

Reduced efficacies of diclazuril and toltrazuril against *Eimeria ovinoidalis* and *Eimeria crandallis* in two French sheep-meat farms

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Abstract

Background: Lamb coccidiosis, caused by intestinal parasites of the *Eimeria* genus, has pronounced health and economic impacts throughout the world. *Eimeria ovinoidalis* and *Eimeria crandallis* are the most pathogenic species in sheep. Control of these parasites requires the use of anticoccidial drugs such as sulfonamides, diclazuril, and toltrazuril. In this study, resistance to anticoccidial drugs was suspected in two farms as clinical signs (diarrhea) persisted after treatment.

Method: On each farm, 4.5-month-old rams were divided into three groups so that they were either (i) left untreated (Control group), (ii) treated with diclazuril (1 mg/kg body weight), or (iii) treated with toltrazuril (20 mg/kg body weight). Animals were treated at day 0 (D0) and fecal samples were collected at D0 and eight days later (D8) to evaluate the reduction in intensity of *Eimeria* oocyst excretion. Oocyst morphology and morphometry were used to identify *Eimeria* species at both sampling dates.

Results: Reduction of oocyst shedding was incomplete in both farms (92.44% and 93.58%) after diclazuril treatment. More specifically, the efficacy was reduced in both farms for *E. ovinoidalis/Eimeria marsica* (92.59% and 91.87%) and *E. crandallis/Eimeria weybridgensis* (75.34% and 80.10%). The general efficacy of toltrazuril was high in both farms (97.6% and 97.96%). However, a slightly reduced efficacy was noted in farm 1 for *E. crandallis/E. weybridgensis* (93.26%) while this efficacy was high in farm 2 (98.88%).

Conclusions: We suggest a simple protocol to investigate the efficacy of anticoccidial treatments in sheep and to rapidly identify potentially resistant species. In these two farms, treating animals with diclazuril will select pathogenic species, and toltrazuril could favor resistant *E. crandallis/E. weybridgensis* in one of them.

Background

Coccidiosis is a common and cosmopolitan animal parasitosis, and the causative agents are Apicomplexa protozoa of the genus *Eimeria*. These obligatory parasites are very specific to their host, rendering cross-infection between different animal species impossible^{1,2}. Eleven *Eimeria* species have been described as etiological agents of lamb coccidiosis³, among which, due to their ability to develop in the Lieberkühn-crypts cells, *Eimeria ovinoidalis* and *Eimeria crandallis* are considered to be the most pathogenic species^{4,5}. After multiplication in the intestinal cells of their host, *Eimeria* oocysts are produced and shed in the feces, thereby contaminating the animal environment. Other animals sharing the living environment become infected by the fecal-oral route. For example, oocysts are usually found in litter and on the ewes' udder, leading to possible lamb infection as soon as they first feed. The clinical signs of coccidiosis are characterized by diarrhea, which can be hemorrhagic, causing dehydration and dieback of the lamb², generally between the first and the third month of life. In case of severe infection, there is a significant increase in morbidity and mortality rates, causing substantial financial losses to farmers^{6,7}. In subclinical infection, the economic losses caused by reduction of the weight gain of lambs are impactful, especially for the sheep-meat industry. As control of these parasites is important for the economic sustainability of a farm⁸, anticoccidial drugs are used for both preventive and curative purposes. In France, the main drugs that are used are sulfonamides, toltrazuril, and diclazuril. Sulfonamides can be used in the feed or water as a preventive or curative treatment. For preventive and curative treatments, toltrazuril and diclazuril can be used as a single administration to the animal⁹. While resistance to anticoccidial drugs in poultry farms has been documented relatively well¹⁰⁻¹³, this is not the case for sheep coccidiosis. However, the first reports of *E. ovinoidalis* resistance to toltrazuril in Norway were published recently^{14,15}.

Evaluation of the efficacy of anticoccidial treatments is an issue regularly encountered in the field. There are currently no validated guidelines for ruminants to evaluate the efficacy of anticoccidials in the field. However, efficacy evaluation studies have already been published, and each proposed a protocol^{8,14,16-22}. These protocols have aspects in common, such as the period between treatment and the post-treatment analyses, but also differences, such as the number of animals per group, the age of the animals at the time of the test, calculation of the efficacy, etc.

Another issue in field evaluation of the efficacy of anticoccidials is the identification of *Eimeria* species. Indeed, oocysts of *E. ovinoidalis* and *E. crandallis* share morphological and morphometric properties with less pathogenic species such as *Eimeria marsica* and *Eimeria weybridgensis*, respectively³. These similarities render identification difficult in fecal samples from naturally infected animals, where a wide diversity of species is usually found. Moreover, molecular identification is still underdeveloped for ovine *Eimeria* species, even though partial genomic sequencing of the ITS-1 region is now available²³.

Thus, this study aimed to i) propose a simple protocol to evaluate the efficacy of toltrazuril and diclazuril in the treatment of lamb coccidiosis in field conditions and ii) propose simple keys for morphological identification of oocysts of sheep *Eimeria* species. In this study, we investigated two farms with coccidiosis management issues.

Methods

Breeding selection, farming context, and necessity to control coccidiosis

In France, the sheep-meat selection scheme is based on a breeding program supervised by a breeders' association. It is based on the use of a core group of farms selected in the breed population. Each year, within a breed, crossbreeding is carried out between dams of sires and sires of sires, and the best male lambs resulting from these crossbreedings are collected after weaning in a common breeding center for evaluation called an "individual control station" (ICS). In an ICS, lambs are tested on criteria of interest for sheep-meat production such as average daily weight gain and fat and muscle thickness.

During 2021, two of these ICS were investigated in Berrichon du Cher (BC) and in Rouge de l'Ouest (RO) breeds, both related to the breeders' association GEODE. Lambs were collected in the ICS at three months of age. On arrival, they received a diclazuril treatment (Vecoxan ND, MSD Santé Animale, 1 mg/kg body weight (BW) and an ivermectin treatment (Baymec ND, Norbrook Laboratories, 200 µg/kg BW) to control coccidiosis and *Strongyloides papillosus* infections, respectively. The lambs came from different farms of origin: in 2021, we enumerated 9 different origins for a total of 55 BC lambs entering the ICS and 12 different origins for the 52 RO lambs.

Throughout the evaluation period in the ICS, the lambs were kept exclusively indoors and fed *ad libitum*. During their growth phase, they were regularly weighed as part of the protocol to evaluate their growth abilities, and when signs of diarrhea appeared on several lambs, an anticoccidial treatment was carried out on all lambs with diclazuril (Vecoxan ND, MSD Santé Animale, 1 mg/kg BW) or sulfonamides (Sulfadimerazine 33% ND, Huvepharma, 90 mg/kg BW over three successive days).

In this very particular breeding context, where coccidiosis must be controlled as much as possible to allow the lambs to express their full genetic background, BC and RO breeders noticed the persistence of diarrhea after the anticoccidial treatment and observed that it was necessary to treat more frequently than in previous years. These observations have been recurrent for several years, despite implementation of a sanitary vacuum and disinfection of the breeding center between two years of male lamb evaluation.

A protocol to evaluate the efficacy of anticoccidial treatments was then implemented in both ICS.

Sampling and analysis

The Fecal Oocyst Count Reduction Test (FOCRT) started 43 days after the animals were recruited in the ICS. The protocol to test the efficacy of anticoccidial drugs was based largely on the protocol proposed by Odden et al. ¹⁴, with some changes to adapt it to our breeding context. The animals were divided into three groups of 11 to 16 lambs, with one group for each treatment that was tested. The groups were constituted so as to be homogeneous in terms of weight and farm of origin. The treatments under evaluation were: diclazuril (Vecoxan ND, MSD Santé Animale, 1 mg/kg BW orally) and toltrazuril (Baycox ND, Elanco, 20 mg/kg BW or Toltranil ND, KRKA, 20 mg/kg BW, both orally). An untreated group was added in both breeds. Individual fecal samples were taken from each lamb on the day of treatment (D0) and eight days after the treatment (D8).

Individual microscopic analyses were performed on all series of fecal samples. *Eimeria* spp. oocysts were counted using a modified Raynaud's method ²⁴. Briefly, three grams of feces were diluted in a saturated sodium chloride solution with a density of 1.2 g/mL before being filtered three times through a tea strainer. The filtrate was then analyzed on a McMaster slide. The oocysts in both grids were counted, summed, and multiplied by 50 to obtain the number of *Eimeria* spp. oocysts per gram of feces. The detection limit of this technique was 50 oocysts per gram (OPG) of fecal material.

In parallel, identification of the *Eimeria* species was performed per treatment group and per date (D0 and D8). To do so, all individual filtrates of a group were mixed in a glass flask. After homogenization, an aliquot of the total suspension was placed in a test tube, filled to the brim, and covered with a coverslip. The flotation time lasted 15 minutes before examination of the slide. The *Eimeria* species were then identified by the morphological and morphometric criteria of their oocysts as defined by Eckert (Eckert, 1995) at

400x magnification. The oocyst measurements were performed with Zeiss image processing software Zen (Zen 2.6 blue edition, Carl Zeiss Microscopy GmbH, 2018).

Species identification was performed on simple and relevant criteria on non-sporulated oocysts. The identification pathway is presented in Fig. 1. First, we checked the presence/absence of a polar cap on the oocyst. When a polar cap was present on the oocyst, the total length of the oocyst was measured to distinguish two sub-categories of *Eimeria*: species with large oocysts (> 30 µm) and species with oocysts of medium size (< 30 µm). Among the *Eimeria* species that have large oocysts with a polar cap, two species were readily distinguishable: *Eimeria intricata*, with a brown, thick wall, and *Eimeria ahsata*, with a very prominent polar cap. When oocysts had a medium size and a polar cap, the total length of the oocysts (Fig. 2) could differentiate the two following clusters: *Eimeria crandallis*/*Eimeria weybridgeensis* with smaller oocysts (total length ranging from 17 to 31 µm for a mean size of 22–24 µm) versus *Eimeria granulosa*/*Eimeria bakuensis* (from 22 to 37 µm for a mean size of 30–32 µm).

When the oocyte did not have a polar cap, the presence/absence of a micropyle was checked. Oocysts of *Eimeria pallida* and *Eimeria parva* species were readily identified as their oocysts lacked both a polar cap and a micropyle, had a round shape, and were small in size (12 to 22 µm) compared to all the other species. When a micropyle was present, the shape of the oocyst was of interest: an oval shape indicated the *Eimeria ovinoidalis*/*Eimeria marsica* cluster whereas a poultry egg shape was characteristic of *Eimeria faurei*.

In each group, we identified one hundred oocysts before and after treatment in order to have an accurate proportion of the species composition. However, in some groups after treatment, a smaller number of oocysts were identified due to the low number excreted after treatment. In this case, we identified as many oocysts as possible.

Calculation of the efficacy of anticoccidial treatments

The percentages reduction of the intensities of *Eimeria* oocyst excretion were estimated by the Kochapakdee formula ²⁵:

$$\text{Efficacy} = (1 - \text{arithmetic mean OPG of the treated group at D8} / \text{arithmetic mean OPG of the treated group at D0}) * 100$$

First, this formula was used on the total number of oocysts counted on McMaster slides at D0 and D8 to obtain the overall efficacy of the drug. According to the WAAVP guidelines for anthelmintic resistance, treatment resistance occurs when the percentage of reduction is less than 95% and the lower 95% confidence interval (CI) is less than 90%. To calculate the confidence intervals, we used the following formula:

$$\text{CI (95\%)} = (\text{mean of efficacy in treated group} \pm 1.96 (\text{Standard Variation of efficacy in treated group} / \sqrt{\text{number of animals in treated group}})$$

Secondly, we evaluated the efficacy of each drug against individual *Eimeria* species or clusters. Following the species identifications, the proportion of each species was calculated in each treatment group. This proportion was plotted against the total number of oocysts counted to obtain an estimate of the number of oocysts of each species in each group. Then, the same formula ²⁵ was used for these estimated numbers of oocysts at D0 and D8 to obtain the efficacy of a given drug against each *Eimeria* species.

Ethic Statement

Stool and blood collection and anthelmintic treatments are a part of routine veterinary procedures without any traumatic method. Such procedures are not qualified as animal experimentation involving vertebrates according to French laws, so no specific ethical clearance was required.

Results

The overall efficacies of the different drugs are listed in Table 1. At D0, all individuals excreted *Eimeria* oocysts, but the intensities of the oocyst excretions exhibited high individual variabilities in each group (see Supplementary Table S1). The mean intensities of the oocyst excretions at D0 were very similar in the control and treated groups for each ISC (between 4 518 OPG and 7 514 OPG for BC lambs and 15 018 and 16 850 OPG for RO lambs). At D8, the average intensities of the oocyst excretions in the control groups were equal or slightly higher than at D0 (5 477 OPG at D8 compared to 4 518 OPG at D0 in BC rams and 25 453 OPG compared to 16 075 OPG in RO rams). All groups treated with the various anticoccidial drugs exhibited a reduction in the intensity of excretions at D8,

including some lambs that were no longer excreting *Eimeria* oocysts. In BC lambs, the efficacy of toltrazuril was high, at 97.6% [96.7–99.3%], whereas the efficacy of diclazuril was 92.44% [76.3–95.6%]. In RO rams, the efficacies of toltrazuril and diclazuril were 97.96% [89–102%] and 93.58% [89.6–99.3%], respectively.

Table 1

Eimeria oocyst excretion intensity for each treatment group in the two breeding centers (mean, SD, median, minimum and maximum values) before treatment at day 0 (D0) and after treatment at day 8 (D8). In bold, the final results of the Fecal Oocyst Count Reduction Test (FOCRT), which is conclusive as to whether the anticoccidian treatment tested was effective or not. ISC = Individual Station Control; BC = Berrichon du Cher breed; RO = Rouge de l'Ouest breed

ISC	Group	N	D0				D8				% reduction and 95% CI
			Mean (OPG)	SD	Median	Min-Max	Mean (OPG)	SD	Median	Min-Max	
BC	Untreated	11	4518	5630	2300	500–19 800	5477	3386	6300	1000–10 800	-
	Diclazuril	14	7514	6177	5475	1050–21 800	568	589	325	0–2000	92.44 [76.3–95.6]
	Toltrazuril	14	7436	3877	6650	2600–15 800	179	350	75	0–1350	97.6 [96.7–99.3]
Ro	Untreated	16	16 075	20790	5725	1250–58 800	25 453	43 344	6800	600–120 600	-
	Diclazuril	14	15 018	16401	9325	1500–60 100	964	1599	225	0–5650	93.58 [89.6–99.3]
	Toltrazuril	15	16 850	15169	15 150	1450–58 000	343	912	50	0–3550	97.96 [89–102]

The number of oocysts per *Eimeria* species per group and per date (D0 and D8) is shown in Table 2. These proportions were then used to obtain an estimated number of oocysts of each species in the group (see Supplementary Table S2). Within the same ISC, the same species were identified in the different groups at D0. In the BC groups, the majority of the identified species were the clusters *Eimeria pallida*/*Eimeria parva*, *Eimeria ovinoidalis*/*Eimeria marsica*, *Eimeria granulosa*/*Eimeria bakuensis* and *Eimeria ahsata*. In the RO groups, the dominant clusters or species were *E. ovinoidalis*/*E. marsica*, *E. granulosa*/*E. bakuensis* and *E. ahsata*. A smaller proportion of oocysts identified as *Eimeria crandallis*/*Eimeria weybridgensis* was also found in all groups of both ISC. In contrast, *Eimeria faurei* and *Eimeria intricata* were only present in small proportions or even absent from some groups at D0, such as in the diclazuril BC group.

Table 2

Estimated number of oocysts per *Eimeria* species per treatment group and date. In bold, the FOVRT results lower than 95% showing a lack of efficacy of the drug on these species. ISC = Individual Station Control; BC = Berrichon du Cher breed; RO = Rouge de l'Ouest breed

ISC	Group	Date	<i>Eimeria pallida</i> / <i>Eimeria parva</i>	<i>Eimeria ovinoidealisis</i> / <i>Eimeria marsica</i>	<i>Eimeria crandallis</i> / <i>Eimeria weybridgensis</i>	<i>Eimeria faurei</i>	<i>Eimeria granulosa</i> / <i>Eimeria bakuensis</i>	<i>Eimeria ahsata</i>	<i>Eimeria intricata</i>	Total number of identified oocysts
BC	Untreated	D0	370	741	370	148	1555	1333	0	61
		D8	518	2072	888	740	1184	0	74	74
	Diclazuril	D0	1307	3697	714	0	1073	714	0	63
		D8	39	274	176	0	78	0	0	29
		FOCR (%)	97	92.59	75.34	ND	92.70	100	ND	
	Toltrazuril	D0	967	1614	1294	126	1487	1747	193	115
D8		22	26	87	13	31	0	0	41	
FOCR (%)		97.75	98.38	93.26	89.69	97.95	100	100		
RO	Untreated	D0	804	5305	1608	0	3376	4823	161	100
		D8	3563	6363	1782	1273	4327	7890	255	100
	Diclazuril	D0	1051	5707	1352	300	4806	1802	0	100
		D8	134	464	269	0	97	0	0	79
		FOCR (%)	87.25	91.87	80.10	100	97.97	100	100	
	Toltrazuril	D0	1854	5055	1685	1180	5224	1685	169	100
D8		153	172	19	0	0	0	0	18	
FOCR (%)		91.76	96.60	98.88	100	100	100	100		

In the BC ICS, diclazuril showed a lack of efficacy against *E. ovinoidealisis*/*E. marsica* (92.59%), *E. crandallis*/*E. weybridgensis* (75.34%), and *E. granulosa*/*E. bakuensis* (92.7%). These results were very similar to those of the diclazuril-treated group in the RO ICS: 91.87% efficacy against *E. ovinoidealisis*/*E. marsica*, 80.10% for *E. crandallis*/*E. weybridgensis*, and 87.25% for the *E. parva*/*E. pallida* cluster. Regarding the toltrazuril treated groups, the efficacies were high in both ICS, except against *E. pallida*/*E. parva* (91.76%) in the RO ICS and against *E. crandallis*/*E. weybridgensis* (93.26%) and *E. faurei* (89.69%) in the BC ICS.

Discussion

There are currently no guidelines available to evaluate the efficacy of anticoccidial drugs in sheep coccidiosis in the field. Some general recommendations have been made by the WAAVP²⁶, and protocols to evaluate the efficacy of anticoccidial drugs have been proposed^{8,14,16,19,20} with substantial variations in terms of group constitution, age of the animals at the time of testing, and natural infection levels.

In our context, the need for effective anticoccidial treatment is important not only to allow the lambs to express their growth potential, but also because, after three months together in the ICS, they are sold to different breeders. If previous treatments have not been effective, then they may contaminate their new farm with resistant and potentially pathogenic *Eimeria* species.

In our study, male lambs from different origins were corralled in the breeding center at three months of age and were treated at the time of their arrival with diclazuril. Therefore, it was not possible to perform the FOVRT immediately upon their entry. Odden et al.¹⁴

proposed the use of twin lambs, one in a treated group and the other one in the control group. In our study, this was not possible. However, other studies did not take this into account^{8,16,19,20}, but the efficacies measured were reliable because their groups were well balanced in terms of age, sex ratio, and weight. As coccidiosis is a disease that occurs in lambs, the age of the animal is important when performing the FOCRT. Compared to many studies^{14,16,19,20}, we performed the test on older animals (four months versus one month for the above-mentioned studies) due to our experimental setting. However, this age is not necessarily a bias if the intensities of *Eimeria* oocyst excretions are still high at the time of the test due to a high level of contamination of the environment, as demonstrated by Alzieu et al.,⁸ on 2- to 3.5-month-old male lambs at the beginning of the FOCRT. Nevertheless, although evaluation of the overall efficacy of a drug is easy, estimation of the efficacy against each *Eimeria* species is nonetheless an onerous undertaking and the pathogen must be present at the beginning of the test and at a sufficient level in the treated groups. Indeed, progressive immunity of the animal is established for the majority of *Eimeria* species². It is then possible that FOCRT cannot be performed on all species because they may be absent at the beginning of the test or present at low levels due to the age of the animals and possible immunity.

Taking into account all these considerations, we propose a simple protocol to evaluate the efficacy of anticoccidial drugs in farms, as well as an easy-to-use key for the identification of *Eimeria* species by microscopy.

At the beginning of our study, the intensities of excretion were relatively close between groups within the same farm and high enough to determine treatment efficacy. The untreated groups ensured the natural dynamics of *Eimeria* oocyst excretion as well as the natural change in the proportion of species in the two breeding centers during the duration of the test. In our case, the intensities of *Eimeria* oocyst excretions were equal or even slightly higher in the two control groups of the ISC from D0 to D8.

Odden et al.,¹⁴ proposed in their protocol that the best period to evaluate the efficacy of anticoccidial treatments is when the intensity of oocyst excretion increases significantly from the beginning of the trial to the end. However, in their case, the animals were younger ("more than 14 days old") and had never received anticoccidial drugs before the test. In our study, we performed the test on older animals that had undergone anticoccidial treatment 43 days before the beginning of the test. In our case, it seems difficult to obtain an excretion intensity that increases significantly between the beginning and the end of the FOCRT, but the maintenance of this excretion intensity between the two dates seems like an acceptable compromise that still allows interpretation of the FOCRT.

Our results show that in both ISC, the toltrazuril treatment was effective at reducing the overall intensity of *Eimeria* oocyst excretion. These results are very similar to those obtained in previous studies^{8,16,20-22,27}. The percentages of reduction of oocyst excretions were 92.44% and 93.58% with diclazuril, which is lower than the minimum threshold of efficacy (95%) required for anthelmintic treatments²⁸.

In studies where the efficacy of diclazuril was not investigated, this drug exhibited an efficacy higher than 97% at four to seven days after treatment^{8,18,27,29}. In our case, the efficacy was slightly lower.

Regarding the efficacies of the drugs against each species, we noticed that although the general efficacy of toltrazuril was higher than 95%, some FOCRT were lower for some species. This was the case in the BC ICS, where the reduction was below 95% for the species *E. crandallis/E. weybridgensis* and *Eimeria faurei*. In this group, only a few oocysts of *E. faurei* were identified at D0 and D8, which likely led to inaccuracy in the calculation of the FOCRT, and the result should hence be interpreted with a degree of caution. By contrast, the number of oocysts identified as *E. crandallis/E. weybridgensis* was substantial at the time of the toltrazuril treatment, and we can assume that there was a real lack of reduction for this cluster of species. In the RO ICS, toltrazuril exhibited a loss of efficacy toward *E. parva/E. pallida* but remained effective toward the pathogenic species.

In the BC ICS, reductions of oocyst excretions below 95% were noted for the species *E. ovinoidalis/E. marsica*, *E. crandallis/E. weybridgensis*, and *E. granulosa/E. bakuensis* after diclazuril treatment. At D8, only a few oocysts (29 oocysts) were identified in this group because the treatment drastically reduced the total intensity of excretion. The proportions were, therefore, less accurate than if they had been estimated with 100 oocysts. Nevertheless, the majority of identified oocysts at D8 belonged to the pathogenic clusters in the diclazuril group. In the RO ICS, diclazuril no longer appeared to be fully effective against *E. parva/E. pallida* as well as against the pathogenic species *E. ovinoidalis/E. marsica* and *E. crandallis/E. weybridgensis*. In these situations, it is difficult to conclude that one of the pathogenic species is resistant due to the difficulty distinguishing it from a less pathogenic morphologically related

species. The development of molecular tools could fill this gap. For the time being, in sheep, molecular techniques, especially real-time Polymerase Chain Reaction, are not as advanced as in poultry³⁰. Although some genomic sequences are available^{23,31}, to our knowledge, no routine technique has been developed to date.

Thus, we cannot presently draw firm conclusions regarding the actual resistance of these species of sheep *Eimeria* to diclazuril or toltrazuril, although it is clear that there is a lack of efficacy in these two breeding centers. Indeed, resistance is normally confirmed by experimental infections in naive lambs under coccidia-free conditions, with oocysts recovered post-treatment and submitted to a new experimental FOCRT¹⁵. Unfortunately, we do not have the resources to validate the resistance by this method. Nevertheless, the maintenance of clinical signs after treatment observed by farmers (diarrhea and slowing of weight and mass gain) seems to indicate a significant persistence of pathogenic species after treatment.

Conclusion

In this study, we evaluated the efficacy of diclazuril and toltrazuril on two sheep-meat farms in France. Using an identification protocol for the ovine *Eimeria* species, we estimated the overall efficacy of the treatments based on reduction of the total intensity of oocyst excretion, as well as by species. The results of this study show that in the two investigated farms, there was an overall lack of efficacy of diclazuril and more particularly in the control of the two pathogenic species *E. ovinoidalis* and *E. crandallis*. In addition, in one of the farms, the overall efficacy of toltrazuril was high but seemed to be slightly reduced in regard to the pathogenic species *E. crandallis*. However, to definitively attribute these reduced efficacies to resistance of *Eimeria* species to these drugs, additional experiments are required. In addition, molecular identification of oocyst species after treatment would further assist with reaching definitive conclusions regarding efficacies. Nevertheless, the approach developed in this study could allow rapid and simple testing of the overall efficacy of anticoccidial drugs and of the various sheep *Eimeria* species, in particular *E. ovinoidalis* and *E. crandallis* in field conditions.

Declarations

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Author contributions

LB, AL, SJ, CG, and PJ: developed and optimized the protocol, analyzed samples in the laboratory, and interpreted results. Contributed mainly to the writing of the manuscript.

CS, AB, AC, and GB: Provided the animals and performed the grouping, sampling, and treatment. Monitored animal welfare.

All authors read and approved the final manuscript.

Availability of data and materials

All data generated or analysed during this study are included in this published article and its supplementary information files.

Competing interests

The authors declare that they have no competing interests.

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Figures

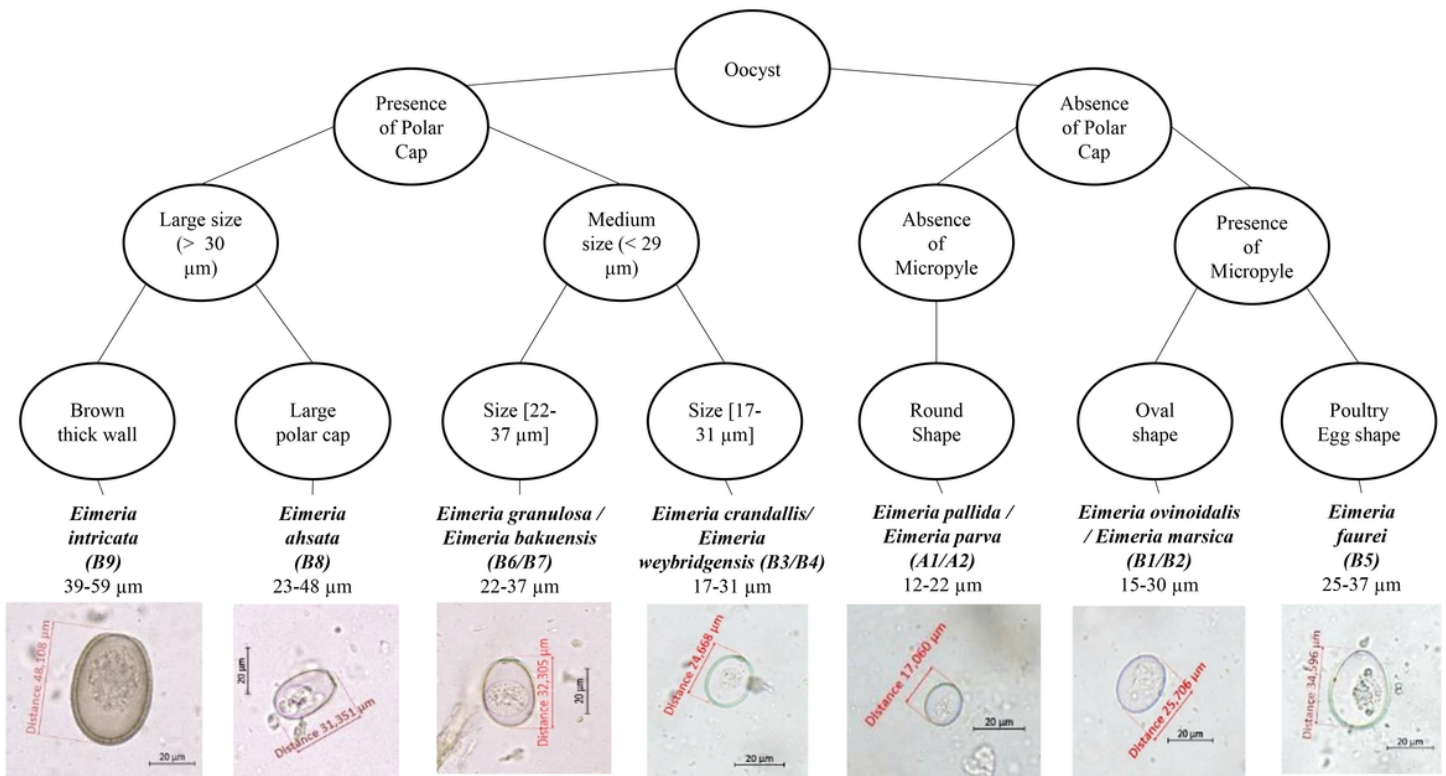


Figure 1

Identification keys for oocysts of ovine *Eimeria* species according to the morphological criteria of Eckert³.

A1 : *E. parva*
 A2 : *E. pallida*
 B1 : *E. marsica*
 B2 : *E. ovinoidalis*
 B3 : *E. weybridgensis*
 B4 : *E. crandallii*
 B5 : *E. faurei*
 B6 : *E. granulosa*
 B7 : *E. bakuensis*
 B8 : *E. ahsata*
 B9 : *E. intricata*

* Micropyle
 ° Polar Cap
 °° Prominent Polar Cap
 Black bar : average size of species

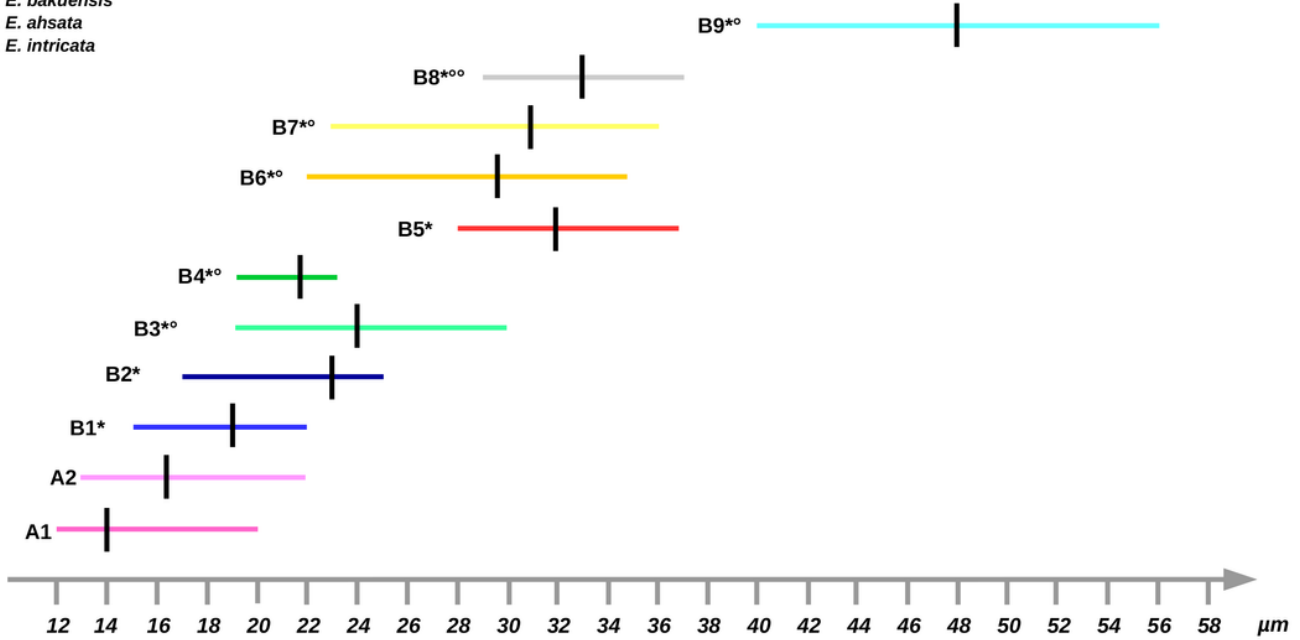


Figure 2

Size scale of oocysts of sheep *Eimeria* species. The grey line below indicates the sizes in micrometers; * denotes the presence of a micropyle in the species; ° presence of a polar cap in the species; °° presence of a prominent polar cap in the species; black bar: average size in micrometers for each species.

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