular, causes the disruption of the tegument of *Fasciola* spp. (Fairweather, 2009) and both triclabendazole and albendazole cause severe damage to the reproductive system of flukes (Fairweather and Boray, 1999). Triclabendazole metabolites reach peak plasma levels at one and three days after administration in cattle (sulfoxide and sulfone, respectively) and are virtually completely eliminated after 10 days (Health Products Regulatory Authority: Ireland, 2018). Triclabendazole-resistance in livestock was first documented in 1995 (Overend and Bowen, 1995), and has since been reported in many countries (Kelley et al., 2016).

Triclabendazole is not registered for use in livestock or humans in many West (Elelu and Eisler, 2018), Central (Greter et al., 2016) and East African countries (Keyyu et al., 2009; Nzalawahe et al., 2018a). In Côte d'Ivoire, despite the high prevalence of fascioliasis (Kouadio et al., 2020), farmers have only two options for treatment: albendazole and nitroxynil, both anthelminthics which only act on adult *Fasciola* spp. Access to efficacious anthelminthic drugs thus remains an issue in this fascioliasis endemic country. Condemnation of *Fasciola*-infected bovine livers from both sedentary and transhumant herds is carried out in slaughterhouses and represent a significant loss of income for livestock producers in Côte d'Ivoire (Achi et al., 2003). Hence, it is in the public interest to control livestock fascioliasis effectively. To our knowledge, there have been no controlled studies of triclabendazole use against fascioliasis in naturally infected cattle in West Africa. We designed a randomised controlled trial to assess the efficacy of triclabendazole against *Fasciola* spp. in naturally infected cattle in Côte d'Ivoire. Triclabendazole was compared to the approved anthelminthic albendazole.

2. Material and methods

2.1. Study design, sampling and safety

This randomised controlled clinical trial was conducted with cattle in Ferkessédougou (9° 35' 37'' N latitude, 5° 11' 50'' W longitude), in the northern part of Côte d'Ivoire (650 km north of Abidjan, the economic capital of the country), from June 2019 to January 2020. The sample size calculation was based on the primary analysis comparing the proportion of non-egg shedding cattle (PNES) of triclabendazole and albendazole. PNES was defined as the proportion of animals who were egg-positive at baseline but became egg-negative at treatment follow-up. Recent randomised trials investigating the efficacy of albendazole and triclabendazole against *F. hepatica* in cattle estimated the proportion of egg-shedding cattle who stop shedding eggs after treatment (apparent cure rate) at 50-70% and 100%, respectively (Nzalawahe et al., 2018b; Shokier et al., 2013). However, the sample size in both trials were low and therefore, we assumed conservatively conversion of egg-shedding cattle to non-egg shedding to be 70% for albendazole and 90% for triclabendazole. To detect a difference with 80% power at a two-sided 5% significance level, a minimum of 60 positive animals per study arm was required. The sample size of the control group was fixed at 20 animals. Assuming a 10% loss to follow-up, we calculated a final sample size of 154 positive cattle (66 per treatment arm and 22 in the untreated control group). The trial statistician (J.H), who was not involved in any field or laboratory work, provided a computer-generated, stratified (by herd), block randomisation code (blocks of size seven) for randomisation of the

cattle into treatment arms.

At baseline, faeces were obtained from the rectums of all cattle that could be handled by our team and the cattle were marked with uniquely numbered ear tags. Demographic information (i.e. sex, age, breed and herd location) were recorded. Cattle faeces were transferred to a nearby laboratory and subjected to the sedimentation technique (Sirois, 2017). Cattle were eligible for inclusion if they were 1 year of age or older, were not visibly ill (assessed by a veterinarian) and had *Fasciola* spp. eggs in the faeces. Cattle that met our inclusion criteria were allocated 3:3:1 to the triclabendazole, albendazole and control arms, respectively. Animals were treated over two consecutive days in July 2019. As there is no control programme for fascioliasis in Côte d'Ivoire, the pastures were not and had not been treated. After treatment, faeces were collected from the cattle in the three groups again on days 21, 28, 90 and 188 post-treatment. Farmers and technicians of the veterinary services in Ferkessédougou, assisted the study team catching and treating the animals.

Cattle were assessed by a veterinarian for diarrhoea and other visible signs of serious illness prior to receiving treatment. Farmers were asked to report adverse events, such as diarrhoea, severe illness or death, occurring within 3 weeks after treatment to the researchers. Veterinary technicians were not blinded, however, the microscopists who examined faecal samples for *Fasciola* spp. infection, were blinded as to the treatment group of the cattle. At the end of the study, *Fasciola* spp. positive cattle from the treatment and control arms were given triclabendazole (12 mg/kg) as per the drug marketer's instructions (Agridirect, 2021a).

2.2. Sedimentation method

Three grams of faeces were homogenised with 30 ml of tap water in a conical beaker. The mixture was left to sediment for 3 min, after which the supernatant was decanted. This process was repeated twice, for a total of 6 min of sedimentation. Then, a drop of methylene blue (1%) was added to the sediment, which was placed on a slide for microscopic examination (Giovanoli Evack et al., 2020; Sirois, 2017). Microscopists examined all sediments for *Fasciola* spp. eggs, using a compound microscope at 100X magnification. The number of eggs was quantified per gram of stool (EPG), by dividing the faecal egg count by a factor of 3 to obtain an estimate of EPG. Microscopists did not differentiate *Fasciola* eggs by species (*F. gigantica* or *F. hepatica*) as the size of eggs overlap between the two species and it was not an objective of this investigation to determine infecting *Fasciola* species (Periago et al., 2006; Valero et al., 2009).

2.3. Treatment

Two molecules were included in the trial: triclabendazole (Fasinex, Novartis Animal Health UK Limited; Surrey, UK) dosed at 12 mg/kg body weight and albendazole (Albex, Chanelle Animal Health Limited, Liverpool, UK) dosed at 15 mg/kg body weight, as recommended by the marketer of the anthelminthics (Agridirect, 2021a, Agridirect 2021b). The control group did not receive any molecule or placebo. The weight of each cow was determined by measuring the chest width with a weight tape, and the appropriate dose of medication was calculated and administered orally using a drench gun. The tape was validated by weighing cattle on a scale at the company "Viande de Ferke" in Ferkessédougou, which specializes in livestock breeding and slaughter. No anthelminthic treatment was administered during the follow-ups.

2.4. Endpoints

The primary endpoint of this study was the PNES of *Fasciola* spp. at 21 days post-treatment, using the sedimentation technique. The World Association for the Advancement of Veterinary Parasitology (WAAVP) recommends comparing faecal eggs counts in treated and untreated cattle at baseline and again at least three weeks after treatment by use of

the sedimentation technique (Wood et al., 1995). Therefore, *Fasciola* spp. egg reduction rate (ERR) at day 21 post-treatment was added as a secondary endpoint using faecal egg count reduction test (FECT), in addition to *Fasciola* reinfection rate (RR) at days 90 and 188 post-treatment. Reinfection was assessed in order to determine how quickly cattle become reinfected after treatment. Two time points were chosen, the first 90 days post-treatment was chosen as the time from ingestion of metacercaria to maturity and egg excretion is 7-8 weeks (Andrews, 1999; Mas-Coma et al., 2007). The second at 188 days post-treatment, was chosen to determine the rate of reinfection six months post-treatment.

2.5. Statistical analysis

Data were double entered into a Microsoft Access 2016 database and imported into Microsoft Access 2002-2003 (Redmond, Washington, USA) and cross-checked using the Data Compare tool of EpiInfo version 3.5.4 (Centers for Disease Control and Prevention; Atlanta, Georgia, USA). Statistical analyses were performed using STATA 15.1 (StataCorp; College Station, Texas, USA) and R version 3.6.2 (R Development Core Team; Vienna, Austria).

Descriptive statistics, crude rates (primary analysis) and adjusted logistic regression (adjustment for herd, age, sex and breed) were calculated to determine the efficacy in terms of PNES. The ERR was defined as the percentage of mean reduction at follow-up 1 (21 days post-treatment) compared to baseline and was calculated using both the arithmetic mean (AM) and the geometric mean (GM). Ninety-five percent confidence intervals (CIs) for differences in ERRs were calculated using the bootstrap resampling method with 1,000 replicates and the significance level was set at p <0.05. The PNES and ERR were also calculated on day 28 post-treatment. Reinfection rates were calculated using only animals in the treatment arms that were deemed uninfected on days 21 and 28. Data were analysed using available case population. The PNES, ERR and RR were determined using the following formulas:

$PNES = 100 \times (neg / n)$
neg = number of negative animals in each group on day 21 post-treatment
n = number of animal per group on day 21 post-treatment
$ERR = 100 \times [1 - (T_x / T_0)]$
T_x = arithmetic or geometric mean EPG of each group on day 21 post-treatment
T_0 = arithmetic or geometric mean EPG of each at baseline
$PNES$ and ERR were calculated in the same way for day 28 post-treatment
$RR_1 = 100 \times (P_{90} / N_{21\&28})$
RR_1 = reinfection rate on day 90 post-treatment
P_{90} = positive animals on day 90 post-treatment
$N_{21\&28}$ = negative animals on days 21 and 28 post-treatment
$RR_2 = 100 \times (P_{188} / N_{90})$
RR_2 = reinfection rate on day 188 post-treatment
P_{188} = positive animals on day 188 post-treatment
N_{90} = negative animals on day 90 post-treatment

2.6. Ethical considerations

This study was part of a larger project pertaining to the transmission dynamics and hybridization of human and animal trematode infections in Côte d'Ivoire. Ethical approval was obtained from the National Ethics and Research Committees of Côte d'Ivoire (reference no. 035/MSH /CNER-kp) and Switzerland (reference no. UBE-2016-00707). In addition, the "Direction des Services Vétérinaires" of the "Ministère des Ressources Animales et Halieutiques en Côte d'Ivoire" gave authorization to conduct the research. Farmers signed an informed consent form for the sampling and treatment of their cattle and a veterinary technician carried out the treatments.

3. Results

3.1. Baseline characteristics

One hundred and ninety-six (196) cattle were screened for fascioliasis and other livestock parasitic infections. *Fasciola* spp. were found in 79.1% (95% confidence interval (CI): 72.7-84.6%), *Paramphistomum* spp. in 98.5% (95% CI: 95.6-99.7%), *Dicrocoelium hospes* in 2.6% (95% CI: 0.8-5.9%), *Schistosoma bovis* in 2.0% (95% CI: 0.6-5.1%), *Strongilyda* in 55.1% (95% CI: 47.9-62.2%) and *Moniezia* spp. in 1.0% (95% CI: 0.1-3.6%). Cattle positive for *Fasciola* spp. (n= 155) were randomly allocated to the three study arms: triclabendazole (n = 66), albendazole (n = 67) and control (n = 22) (Fig. 1). Faecal specimens could not be collected from all cattle at follow-ups (21, 28, 90 and 188 days post-treatment), either because the animals were no longer available (absent, sold or slaughtered) or because the animals had already defecated. The cattle from whom faeces could be collected at follow-up, were included in the analysis of that follow-up (Fig. 1). At baseline, the treatment groups did not differ considerably in terms of sex, age, breed, chest size, weight and *Fasciola* spp. EPG (Table 1).

3.2. PNES, ERR and safety

The PNES of triclabendazole against *Fasciola* spp. was significantly higher than that of albendazole at day 21 post-treatment (95.4% vs. 70.3%; odds ratio [OR] 8.73, 95% CI 2.43-31.28, p <0.001). Likewise, the arithmetic mean ERR was significantly higher in the triclabendazole group compared to the albendazole group (99.8% vs. 92.2%, respectively; difference: 7.6%-points, 95% CI: 0.9-14.5%-points, p=0.026). In terms of the geometric mean based ERR, triclabendazole was superior to albendazole (99.6% vs. 91.0%; difference: 8.6%-points, 95% CI: 2.4-15.0%-points, p=0.007) (Table 2). A similar pattern was observed at day 28 post-treatment, the PNES of triclabendazole against *Fasciola* spp. was significantly higher compared to albendazole (95.3% vs. 61.2%; OR 12.89, 95% CI 3.66-45.41, p <0.001). The arithmetic mean ERR was significantly higher in the triclabendazole group compared to the albendazole group (99.8% vs. 93.0%, respectively; difference: 6.4%-points, 95% CI: 1.5-11.4%-point, p=0.011) (Table 2). Unexpectedly, the apparent PNES in the control group at days 21 and 28 post-treatment were 50.0% (95% CI 27.2-72.8%) and 38.1% (95% CI 18.1-61.6%), respectively. None of the cattle was visibly ill at baseline and no adverse events were observed by the farmers in either the triclabendazole (12 mg/kg) or albendazole (15 mg/kg) treatment groups during the 3 weeks after treatment.

3.3. Rate of reinfection

Reinfection was assessed at 90 and 188 days post-treatment. Ninety-three cattle who were in the treatment arms and were negative on days 21 and 28 were included in the analysis (Fig. 2). There were fewer infected cattle in the triclabendazole arm compared to the albendazole arm at both 90 and 188 days post-treatment (Fig. 3). Ninety days after treatment, we observed an infection rate of 3/57 (5.3%) in the triclabendazole arm and 8/34 (23.5%) in the albendazole arm. At 188 days, we found 10/54 (18.5%) and 11/33 (33.3%) of cattle were *Fasciola* spp. positive in the triclabendazole and albendazole arms, respectively. *Fasciola* spp. faecal egg counts were lower in the triclabendazole arm compared to albendazole (Fig. 4). Triclabendazole outperformed albendazole in terms of ERR at day 90 (ERRs 99.0% vs 89.9%, difference 9.0%-points, 95% CI: 4.0-16.6%) and day 180 (ERRs 97.4% vs 92.3%, difference 5.1%-points, 95% CI: 1.3-10.0%).

4. Discussion

Triclabendazole has been used in veterinary medicine since the early 1980s and is the recommended treatment for fascioliasis because of its
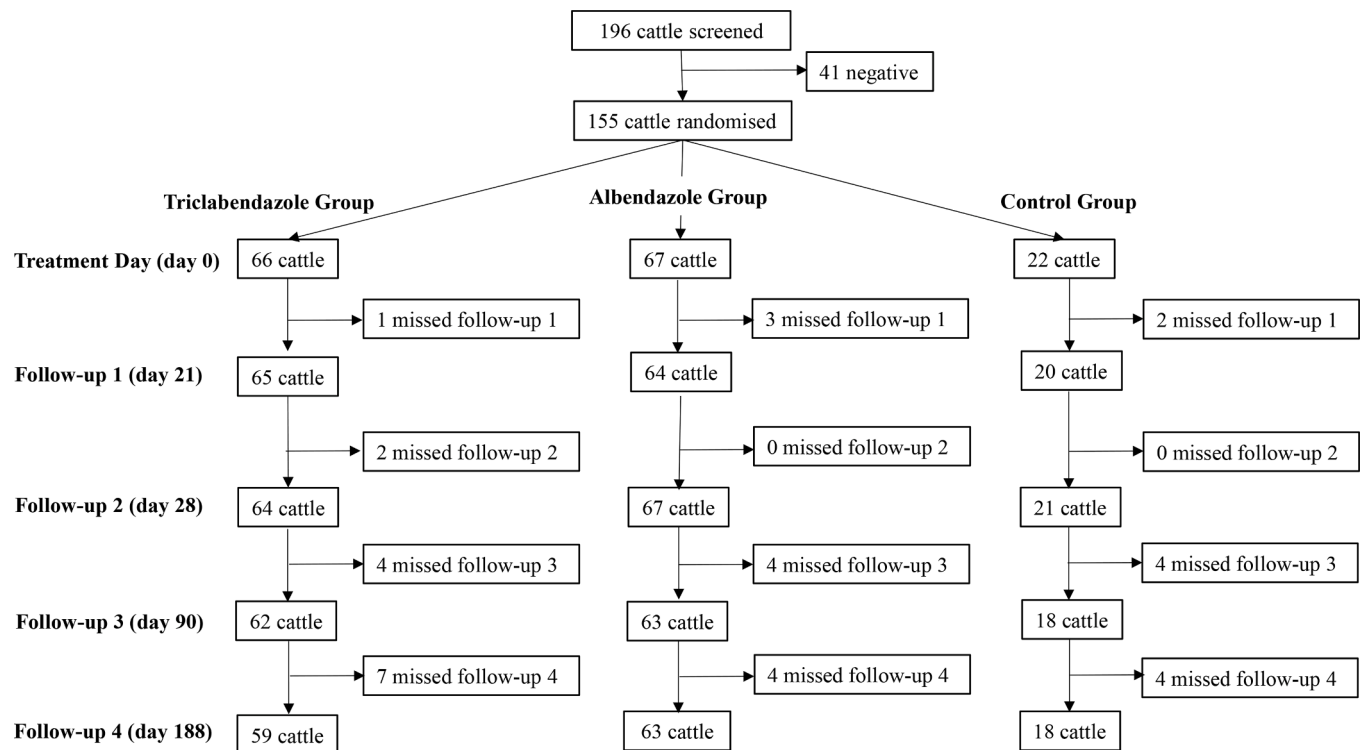
Fig. 1. Trial profile. T1: received triclabendazole; T2: received albendazole; CG: control group (no treatment). Follow-up 1, 2, 3 and 4 correspond to the follow-up faecal sampling and coproscopy on days 21, 28, 90 and 188 post-treatment, respectively.

Table 1
Baseline characteristics of randomised cattle from Côte d'Ivoire in June 2019, stratified by treatment arm. Mean values (standard deviation), EPG = egg per gram of faeces, IQR = interquartile range.

	Triclabendazole	Albendazole	Control
Cattle treated	66	67	22
Sex n (%)			
Female	50 (75.8)	50 (74.6)	14 (63.6)
Male	16 (24.2)	17 (25.4)	8 (36.4)
Breed n (%)			
Zébu n (%)	3 (4.6)	5 (7.5)	3 (13.6)
Taurin x Zébu n (%)	63 (95.4)	62 (92.5)	19 (86.4)
Age [years] mean (SD)	4.8 (2.2)	5.1 (2.3)	5.0 (2.2)
Chest size [cm] mean (SD)	139.0 (15.3)	140.7 (13.5)	146.3 (7.2)
Weight [kg] mean (SD)	232.0 (61.4)	236.0 (58.9)	261.9 (34.7)
Fasciola			
EPG median (IQR)	3.2 (0.7, 6.3)	3.0 (1.0, 7.3)	5.5 (1.0, 11.3)
EPG arithmetic mean	9.2	8.0	8.8
EPG geometric mean	2.9	2.7	3.8

good safety and efficacy profile in animals at all stages and forms of infection (Boray et al., 1983; Gandhi et al., 2019; Kelley et al., 2016). Yet, triclabendazole has not been approved for use in all countries. We present results from the first randomised controlled trial in Côte d'Ivoire and demonstrated that the known efficacy of triclabendazole against fascioliasis in cattle holds true for this West Africa country.

Our findings show that triclabendazole is more efficacious against *Fasciola* spp. infections than albendazole, both in terms of PNES and ERR at 21 and 28 days post-treatment. Our findings corroborate previous studies, which demonstrated high efficacy of triclabendazole (Craig and Huey, 1984; Lecuyer et al., 1985; Rapic et al., 1988; Richards et al., 1990; Stansfield et al., 1987; Suhardono et al., 1991).

The activity of albendazole is restricted to adult *Fasciola* spp. flukes (Johns and Dickeson, 1979; Knight and Colglazier, 1977); hence, immature flukes survive treatment and continue to develop to adulthood. This might explain why the ERR was considerably lower in the albendazole arm compared to the triclabendazole arm, as immature flukes that survived albendazole treatment likely developed into egg-laying, adult flukes. The lower ERR in the albendazole arm might also be explained by drug-resistance developed in flukes as albendazole has been used for many years in Ivorian cattle as an anthelminthic drug. However, this seems unlikely in our study area, as albendazole was still efficacious with an ERR of >90%.

Fifty percent (50%) of the cattle in the control group were found negative at day 21 post-treatment and 38% were negative at day 28. This finding was unexpected. A decrease in PNES and ERR in the control arm is commonly observed in veterinary and human drug trials (Moser et al., 2017). This can be attributed to a regression to the mean phenomenon because only apparent positive animals will be enrolled. However, the effect in the trial reported here was rather high. In theory, the effect should be more pronounced if the day-to-day fluctuation of the parasite is high and the average baseline infection intensity is low. Both are certainly true in this trial, but given the small sample size in the control group, an impact of chance cannot be ruled out. Consequently, the true effect of triclabendazole is likely smaller than estimated but it is important to note that the conclusion of the primary hypothesis "triclabendazole is more efficacious than albendazole" remains valid independently of any potential placebo effect.

Additional factors contributing to the under-estimation of the true prevalence associated with coprology include: variation in the distribution of eggs within a single faecal specimen, daily fluctuations of faecal production and consistency in the host, and daily fluctuations related to oviposition patterns of the parasite (Mas-Coma et al., 2014; Valero et al., 2011, Valero et al., 2002). The Kato-Katz method is

Table 2

Proportion of non-egg shedding cattle (PNES) and egg reduction rate (ERR) against *Fasciola* spp. at days 21 and 28 after the administration of triclabendazole or albendazole in cattle from Côte d'Ivoire. CI = confidence interval, EPG = eggs per gram of faeces.

|	Triclabendazole		Albendazole		Control group	
	Day 21	Day 28	Day 21	Day 28	Day 21	Day 28
Resampled cattle	65	64	64	67	20	21
Non-egg shedding cattle after treatment	62	61	45	41	10	8
PNES (95% CI)	95.4% (87.1-99.0%)	95.3% (86.9-99.0%)	70.3% (57.6-81.1%)	61.2% (48.5-72.9%)	50.0% (27.2-72.8%)	38.1% (18.1-61.6%)
EPG arithmetic mean						
Before treatment	9.1	9.5	8.2	8	7.3	7.1
After treatment	0.02	0.03	0.7	0.6	1.7	1.6
ERR (95% CI)	99.8% (99.4-100%)	99.7% (99.1-100%)	92.2% (83.0-96.9%)	93.0% (86.8-96.4%)	76.7% (50.5-92.2%)	78.0% (55.1-89.7%)
EPG geometric mean						
Before treatment	3.6	3.8	3.5	3.5	4.4	4.2
After treatment	0.02	0.02	0.3	0.4	0.9	0.9
ERR (95% CI)	99.6% (98.9-100%)	99.4% (98.5-100%)	91.0% (82.9-95.9%)	89.6% (82.8-94.2%)	80.2% (53.7-93.1%)	77.8% (55.7-89.2%)
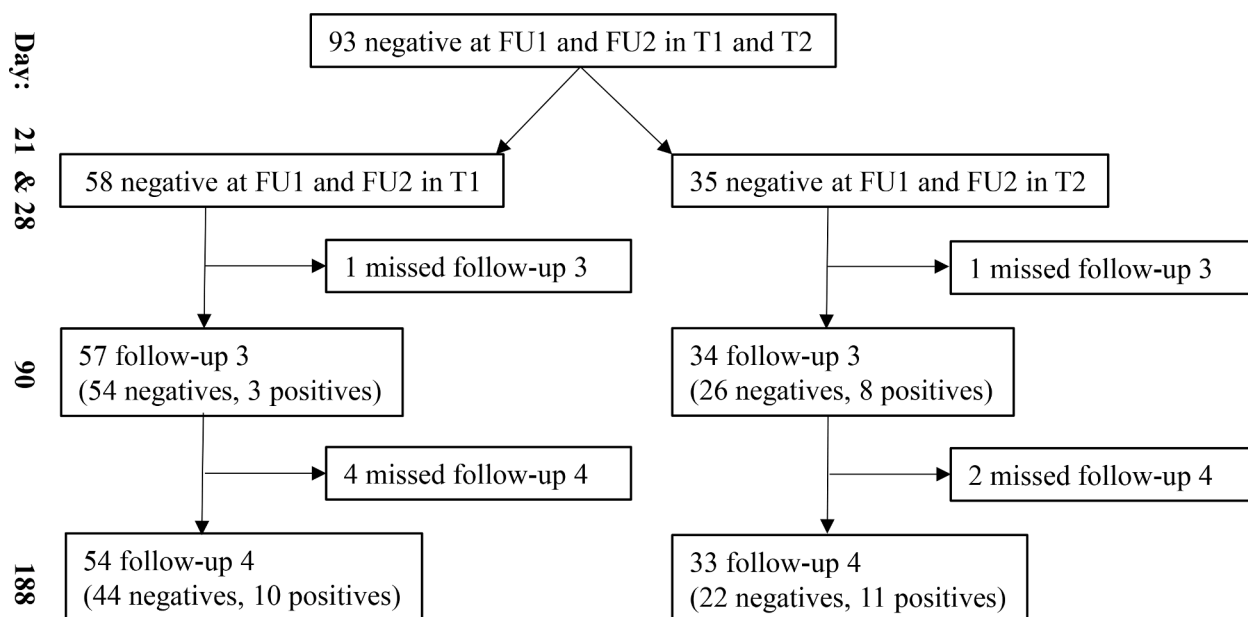
Fig. 2. Flow chart of reinfection. FU1: follow-up 1; FU2: follow-up 2; T1: received triclabendazole; T2: received albendazole. Follow-up 1, 2, 3 and 4 correspond to the follow-up faecal sampling and coproscopy on days 21, 28, 90 and 188 post-treatment, respectively.

commonly used for diagnosis of helminths in human trials (Lamberton et al., 2014), where the standard is to examine multiple slides from multiple samples over 2-3 days. Applying such a strategy to livestock trials would increase sensitivity of diagnosis and improve the accuracy of efficacy estimates.

Our findings reveal that triclabendazole had an effect on post-treatment reinfection that was more pronounced than that of albendazole and was visible for several months, even in a setting of high reinfection. At both 90 and 188 days post-treatment, few cattle in the triclabendazole arm were found to be reinfected with *Fasciola* spp. (5.3% and 18.5%, respectively), compared to those in the albendazole arm (23.5% and 33.3%, respectively). Furthermore, the egg counts were also considerably lower in the triclabendazole arm compared to the albendazole arm at days 90 and 188 post-treatment. The higher reinfection rate in the albendazole group may also be due to the lack of activity of albendazole against immature flukes, as mentioned above (Keyyu et al., 2009).This study was conducted on farms under real life conditions in the northern part of Côte d'Ivoire. Hence, animals pastured and consumed water daily from rivers or dams, where they might become infected with *Fasciola* spp. by ingesting metacercariae. It should be noted

that post-treatment reinfection patterns are largely dependent on pasture infestation, and hence, the presence and abundance of inter-mediate host snails (Brunsdon, 1980). Previous studies from northern Côte d'Ivoire reported the presence of *Lymnaea natalensis* and *Physa acuta* snails, which serve as intermediate hosts for *Fasciola* (Krauth et al., 2017). As we can see from the baseline *Fasciola* spp. prevalence of 79.1%, our study area is an endemic zone for *Fasciola* spp. (Kouadio et al., 2020). Considering that cattle treated with triclabendazole experienced both higher efficacy and fewer reinfections, triclabendazole should be the preferred anthelminthic for the treatment and control of fascioliasis in Côte d'Ivoire.

Despite these promising results, alternating anthelminthics to avoid the development and spread of resistance, as per the manufacturer's recommendations, is highly advisable. Drug resistance in livestock has already been reported in some parts of the world (Fairweather, 2011a, Fairweather, 2011b). In fact, lower efficacy and even resistance to tri-clabendazole (efficacy <90%) has been documented in Argentina (Olaechea et al., 2011), Australia (Brockwell et al., 2014; Elliott et al., 2015; Kelley et al., 2020), and Northern Ireland (Hanna et al., 2015). Reduction of efficacy or resistance to albendazole has also been
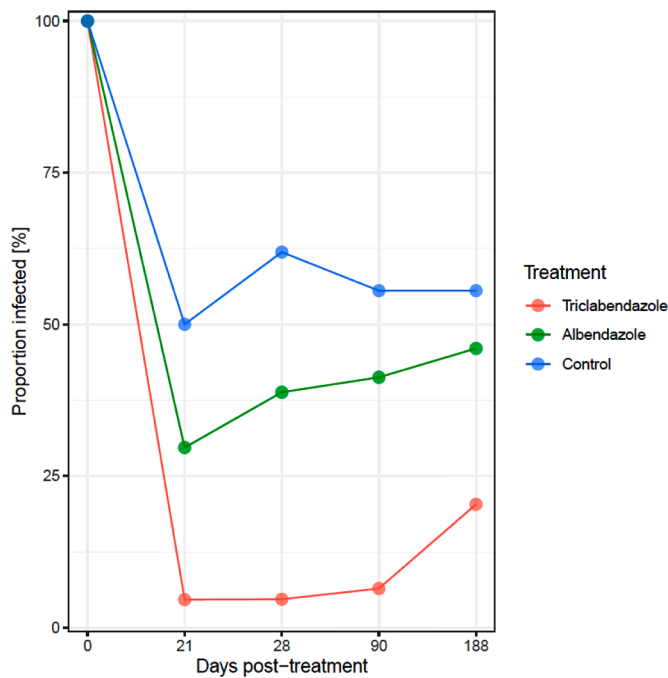
Fig. 3. Variation in the proportion of infected cattle during post-treatment follow-ups.

demonstrated in flukes in Spain (Alvarez-Sanchez et al., 2006), Tanzania (Nzalawahe et al., 2018a) and in a controlled study in Argentina with samples from South America and the UK (Canevari et al., 2014).

Therefore, alternating therapies should be considered as a strategy to avoid or delay the development or spread of resistance (Williams, 1997) and the use of anthelminthics and resistance to them in Ivorian livestock should be further investigated.

Concerning safety, these treatments have been used extensively for many decades and have a well-established safety profile. No adverse events were reported in our study, however under these conditions it is difficult to say if subtler adverse events, such as diarrhoea, occurred. Safety is difficult to assess in the context of real-life field studies with

animals such as cattle, particularly in Africa. These animals are large, unpredictable and somewhat dangerous, and with a lack of restraining equipment, it is difficult to control or observe them for more subtle adverse events. Furthermore, it is common in Africa for the herd to spend the day grazing in the pasture, where it is not possible for the handlers to closely monitor 100 or more head of cattle.

5. Conclusion

This study has shown that triclabendazole is safe and highly efficacious against liver fluke infections in cattle in a setting characterised by high fascioliasis prevalence in Côte d'Ivoire. Albendazole, although less efficacious, still plays an important role in the treatment and control of livestock fascioliasis in this country, as it is the registered treatment. To our knowledge, this is the first rigorously designed and executed trial to demonstrate the efficacy of triclabendazole in livestock in Côte d'Ivoire and it is reasonable to assume that these findings are generalisable to other parts of West Africa. Triclabendazole and albendazole should be used for the control of livestock fascioliasis in Côte d'Ivoire as part of a strategic seasonal approach, including intermediate host snail control, as well as raising farmers' awareness of pasture and livestock management. If the seasonal risk of infection is better understood, the treatment could be timed to prolong the effect to a maximum.

Conflict of interest

None
International and national guidelines for the care and use of animals were followed.

Credit author statement

Jules N. Kouadio, Jennifer Giovanoli Evack, Louise Y. Achi, Oliver Balmer, Jürg Utzinger, Bassirou Bonfoh and Jakob Zinsstag:
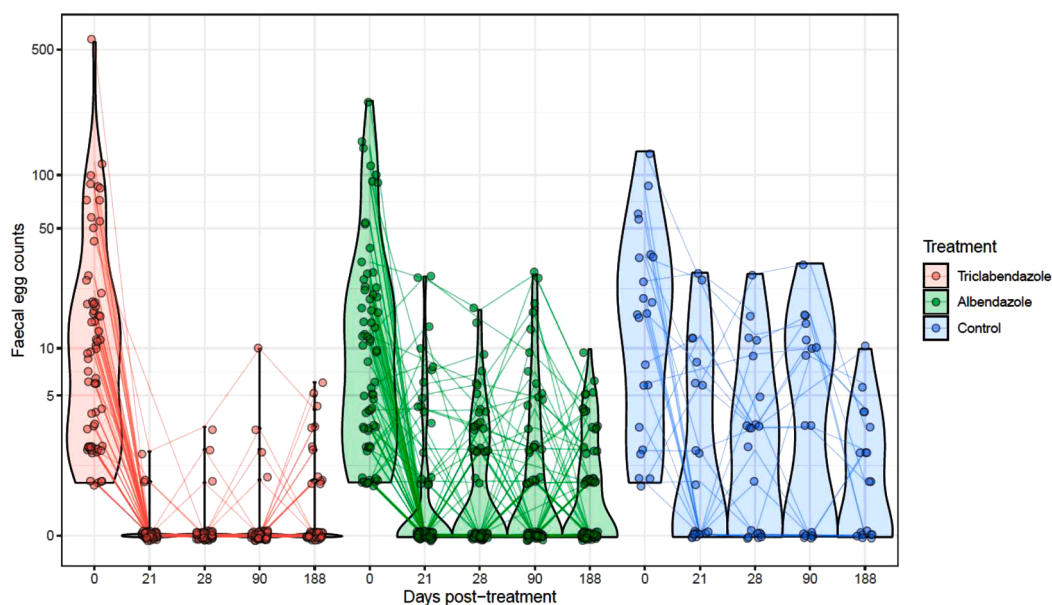
Fig. 4. Density of the distribution of faecal egg (ep3g) counts in the treatment groups at baseline (day 0) and follow-ups (days 21, 28, 90 and 188 post-treatment). ep3g: number of eggs per 3 grams of faeces. Small random noise was added to avoid over plotting.

References

Achi, Y.L., Zinsstag, J., Yéo, N., Dea, V., Dorchies, P.H., 2003. Les nématodes gastro-intestinaux des bovins de la région des savanes de la Côte d'Ivoire: enquête d'abattoir. Rev. Médecine Vét. 154, 105–114.

Agridirect, 2021a. Fasinex 240 (Triclabendazole). URL https://www.agridirect.ie/product/fasinex-240-22-ltr# (accessed 26 Fevruary 2021).

Agridirect, 2021b. Albex 2.5% (Albendazole). URL https://www.agridirect.ie/product/albex-25 (accessed 26 February 2021).

Alvarez-Sanchez, M.A., Mainar-Jaime, R.C., Perez-Garcia, J., Rojo-Vazquez, F.A., 2006. Resistance of *Fasciola hepatica* to triclabendazole and albendazole in sheep in Spain. Vet. Rec. 159, 424–425. https://doi.org/10.1136/vr.159.13.424.

Andrews, S.J., 1999. The Life Cycle of *Fasciola hepatica*. In: Dalton, J.P. (Ed.), Fasciolosis. CAB International Publications, Cambridge.

Bennema, S.C., Molento, M.B., Scholte, R.G., Carvalho, O.S., Pritsch, I., 2017. Modelling the spatial distribution of *Fasciola hepatica* in bovines using decision tree, logistic regression and GIS query approaches for Brazil. Parasitology 144, 1677–1685. https://doi.org/10.1017/S0031182017000786.

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. Vet. Rec. 113, 315–317. https://doi.org/10.1136/vr.113.14.315.

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. Int. J. Parasitol. Drugs Drug Resist. 4, 48–54. https://doi.org/10.1016/j.ijpddr.2013.11.005.

Brunsdon, R.V., 1980. Principles of helminth control. Vet. Parasitol. 6, 185–215. https://doi.org/10.1016/0304-4017(80)90043-6.

Canevari, J., Ceballos, L., Sanabria, R., Romero, J., Olaechea, F., Ortiz, P., Cabrera, M., Gayo, V., Fairweather, I., Lanusse, C., Alvarez, L., 2014. Testing albendazole resistance in *Fasciola hepatica*: validation of an egg hatch test with isolates from South America and the United Kingdom. J. Helminthol. 88, 286–292. https://doi.org/10.1017/S0022149x13000163.

Craig, T.M., Huey, R.L., 1984. Efficacy of triclabendazole against *Fasciola hepatica* and *Fascioloides magna* in naturally infected calves. Am. J. Vet. Res. 45, 1644–1645.

Elelu, N., Eisler, M.C., 2018. A review of bovine fasciolosis and other trematode infections in Nigeria. J. Helminthol. 92, 128–141. https://doi.org/10.1017/S0022149x17000402.

Elliott, T.P., Kelley, J.M., Rawlin, G., Spithill, T.W., 2015. High prevalence of fasciolosis and evaluation of drug efficacy against *Fasciola hepatica* in dairy cattle in the Maffra and Bairnsdale districts of Gippsland. Victoria, Australia. Vet. Parasitol. 209, 117–124. https://doi.org/10.1016/j.vetpar.2015.02.014.

Fairweather, I., 2009. Triclabendazole progress report, 2005–2009: an advancement of learning? Journal of helminthology 83, 139–150.

Fairweather, I., 2011a. Raising the bar on reporting "triclabendazole resistance. Vet. Rec. 168, 514–515. https://doi.org/10.1136/vr.d2867.

Fairweather, I., 2005. Triclabendazole: new skills to unravel an old (ish) enigma. J. Helminthol. 79, 227–234.

Fairweather, I., 2011b. Liver fluke isolates: a question of provenance. Vet. Parasitol. 176, 1–8. https://doi.org/10.1016/j.vetpar.2010.12.011.

Fairweather, I., Boray, J.C., 1999. Fasciolicides: efficacy, actions, resistance and its management. Veterinary Journal 158, 81–112. https://doi.org/10.1053/tvjl.1999.0377.

Fürst, T., Keiser, J., Utzinger, J., 2012. Global burden of human food-borne trematodiasis: a systematic review and meta-analysis. Lancet Infect. Dis. 12, 210–221. https://doi.org/10.1016/S1473-3099(11)70294-8.

Gandhi, P., Schmitt, E.K., Chen, C.-W., Samantray, S., Venishetty, V.K., Hughes, D., 2019. Triclabendazole in the treatment of human fascioliasis: a review. Trans. R. Soc. Trop. Med. Hyg. 113, 797–804. https://doi.org/10.1093/trstmh/trz093.

Giovanoli Evack, J., Kouadio, J.N., Achi, L., Balmer, O., Hattendorf, J., Bonfoh, B., Zinsstag, J., N'Goran, E.K., Utzinger, J., 2020. Accuracy of the sedimentation and filtration methods for the diagnosis of schistosomiasis in cattle. Parasitol. Res. 119, 1707–1712. https://doi.org/10.1007/s00436-020-06660-0.

Greter, H., Batil, A.A., Alfaroukh, I.O., Grimm, F., Ngandolo, B.N., Keiser, J., Utzinger, J., Zinsstag, J., Hattendorf, J., 2016. Re-infection with *Fasciola gigantica* 6-month post-treatment with triclabendazole in cattle from mobile pastoralist husbandry systems at Lake Chad. Vet. Parasitol. 230, 43–48. https://doi.org/10.1016/j.vetpar.2016.10.019.

Greter, H., Batil, A.A., Ngandolo, B.N., Alfaroukh, I.O., Moto, D.D., Hattendorf, J., Utzinger, J., Zinsstag, J., 2017. Human and livestock trematode infections in a mobile pastoralist setting at Lake Chad: added value of a One Health approach beyond zoonotic diseases research. Trans. R. Soc. Trop. Med. Hyg. 111, 278–284. https://doi.org/10.1093/trstmh/trx051.

Hanna, R.E.B., McMahon, C., Ellison, S., Edgar, H.W., Kajugu, P.-E., Gordon, A., Irwin, D., Barley, J.P., Malone, F.E., Brennan, G.P., Fairweather, I., 2015. *Fasciola hepatica*: a comparative survey of adult fluke resistance to triclabendazole, nitroxynil and closantel on selected clopland and lowland sheep farms in Northern Ireland using faecal egg counting, coproantigen ELISA testing and fluke histology. Vet. Parasitol. 207, 34–43. https://doi.org/10.1016/j.vetpar.2014.11.016.

Health Products Regulatory Authority: Ireland, 2018. Summary of Product Characteristics. Triclabendazole.

Johns, D.R., Dickeson, S.J., 1979. Efficacy of albendazole against *Fasciola hepatica* in sheep. Aust. Vet. J. 55, 431–432. https://doi.org/10.1111/j.1751-0813.1979.tb05599.x.

Kaplan, R.M., 2001. *Fasciola hepatica*: a review of the economic impact in cattle and considerations for control. Vet. Ther. Res. Appl. Vet. Med. 2, 40–50.

Keiser, J., Engels, D., Büscher, G., Utzinger, J., 2005. Triclabendazole for the treatment of fasciliasis and paragonimiasis. Expert Opin. Investig. Drugs 14, 1513–1526. https://doi.org/10.1517/13543784.14.12.1513.

Keiser, J., Utzinger, J., 2004. Chemotherapy for major food-borne trematodes: a review. Expert Opin. Pharmacother. 5, 1711–1726. https://doi.org/10.1517/14656566.5.8.1711.

Kelley, J.M., Elliott, T.P., Beddoe, T., Anderson, G., Skuce, P., Spithill, T.W., 2016. Current threat of triclabendazole resistance in *Fasciola hepatica*. Trends Parasitol 32, 458–469. https://doi.org/10.1016/j.pt.2016.03.002.

Kelley, J.M., Rathinasamy, V., Elliott, T.P., Rawlin, G., Beddoe, T., Stevenson, M.A., Spithill, T.W., 2020. Determination of the prevalence and intensity of *Fasciola hepatica* infection in dairy cattle from six irrigation regions of Victoria, South-eastern Australia, further identifying significant triclabendazole resistance on three properties. Vet. Parasitol. 277, 109019 https://doi.org/10.1016/j.vetpar.2019.109019.

Keyyu, J.D., Kyvsgaard, N.C., Monrad, J., Kassuku, A.A., 2009. Effectiveness of strategic anthelmintic treatments in the control of gastrointestinal nematodes and *Fasciola gigantica* in cattle in Iringa region. Tanzania. Trop. Anim. Health Prod. 41, 25–33. https://doi.org/10.1007/s11250-008-9150-5.

Knight, R.A., Colglazier, M.L., 1977. Albendazole as a fasciolicide in experimentally infected sheep. Am. J. Vet. Res. 38, 807–808.

Kouadio, J.N., Giovanoli Evack, J., Achi, L.Y., Fritsche, D., Ouattara, M., Silué, K.D., Bonfoh, B., Hattendorf, J., Utzinger, J., Zinsstag, J., Balmer, O., N'Goran, E.K., 2020. Prevalence and distribution of livestock schistosomiasis and fascioliasis in Côte d'Ivoire: results from a cross-sectional survey. BMC Vet. Res. 16, 446. https://doi.org/10.1186/s12917-020-02667-y.

Krauth, S.J., Wandel, N., Traoré, S.I., Vounatsou, P., Hattendorf, J., Achi, L.Y., McNeill, K., N'Goran, E.K., Utzinger, J., 2017. Distribution of intermediate host snails of schistosomiasis and fascioliasis in relation to environmental factors during the dry season in the Tchologo region, Côte d'Ivoire. Adv. Water Resour. 108, 386–396. https://doi.org/10.1016/j.advwatres.2016.11.016.

Lamberton, P.H.L., Kabatereine, N.B., Oguttu, D.W., Fenwick, A., Webster, J.P., 2014. Sensitivity and specificity of multiple Kato-Katz thick smears and a circulating cathodic antigen test for *Schistosoma mansoni* diagnosis pre- and post-repeated-praziquantel treatment. PLoS Negl. Trop. Dis. 8, e3139. https://doi.org/10.1371/journal.pntd.0003139.

Lecuyer, B., Bordas, C.H., Marchand, C., Nguyen, H.N., Sourd, C.H., 1985. Investigation of the fascilicide activity of triclabendazole in cattle in Nièvre. France. Bull. Soc Vet Prat Fr. 69, 507–513.

Malatji, M.P., Pfukenyi, D.M., Mukaratirwa, S., 2019. *Fasciola* species and their vertebrate and snail intermediate hosts in East and Southern Africa: a review. J. Helminthol. 94, e63. https://doi.org/10.1017/S0022149x19000531.

Mas-Coma, S., Bargues, M.D., Valero, M.A., 2018. Human fascioliasis infection sources, their diversity, incidence factors, analytical methods and prevention measures. Parasitology 145, 1665–1699. https://doi.org/10.1017/S0031182018000914.

Mas-Coma, S., Bargues, M., Valero, M., 2007. Plant-borne trematode zoonoses: fascioliasis and fasciolopsiasis. In: Darwin Murrell, K., Fried, B. (Eds.), Food-Borne Parasitic Zoonoses: Fish and Plant-Borne Parasites, World Class Parasites, II. Springer Science, New York, USA, pp. 293–334.

Mas-Coma, S., Bargues, M.D., Valero, M.A., 2014. Diagnosis of human fascioliasis by stool and blood techniques: update for the present global scenario. Parasitology 141, 1918–1946. https://doi.org/10.1017/S0031182014000869.

Moser, W., Schindler, C., Keiser, J., 2017. Efficacy of recommended drugs against soil transmitted helminths: systematic review and network meta-analysis. BMJ j4307. https://doi.org/10.1136/bmj.j4307.

Nzalawahe, J., Hannah, R., Kassuku, A.A., Stothard, J.R., Coles, G., Eisler, M.C., 2018a. Evaluating the effectiveness of trematocides against *Fasciola gigantica* and amphistomes infections in cattle, using faecal egg count reduction tests in Iringa Rural and Arumeru Districts. Tanzania. Parasit. Vectors 11. https://doi.org/10.1186/s13071-018-2965-7.

Nzalawahe, J., Hannah, R., Kassuku, A.A., Stothard, J.R., Coles, G., Eisler, M.C., 2018b. Evaluating the effectiveness of trematocides against *Fasciola gigantica* and amphistomes infections in cattle, using faecal egg count reduction tests in Iringa Rural and Arumeru Districts. Tanzania. Parasit. Vectors 11, 384. https://doi.org/10.1186/s13071-018-2965-7.

Olaechea, F., Lovera, V., Larroza, M., Raffo, F., Cabrera, R., 2011. Resistance of *Fasciola hepatica* against triclabendazole in cattle in Patagonia (Argentina). Vet. Parasitol. 178, 364–366.

Overend, D.J., Bowen, F.L., 1995. Resistance of *Fasciola hepatica* to triclabendazole. Australian Veterinary Journal 72, 275–276. https://doi.org/10.1111/j.1751-0813.1995.tb03546.x.

Parkinson, M., O'Neill, S.M., Dalton, J.P., 2007. Endemic human fasciolosis in the Bolivian Altiplano. Epidemiol. Infect. 135, 669–674. https://doi.org/10.1017/S095026880600728X.

Periago, M.V., Valero, M.A., Panova, M., Mas-Coma, S., 2006. Phenotypic comparison of allopatric populations of *Fasciola hepatica* and *Fasciola gigantica* from European and African bovines using a computer image analysis system (CIAS). Parasitol. Res. 99, 368–378. https://doi.org/10.1007/s00436-006-0174-3.

Rapic, D., Dzakula, N., Sakar, D., Richards, R.J., 1988. Comparative efficacy of triclabendazole, nitroxynil and rafoxanide against immature and mature *Fasciola hepatica* in naturally infected cattle. Vet. Rec. 122, 59–62.

Richards, R.J., Bowen, F.L., Essenwein, F., Steiger, R.F., Büscher, G., 1990. The efficacy of triclabendazole and other anthelmintics against *Fasciola hepatica* in controlled studies in cattle. Vet. Rec. 126, 213–216.

Shokier, K.M., Aboelhadid, S.M., Waleed, M.A., 2013. Efficacy of five anthelmintics against a natural *Fasciola* species infection in cattle. Beni-Suef Univ. J. Basic Appl. Sci. 2, 41–45. https://doi.org/10.1016/j.bjbas.2013.09.006.

Sirois, M., 2017. Principles and Practice of Veterinary Technology, 4th Ed. Elsevier, St. Louis, Missouri; USA.

Smeal, M.G., Hall, C.A., 1983. The activity of triclabendazole against immature and adult *Fasciola hepatica* infections in sheep. Aust. Vet. J. 60, 329–331. https://doi.org/10.1111/j.1751-0813.1983.tb02833.x.

Stansfield, D.G., Lonsdale, B., Lowndes, P.A., Reeves, E.W., Schofield, D.M., 1987. Field trials of triclabendazole against mixed age infections of *Fasciola hepatica* in sheep and cattle. Vet. Rec. 120, 459–460. https://doi.org/10.1136/vr.120.19.459.

Stevenson, C., Mahoney, R., Fisara, P., Strehlau, G., Reichel, M., 2002. The efficacy of formulations of triclabendazole and ivermectin in combination against liver fluke (*Fasciola hepatica*) and gastro-intestinal nematodes in cattle and sheep and sucking lice species in cattle. Aust. Vet. J. 80, 698–701. https://doi.org/10.1111/j.1751-0813.2002.tb11303.x.

Suhardono, Widjajanti, S., Stevenson, P., Carmichael, I.H., 1991. Control of *Fasciola gigantica* with triclabendazole in Indonesian cattle. Trop. Anim. Health Prod. 23, 217–220.

Suleiman, A., Jackson, E.L., Rushton, J., 2015. Challenges of pastoral cattle production in a sub-humid zone of Nigeria. Trop. Anim. Health Prod. 47, 1177–1185. https://doi.org/10.1007/s11250-015-0845-0.

Valero, M.A., Panova, M., Comes, A.M., Fons, R., Mas-Coma, S., 2002. Patterns in size and shedding of *Fasciola hepatica* eggs by naturally and experimentally infected murid rodents. J. Parasitol. 88, 308–313. https://doi.org/10.1645/0022-3395(2002)088[0308:PISASO]2.0.CO;2.

Valero, M.A., Panova, M., Pérez-Crespo, I., Khoubbane, M., Mas-Coma, S., 2011. Correlation between egg-shedding and uterus development in *Fasciola hepatica* human and animal isolates: applied implications. Vet. Parasitol. 183, 79–86. https://doi.org/10.1016/j.vetpar.2011.07.003.

Valero, M.A., Perez-Crespo, I., Periago, M.V., Khoubbane, M., Mas-Coma, S., 2009. Fluke egg characteristics for the diagnosis of human and animal fascioliasis by *Fasciola hepatica* and *F. gigantica*. Acta Trop 111, 150–159. https://doi.org/10.1016/j.actatropica.2009.04.005.

Williams, J.C., 1997. Anthelmintic treatment strategies: current status and future. Vet. Parasitol. 72, 461–470. https://doi.org/10.1016/s0304-4017(97)00111-8 discussion 470-477.

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 (3), 181–213. https://doi.org/10.1016/0304-4017(95)00806-2.

Yusuf, M., Ibrahim, N., Tafese, W., Deneke, Y., 2016. Prevalence of bovine fasciolosis in municipal abattoir of Haramaya. Ethiopia. Food Sci. Qual. Manag. 48, 38–43.