Journal of Helminthology

cambridge.org/jhl

Comparison of two coprological methods for the diagnosis of *Eurytrema* ssp. in cattle and sheep

L.H. Olmos[1,2], A. Pantiu[3], A. Avellaneda-Cáceres[1,2,4], P.N. Valencia[5], P.N. Cayo[5], M. Signorini[6] and J.F. Micheloud[1,2,4]

[1]Instituto Nacional de Tecnología Agropecuaria (INTA), CIAP, Instituto de Investigación Animal del Chaco Semiárido, Área de Investigación en Salud Animal, EEA Salta, Salta, Argentina; [2]Facultad de Ciencias Agrarias y Veterinarias, Universidad Católica de Salta, Salta, Argentina; [3]Instituto Nacional de Tecnología Agropecuaria (INTA), EEA Montecarlo, Misiones, Argentina; [4]Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), EEA Salta, Salta, Argentina; [5]Instituto San Cayetano N° 8092 Tecnicatura Superior en Laboratorio– Salta Capital, Salta, Argentina and [6]Instituto de Investigación de la Cadena Láctea (CONICET – INTA), Ruta 34 Km 227, Rafaela, Santa Fe, Argentina

Short Communication

Cite this article: Olmos LH, Pantiu A, Avellaneda-Cáceres A, Valencia PN, Cayo PN, Signorini M, Micheloud JF (2022). Comparison of two coprological methods for the diagnosis of *Eurytrema* ssp. in cattle and sheep. *Journal of Helminthology* **96**, e53, 1–4. https://doi.org/10.1017/S0022149X22000414

Received: 19 April 2022
Revised: 22 June 2022
Accepted: 25 June 2022

Key words:
Eurytrema; diagnosis; coprology; sedimentation

Author for correspondence:
L.H. Olmos,
E-mail: olmos.leandro@inta.gob.ar

Abstract

Eurytrematosis is a disease caused by flukes of the genus *Eurytrema*. These parasites infect the pancreatic ducts of a wide variety of species, including cattle, sheep and humans. Diagnosing eurytrematosis through the analysis of faecal samples can be difficult because most of the available techniques are considered of low sensitivity. In this context, a modification of the Dennis, Stone and Swanson technique (Belem Sedimentation Technique, BST) was previously developed to increase the probability of detecting infected animals; nevertheless, the values of eggs per gram obtained using the modified technique are generally low. We proposed a modification of the this technique (MBST), to increase the sensitivity and detection rate of infected animals. The objective of this work was to describe MBST and compare it with BST. Faecal samples of 212 clinically healthy animals (174 from cattle and 38 from sheep) from 20 farms were taken by the intra-rectal route and stored at 4°C. The samples were processed using BST and MBST. Positive samples amounted to 55 (25.9%) using BST and 121 (57.1%) using MBST. In the simples from cattle, 52 (29.8%) and 107 (61.4%) were positive in BST and MBST, respectively. In sheep, three (7.8%) and 14 (36.8%) positive samples were obtained in BST and MBST, respectively.The results obtained using the two methods were significantly different, indicating a lack of agreement between their findings. The results suggest that MBST is a more sensitive method to detect *Eurytrema spp. eggs* in faeces than BST.

Introduction

Eurytrematosis is a disease caused by flukes of the genus *Eurytrema* (Bassani *et al.*, 2006). These parasites infect the pancreatic ducts of a wide variety of species, including cattle, sheep and humans (Yeh *et al.*, 2019; Leite *et al.*, 2020; De Sousa *et al.*, 2021). In South America, there are several records of the presence of this parasite, mainly in Brazil (Brant, 1962; Azevedo *et al.*, 2004). In Argentina, only one study reported the occurrence of this parasite in Misiones province (Moriena *et al.*, 1996).

Diagnosing eurytrematosis through the analysis of faecal samples can be difficult because most of the available techniques are very laborious and generally considered of low sensitivity (Chinone & Itagaki, 1976; Sakamoto *et al.*, 1980; Viana, 1985). The Dennis, Stone and Swanson technique was modified by Belém *et al.* (1992) to increase the probability of detecting animals positive for *Eurytrema* spp. in faecal samples. According to these authors, the modified technique had a probability of detection of 94.2%. Nevertheless, they observed that the values of eggs per gram (EPG) are generally low. In this regard, some authors propose that the low loads of eggs in faeces, as well as the number of false negatives that occur using the different techniques, may be due to egg laying fluctuations or to a low parasitic load at sampling (Martin, 1972; Chinone & Itagaki, 1976; Belém *et al.* 1992).

We proposed a modification of the technique described by Belém *et al.* (1992), to increase the sensitivity and detection rate of infected animals. The objective of this work was to describe the new technique and to compare it with that provided by Belém *et al.* (1992).

Materials and methods

Sampling was conducted in Misiones province, Argentina (north-eastern Argentina on the border with Brazil) between March 2021 and November 2021. Faecal samples of 212 clinically healthy

animals from 20 farms were collected by the intra-rectal route and stored at 4°C. Subsequently, the samples were transported to the Parasitology Laboratory of the Animal Research Institute of Chaco Semiarid (IIACS) under low-temperature conditions for processing. Of the total samples, 174 were from cattle (calves, heifers, steers and cows) and 38 from sheep (adult females). The farms used for this study were selected based on reports of the presence of *Eurytrema* spp. in the region and information provided by veterinarians working at abattoirs about the presence of the parasite in the slaughtered animals (Servicio Nacional de Sanidad y Calidad Agroalimentaria (SENASA), pers. comm.). On the other hand, and considering the similarity in the morphology of the eggs of *Eurytrema* spp. and *Dicrocoelium* spp., no specimen of *Dicrocoelium* spp. was found in the slaughter of the animals and the necropsies carried out in several of the establishments, *Eurytrema* spp. being the only trematode of the family Dicrocoeliidae in the sampled herds. Therefore, it can be assumed that the eggs observed in the coprological studies belong to the genus *Eurytrema*.

Samples were processed using two sedimentation techniques: The technique described by Belém *et al.* (1992) the first one (Belém *et al.* (1992) Sedimentation Technique, BST), and the second one was developed by us, which we named 'Modification of the technique described by Belém *et al.* (1992)' (MBST). BST is one of the most widely applied techniques to diagnose *Eurytrema coelomaticum* infection in faeces at the regional level (Araújo & Belém, 1993, 1994; Belém *et al.* 1994; Bassani, 2005; Lucca *et al.*, 2015). It is a modification of the technique of Dennis *et al.* (1954) and uses a 60-µm sieve, 10 min of sedimentation and 1 g of sample. BST consists of diluting 1 g of faecal matter in a 50 ml 0.5% dishwashing detergent solution (DS) and filtering the contents through a 60-µm sieve into a 50 ml tube; then this solution is allowed to settle for 10 min. Subsequently, the supernatant is removed and only the sediment is left. Finally, using a Pasteur pipette, the sediment is transferred to a slide and observations are made at a magnification of 100 or 400. The result is expressed as EPG of faecal matter (final dilution 1 g/50 ml). According to Belém *et al.* (1992), this technique has a high probability of detecting infection by *Eurytrema* spp. (94.2%).

On the other hand, our technique (MBST) combines the sedimentation techniques described by Dennis *et al.* (1954), Belém *et al.* (1992) and Viñabal *et al.* (2015). MBST consists of homogenization of 5 g of faeces in 250 ml of DS. First, homogenization is carried out in 50 ml of 0.5% DS in a laboratory mortar and then, after filtration with a strainer, the homogenized solution is transferred to a 250-ml graduated conical beaker; then the DS is added until the 250-ml graduated conical beaker is full. This solution is filtered through two sieves (150 µm and 180 µm). The content is poured into another 250-ml graduated conical beaker and then allowed to settle for 10 min. After that, the supernatant is removed, leaving 50 ml of the solution, which is homogenized by shaking, and transferred to another 250-ml conical beaker using a 60-µm sieve. Subsequently, to increase the chances of collecting *Eurytrema* spp. eggs, DS is added through the 60-µm sieve until the 250-ml graduated conical beaker is full and allowed to settle for 10 min. After 10 min, 200 ml of supernatant are removed, and the remaining 50 ml are homogenized with the pellet by shaking, transferred to a 50-ml Falcon tube and allowed to settle for 10 min. Finally, the supernatant is removed, and the remaining solution (2 ml) is homogenized, collected with a pipette and deposited in the camera described by Viñabal *et al.* (2015) for its reading in an optical microscope at 100×. The result are expressed as eggs/5 g faecal matter (final dilution 5 g/50 ml).

The results of the techniques were reported as positive/negative because this study intends to compare the ability of both techniques to detect at least one egg in the sample and not the number of EPG as such. The agreement between the techniques was evaluated using the Cohen's kappa coefficient κ.

Results

Of the 212 samples tested, 55 were positive for BST (25.9%) and 121 (57.1%) were positive for MBST. The analysis of positive results for each species showed 52 (29.8%) and 107 (61.4%) positive bovine samples by BST and MBST, respectively, and three (7.8%) and 14 (36.8%) positive sheep samples by BST and MBST, respectively. Some samples (11 sheep and 59 bovine samples) were positive by MBST and negative by BST. On the other hand, four bovine samples were positive by BST and negative by MBST, while all the sheep samples positive by BST were positive by MBST (table 1).

The Kappa coefficient values were 0.223 ($P = 0.018$) for sheep samples, 0.337 ($P < 0.001$) for bovine samples and 0.346 ($P < 0.001$) for all samples (table 2).

Discussion

MBST differs from BST in the followings aspects: the sample size is larger (5 g vs. 1 g); it uses an additional wash and passage through two sieves (150 µm and 180 µm) before the passage through a 60-µm sieve; and it uses the camera described by Viñabal *et al.* (2015) for reading. Our hypothesis was that using a larger sample and modifying some aspects of the technique provided by Belém *et al.* (1992) would allow us to increase the detection of positive cases. The results suggest that MBST has a higher sensitivity than BST. These differences can be observed mainly in the analysis of bovine samples. Indeed, many of the bovine samples ($n = 59$) were positive by MBST and negative by BST, suggesting that MBST has a higher negative predictive value than BST. In the case of sheep samples, however, both techniques tended to coincide when the result was negative.

Coprological diagnosis of *Eurytrema* spp. tends to be difficult and of very low sensitivity (Martin, 1972; Belém *et al.*, 1992). These difficulties may be related to the parasite load in the animals and fluctuations in the oviposition of *Eurytrema* spp. (Chinone & Itagaki, 1976). In this regard, Martin (1972) observed an increase in false negatives when loads were below 100 adult specimens in the animal pancreas. Similar difficulties in detecting infected animals with low parasite loads were previously reported (Sakamoto *et al.*, 1980; Viana, 1985). On the other hand, Belém *et al.* (1992) describe a 94.2% probability of detection in infected animals. However, the comparison of the results shows that BST has a lower sensitivity to recognize an infected animal than MBST (25.9% for BST and 57.1% for MBST). The studies conducted with BST in Brazil showed positivity of 37.8% (Bassani, 2005) and 68.9% (Lucca *et al.*, 2015), which is higher than the value recorded in this study (25.9%). However, the elimination of eggs through faeces could be related to the parasitic burden of the pancreas and the seasonal fluctuations in the oviposition of *Eurytrema* spp., which could also influence the results of coproparasitological studies (Bassani *et al.*, 2007). These factors may be responsible for the differences observed in the studies from Brazil and Argentina.

Epidemiological characterization studies of eurytrematosis in Argentina are necessary to confirm this assumption. Therefore, MBST is more efficient than BST, and can be a useful tool in the

https://doi.org/10.1017/S0022149X22000414 Published online by Cambridge University Press

Table 1. Cross-tabulation table for the Modification of the technique described by Belem *et al.* (1992) (MBST), the technique provided by Belém *et al*. (1992) (BST) and species. The results of the sample by BST are presented in columns and results by MBST are in rows.

				BST		
Species				Negative	Positive	Total
Sheep	MBST	Negative	Counts	24	0	24
			% MBST	100.0%	0.0%	100.0%
		Positive	Counts	11	3	14
			% MBST	78.6%	21.4%	100.0%
	Total		Counts	35	3	38
			% MBST	92.1%	7.9%	100.0%
Bovine	MBST	Negative	Counts	63	4	67
			% MBST	94.0%	6.0%	100.0%
		Positive	Counts	59	48	107
			% MBST	55.1%	44.9%	100.0%
	Total		Counts	122	52	174
			% MBST	70.1%	29.9%	100.0%
Total	MBST	Negative	Counts	87	4	91
			% MBST	95.6%	4.4%	100.0%
		Positive	Counts	70	51	121
			% MBST	57.9%	42.1%	100.0%
	Total		Counts	157	55	212
			% MBST	74.1%	25.9%	100.0%

Table 2. Kappa's coefficient by species and total samples.

Species			Value	Asymptotic standard error	*P*-value
Sheep	Agreement	Kappa	0.256	0.126	0.018
	No. of valid cases		38		
Bovines	Measure of agreement	Kappa	0.337	0.055	<0.001
	No. of valid cases		174		
Total	Measure of agreement	Kappa	0.346	0.050	<0.001
	No. of valid cases		212		

detection of *Eurytrema* spp. as a complement to the techniques currently available to reduce the presence of false-negative animals. Future studies are needed to validate this technique.

In conclusion, the results observed in this study suggest that MBST may be a more sensitive technique in the detection of *Eurytrema* spp. eggs in faeces than BST. However, more studies are necessary to validate this technique and better understand epidemiological aspects of eurytrematosis in Argentina.

Financial support. This work was supported by grants from INTA (PEM-E5-I702-001: 'Caracterización de la Euritremosis en Rumiantes en Misiones').

Conflicts of interest. None.

Ethical standards. This study was conducted in accordance with animal ethics guidelines and approved protocols described in the requirements of the Argentine Animal Protection Policy (Law 14346) and the European Union Directive 2010/63 on the protection of experimental animals were fulfilled. All information is used for research purposes with the consent of the farm manager.

Author contributions. This work was carried out in collaboration with all authors. L.H.O. and J.F.M.: study conception, design, work supervision, data collection, material preparation, analysis and writing – original draft preparation. A.P.: sample collection, work supervision and writing – review. P.N.V., P.N.C. and A.A.-C.: data collection and processing of faecal samples. M.S.: statistical analysis and writing – original draft preparation. All authors read and approved the final manuscript.

References

Araújo JV and Belém PD (1993) Efeito Anti-helmíntico do albendazole sobre a contagem e ovos de *Eurytrema* sp. (Trematoda) em fezes de bovinos. *Arquivo Brasileiro de Medicina Veterinária E Zootecnia* **45**, 111–114.

Araújo JV and Belém PD (1994) Curso natural da eliminação de ovos de *Eurytrema* sp. Looss, 1907 nas fezes de bezerros. *Brazilian Journal of Veterinary Research and Animal Science* **31**, 125–129.

https://doi.org/10.1017/S0022149X22000414 Published online by Cambridge University Press

Azevedo JR, Mannigel RC, Agulhon AZ, Borba TR, Barbiéri AW, de Oliveira DC, Headley SA and Janeiro V (2004) Prevalence and geographical distribution of bovine eurytrematosis in cattle slaughtered in northern Paraná, Brazil. *Pesquisa Veterinária Brasileira* **24**, 23–26. DOI: 10.1590/S0100-736X2004000100006.

Bassani AC (2005) *Estudo epidemiológico e patológico da euritrematose bovina*. Londrina, Tese (Mestrado em Ciência Animal Concentraçâo Sanidade Animal) Universidade Estadual de Londrina.

Bassani CA, Sangioni LA, Saut JPE, Yamamura MH and Headley SA (2006) Epidemiology of eurytrematosis (*Eurytrema* spp. Trematoda: Dicrocoeliidae) in slaughtered beef cattle from the central-west region of the State of Paraná, Brazil. *Veterinary Parasitology* **141**, 356–361.

Bassani CA, Sangioni LA, Saut JPE, Headley SA and Yamamura MH (2007) Euritrematose bovina. *Semina: Ciências Agrárias* **28**, 299–315. DOI: 10.1016/j.vetpar.2006.06.003.

Belém PD, Rodrigues de Oliveira M and Roberto Padovani C (1992) Adaptation of the Dennis, Stone & Swanson's technique for parasitologic diagnostic of *Eurytrema* sp. infection in cattle. *Brazilian Journal of Veterinary Research and Animal Science* **29**, 303–307.

Belém PAD, Oliveira MRD, Padovani CR and Luvizotto MCR (1994) Alterações pancreáticas em bovinos naturalmente infectados por *Eurytrema* sp. e sua associação com a carga parasitária e o número de ovos por grama de fezes (OPG). *Brazilian Journal of Veterinary Research and Animal Science* **31**, 273–281.

Brant PC (1962) Frequencia de algumas parasitoses em carcacas evísceras de bovinos abatidos em Belo Horizonte. *Arquivos da Escola de Veterinaria de Minas Gerais* **14**, 127–132.

Chinone S and Itagaki H (1976) Development of *Eurytrema pancreaticum* (TREMATODA). II. Development in definitive hosts. *Bulletin of the Azabu Veterinary College, Sagamihara* **1**, 173–181.

Dennis WR, Stone WM and Swanson LE (1954) A new laboratory and field diagnostic test for fluke ova in feces. *Journal of the American Veterinary Medical Association* **124**, 47–50.

De Sousa DER, Barbosa EDF, Wilson TM, Machado M, Oliveira WJ, Duarte MA and de Castro MB (2021) *Eurytrema coelomaticum* natural infection in small ruminants: a neglected condition. *Parasitology* **148**, 576–583.

Leite KG, Lopes-Torres EJ, Souza JGR, Neves RH, Gomes DC and Machado-Silva JR (2020) *Eurytrema coelomaticum*: updated morphology of adult worms using advanced microscopy experiments. *Journal of Helminthology* **94**, E122.

Lucca N, Stedille F, Schwertz C, Henker L, Gabriel M, Mendes R, Pappen F and Rosa L (2015) Principais parasitoses gastrointestinais em bovinos provenientes de propriedades leiteiras de municípios do Alto Uruguai, Santa Catarina. *Extensão Tecnológica: Revista de Extensão do Instituto Federal Catarinense* **3**, 63–68.

Martin OC (1972) The incidence of *Eurytrema pancreaticum* (Looss, 1907) in dairy cattle at the DTRI Farm. *Philippine Agriculturist* **56**, 25–34.

Moriena RA, Lombardero OJ and Racioppi O (1996) *Eurytrema coelomaticum* (Gerard & Billet, 1892) (Trematoda, Dicrocoeliidae) nuevo parásito del bovino para la Argentina. *Revista de Medicina Veterinaria (Buenos Aires)* **11**, 247–249.

Sakamoto T, Kono I, Yasuda N, Yamamoto Y and Nakagawa H (1980) Studies on *Eurytrema coelomaticum*. II. The anthelmintic efficiency of nitroxynil and praziquantel against *Eurytrema coelomaticum* in cattle. *Memoirs of the Faculty of Agriculture, Kagoshima University* **16**, 93–101.

Viana SS (1985) *Técnica coproscópica de sedimentação para concentração de ovos de Eurytrema Looss, 1907*. São Paulo, Tese (Doutorado) – Instituto de Ciências Biológicas, Universidade de São Paulo.

Viñabal A, Cafrune Wierna MM, Aguirre DH, Bassanetti AF, Bertoni EA and Suarez VH (2015) Propuesta y evaluación de una técnica de sedimentación y tinción con azul de metileno (y de una variante) para el diagnóstico de *Fasciola hepatica*. *Veterinaria Argentina* **32**, 327.

Yeh HY, Cheng CFJ, Huang C, Zhan X, Wong WK and Mitchell PD (2019) Discovery of *Eurytrema* eggs in sediment from a colonial period latrine in Taiwan. *The Korean Journal of Parasitology* **57**, 595.

https://doi.org/10.1017/S0022149X22000414 Published online by Cambridge University Press