Ciência Rural, Santa Maria, v.52:2, e20210041, 2022
http://doi.org/10.1590/0103-8478cr20210041
ISSNe 1678-4596
PARASITOLOGY

Eurytrema coelomaticum infection: correlation between parasite burden and impairment of pancreatic exocrine enzyme secretion

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ABSTRACT: *Eurytrema coelomaticum is a trematode reported in the pancreatic ducts of ruminants. It is conjectured that may cause disorders in the pancreas, as well as digestive and metabolic processes dependent on them. This study, determined if there is an impairment of exocrine pancreatic function, and correlated it with parasite burden. Pancreas, blood, and fecal samples were collected from 119 bovines at a abattoir. Stool samples were subjected to the gelatin and x-ray film digestion tests (to detect the presence of trypsin in feces). Using blood samples, the following biochemical tests were performed: amylase, lipase, glucose, fructosamine, cholesterol, triglycerides, total protein, albumin, and globulins. Analyses were correlated with pancreatic parasite burden. Cattle with a high parasitic load presented higher incidence of negative tests in both gelatin digestion and x-ray film digestion tests ($P < 0.001$) when compared to non-parasitized animals and those with a low parasitic load. Changes in those tests only occurred if the parasitemia was moderate or severe. The activity of the amylase and lipase enzymes was significantly higher in animals with low parasitemia ($P < 0.05$), compared to non-parasitized animals and with a high parasitic burden. In this study, in cases of high parasitemia, negative results were observed in both gelatin and x-ray film in the feces digestion tests. However, the low infection of E. coelomaticum, higher levels of serum amylase and lipase that also indicated loss of pancreatic exocrine functions were reported.*
Key words: *amylase, Eurytrema coelomaticum, pancreatic insufficiency, lipase, pancreatitis.*

Eurytrema coelomaticum: correlação entre carga parasitária e insuficiência pancreática exócrina

RESUMO: *Eurytrema coelomaticum, um trematódeo de ductos pancreáticos de ruminantes. Conjectura-se que possa ocasionar transtornos nas funções pancreáticas, mais especificamente nos processos digestivos e metabólicos dependentes destas. Neste estudo, o objetivo foi determinar se há comprometimento da função pancreática exócrina, correlacionado-a a carga parasitária. Foram utilizados pâncreas e respectivas amostras de sangue e fezes de 119 bovinos. As amostras de fezes foram submetidas aos testes de digestão da gelatina em tubo e digestão de filme radiográfico, ambos para detecção de tripsina nas fezes. Foram realizados os seguintes exames bioquímicos em amostras de sangue: amilase, lipase, glicemia, frutosamina, colesterol, triglicerídeos, proteínas totais, albumina e globulinas. Após isto, as análises bioquímicas foram correlacionadas com a quantidade numérica de parasitas encontrados no pâncreas (post-mortem). Houve maior quantidade de testes negativos (digestão do filme radiográfico e prova de digestão da gelatina) nos animais com alta carga parasitária ($P < 0.001$), quando comparados aos animais não parasitados e com baixa carga parasitária. Portanto, os exames supracitados se alteram somente se a quantidade de parasitas for moderada ou severa. As atividades das enzimas amilase e lipase foram significativamente maiores nos animais que apresentavam baixa parasitemia ($P < 0.05$), em comparação com os animais com alta carga parasitária e não parasitados. Conclui-se que em quadros de alta parasitemia há alteração significativa nos testes de digestão nas fezes, e que em quadros de baixa parasitemia há alterações significativas nos valores de amilase e lipase séricas, ambos comprovando alterações pancreáticas importantes, de acordo com o quadro de parasitemia.*
Palavras-chave: *amilase, Eurytrema coelomaticum, insuficiência pancreática, lipase, pancreatite.*

INTRODUCTION

In Brazil, bovine eurytrematosis is caused by *Eurytrema coelomaticum*, a trematode reported mainly in the pancreatic ducts and, occasionally, in bile ducts of ruminants and other mammals. It is endemic in some Brazilian states, predominantly in the south of the country (Paraná, Santa Catarina, and Rio Grande do Sul States) (BASSANI et al., 2007; SCHWERTZ et al., 2016). Besides, the parasite is

Received 01.18.21 Approved 05.11.21 Returned by the author 06.24.21
CR-2021-0041.R3
Editor: Rudi Weiblen

widespread, with reports of the presence of species of *Eurytrema* spp. in Europe and Asia (CHAI & JUNG, 2019; HAFIAH et al., 2019), including as zoonotic disease (OGAWA et al., 2019).

Despite some authors pointing out the disease as a cause of death (BASSANI et al., 2007; RACHID et al., 2011; QUEVEDO et al., 2013), it is almost a consensus that the disease does not produce clinical signs, but a silent disease which causes losses in milk and meat production (SILVA JÚNIOR et al., 2018; SU et al., 2018). In addition, the infection of humans with this pancreatic trematode is also possible (Ogawa et al., 2019). Whereupon, CARNEIRO et al. (2019) described that only 0.3% of the 865 diagnoses between 2013 and 2017 at the veterinary pathology laboratory (LPV) of the Catarinense Federal Institute (IFC) at campus Concórdia were parasitic pancreatitis. These three diagnoses were from histopathological samples received by mail, which represent 35% of the 865 diagnoses, not necessarily being the cause of death. As for the remaining 65%, these came from necropsies performed by the LPV, and there were no deaths due to this disease. Similarly, LUCENA et al. (2010) described three (0.07%) diagnoses of parasitic pancreatitis from 4,220 cases.

Endoparasitism causes lesions characterized by fibrosing interstitial pancreatitis and total or partial obliteration of the pancreatic ducts, which may cause disorders, in varying degrees, in the secretory functions of the pancreas (BASSANI et al., 2007; RACHID et al., 2011; SCHWERTZ et al., 2016). In cattle, pancreatic lesions associated with parasitism induced by *Eurytrema* spp. were related to pancreatic atrophy (BASSANI et al., 2007; RACHID et al., 2011). Fecal loss of protein and lipid elements may occur, causing serum, hematological, and fecal changes long before the animals display apparent clinical signs (SAKAMOTO et al., 1980; ILHA et al., 2005; HEADLEY et al., 2009).

To date, there are no anthelmintic drugs capable of combating parasitism by *Eurytrema* spp. (ILHA et al., 2005; BASSANI et al., 2006). Therefore, the disease is going to progress invariably to fibrosis, being the damage irreversible (AZEVEDO et al., 2005; BASSANI et al., 2006).

Most authors constantly report parasitological and histopathological aspects and necropsy descriptions, concealing information on the clinical pathology in severely infected animals (GASTE, 1991; BELÉM et al., 1994). Thus, an accurate clinical and laboratory diagnosis is important for the best elucidation of the disease, since the definitive diagnosis is currently only reached with the necropsy of the animal or post-mortem examinations in slaughterhouses.

Using multiple regression models to estimate the parasitic load (PL) of *Eurytrema* sp. in cattle, BELÉM et al. (1992a) observed a relation with the number of eggs per gram of feces (EPG). These authors predicted that in false-negative cases (EPG = 0) the animals should carry an average of 63.1 parasites in the pancreas.

The present study evaluated the possible correlation between biochemical profile and pancreatic functions and the parasitic burden of cattle naturally infected with *Eurytrema coelomaticum*. These data were correlated with the alterations observed with the parasitic load of the animals.

MATERIALS AND METHODS

Pancreas, blood, and fecal samples were collected randomly from 119 bovines, male or female, of different breeds and farms, slaughtered in the city of Concórdia (SC). The sample size was established using the EPIDAT® 4.2, with 95% confidence level and precision of 0,05%; taking into account the number of bovine population and prevalence of disease in the city (SCHWERTZ et al., 2016).

The blood was collected after stunning with a pneumatic powered stunner and during regular bleeding, stored in vacuum blood collection tubes without additives, being later centrifuged for 5 min at 2000 g. The serum was separated in duplicate, stored in tubes, and frozen at -20 ºC. All biochemical measurements in the serum were performed using commercial reagents, and the reactions were analyzed by a Prietest TOUCH® semi-automatic biochemistry analyzer.

Results of biochemical tests for amylase, lipase, glucose, fructosamine, cholesterol, triglycerides, total protein, albumin, and globulins were compared with data from non-parasitized animals from the same abattoir; subsequently, the biochemical analyses were correlated with the number of parasites detected in the animals' pancreas (*post-mortem*). The renal function of the animals (urea and creatinine) was also evaluated, as renal diseases can increase the enzymes amylase and lipase due to deficiency in excretion, thus, interfering with the results of the exocrine pancreatic function by causing a false positive for pancreatic lesions (STOCKHAM & SCOTT, 2011).

After the evisceration of the animals, the entire pancreas was collected, whether parasitized or not, and 20 g of feces from the rectum ampulla were collected. These were packed in previously identified

individual plastic bags and, finally, transported to the Parasitology Laboratory of the IFC campus Concórdia for processing.

Stool samples were kept in a refrigerator at 4 ºC until they were subjected to the gelatin hydrolysis and x-ray film digestion tests (both to detect the presence of trypsin in feces). For the latter, the feces were diluted in 5% sodium bicarbonate in a 1:9 ratio and incubated with an x-ray film for 1 hour at 37 ºC, according to COLES (1984). For the former, the solution prepared for the x-ray film digestion test was diluted in colorless gelatin at a 1:2 ratio, carried out according to the manufacturer's instructions, placed in an oven for 1 hour at 37 ºC, and kept in the refrigerator for 24 hours for analysis later. Results could be atypical if the feces did not adequately liquefy gelatin, which indicated decreased fecal protease activity (STOCKHAM & SCOTT, 2011). In order to compare the tests performed, a negative control was performed using distilled water instead of the feces.

The pancreases were placed in individual trays, isolated, and washed. All the water used in the washing was sieved through nylon sieves to separate the trematodes. Subsequently, the pancreatic ducts were opened with the aid of a scalpel, forceps, and surgical scissors, and the trematodes were removed using surgical tweezers and counted. The parasites were identified morphologically with a light microscope (LM) (ZAFRA et al., 2010; BOTTARI et al., 2015; SCHWERTZ et al., 2016; GROSSKOPF et al., 2017) and were confirmed as *E. coelomaticum* based on LM according to the size of the suckers and of the body, the specimens had a total body length of 4.43 to 8.95 mm and width of 2.20 a 4.91 mm and the subterminal oral sucker and ventral sucker placed in the forebody (LEITE et al. 2020).

The data were analyzed using the SAS software (Analysis System Institute, Cary, NC, USA, version 9.4). In order to analyze the relationship between the number of *Eurytrema* sp. with blood variables, the data were evaluated using Spearman's correlation analysis (PROC CORR). The degree of parasitemia was defined according to the multiple regression model described in BELÉM et al. (1992a), considering the number of *Eurytrema* sp.,: non-parasitized: 0; low parasitemia group: from 1 to 63; and high parasitemia group: > 95. Since no animal had a count between 63 and 95, the group described by the authors as intermediate was excluded.

The comparison among groups was performed using analysis of variance procedures, MIXED (triglycerides – variable with parametric distribution) and NPAR1WAY (other blood variables – nonparametric distribution). The means of the groups were compared using Tukey's test at a 5% significance level. For the gelatin hydrolysis and x-ray film digestion tests, the chi-square test was used at a 5% significance level. Logistic regression analysis (PROC LOGISTIC) and the odds ratio calculation were carried to identify the odds ratio of the low and high parasitemia groups to present changes in blood variables concerning the non-parasitized group.

RESULTS AND DISCUSSION

Initially, the animals were classified as parasitized or non-parasitized by *Eurytrema coelomaticum*, with 71 positive and 48 negative cases, resulting in a frequency of 59.66%. The mean number of specimens per infected pancreas was 628 parasites, ranging from 6 to 3,829.

A study carried out in the central-western region of the state of Paraná observed a prevalence of almost 50% of the animals parasitized by *E. coelomaticum*, with variations between 26.9% and 72.9% in different locations (BASSANI et al., 2006). SILVA JÚNIOR (2017) demonstrated an average prevalence of 20.7% in different cities in the southern region of the state of Minas Gerais. BELÉM et al. (1992a) detected an infection rate of 8.5% in the southwestern mesoregion of São Paulo. SCHWERTZ et al. (2016) observed a prevalence of 63.5% of the parasitized animals in the region of Concórdia (Santa Catarina), a value very similar to that of this study. The prevalence rate may change in different regions mainly due to rainfall variations, the population of *Bradybaena similaris*, and intermediate host, as air and soil humidity are determining factors for the reproduction of the terrestrial mollusk (BASSANI et al., 2006; BASSANI et al., 2007).

The exocrine pancreatic function tests, x-ray film digestion and gelatin hydrolysis were performed with the collected feces. Most of the non-parasitized animals tested positive for the presence of digestive enzymes, in both x-ray film digestion (95.8%) and gelatin hydrolysis (93.7%) tests. As seen in table 1, there was no statistical difference between the tests performed ($P > 0.05$).

When non-parasitized animals were compared with those with low parasitic load, similar results were observed for both tests. However, there was a greater (statistically significant) number of negative tests in animals with a high parasitic load, for both the x-ray film digestion (63.7%) and gelatin hydrolysis (52.8%) tests ($P < 0.001$). Moreover, a difference was verified in the x-ray film digestion test

results between animals with low and high parasitic loads ($P < 0.001$), demonstrating that the results for both tests change only if the number of parasites is significant (Table 1).

SAKAMOTO et al. (1980) evaluated the x-ray film test for the presumptive diagnosis of infections by *Eurytrema* sp. After verifying that seven out of eight animals proven to be infected had negative results, the authors began to recommend the test as a screening method for diagnosis. Nevertheless, the tests were carried out at room temperature and analyzed up to 24 h after incubation. BELÉM et al. (1992b) tested fecal samples from 29 parasitized bovines and 19 healthy animals (non-parasitized control) and demonstrated that the results of both groups were similar for the x-ray film test. Therefore, even though they did not perform the test referent to the parasitic load, the authors concluded that the test should be disregarded for presumptive diagnosis of infections by *Eurytrema* spp. in cattle.

Results detected in this study confirmed that there is a change in the exocrine secretion of the pancreas in cattle with a high parasitic load, as approximately 52.8% of the animals presented negative results in the gelatin hydrolysis test and 63.7%, in the x-ray film digestion test, thus, demonstrating that there may be alterations in the digestibility of the food consumed by the animals. These data confirmed the observations of SCHWERTZ et al. (2016), who believe that important pancreatic morphological changes have an impact on digestive processes and; consequently, animal production. Similarly, WHITLEY (2014) described fibrosing pancreatitis as a chronic inflammatory process, characterized by irreversible destruction of the architecture of the parenchyma and ductal system, production and increase in fibrous tissue, which can result in loss of exocrine and endocrine functions.

In order to assess the exocrine pancreas, the activity of the enzymes amylase and lipase were determined in blood samples, being significantly higher ($P < 0.05$) in animals with low parasitemia (119.3±87.8 and 23.8±14.6), compared to non-parasitized animals (56.0±30.2 and 14.4±4.6) and those with high parasitic load (77.5±29.0 and 17.9±11.4). In animals with amylase and lipase above the reference values proposed for the species, renal function evaluation was performed to rule out possible interference of renal problems on the results of the tests performed. Mean values detected for urea and creatinine were 31.9±7.5 mg/dL and 1.1±0.3 mg/dL, respectively. Normal value ranges for the species, according to KANEKO et al. (2008), are from 20 to 30 mg/dL for urea and from 1 to 2 mg/dL for creatinine.

These results demonstrate that, initially, with low parasitic load, pancreatic injury and extravasation of enzymes may occur; however, if the number of parasites in the pancreas increases dramatically, with the destruction of the organ and the fibrosis of the pancreatic tissue, the level of serum enzymes returns to normal. This increase in the activity of the enzymes amylase and lipase in cattle in the initial stage of the disease is modest, when compared to that of an acute pancreatitis in other species, such as dogs, during which the enzymes may increase up to ten times their reference values. Nevertheless, such a modest increase was expected, since pancreatic enzymes are not so significant in bovines, (STOCKHAM & SCOTT, 2011).

The pathogenesis of acute pancreatitis is complex and includes the intra-acinar activation of digestive enzymes. This results in the degeneration and necrosis of acinar cells which, in turn, lead to self-digestion of the pancreatic parenchyma and an intense concomitant inflammatory response (WHITLEY, 2014). In general; however, diseases in which secretion abnormalities occur are not readily identified in cattle. These are recognized only as changes in motility, since the digestive system

Table 1 - Comparison between the x-ray film digestion and gelatin hydrolysis tests considering non-parasitized animals and animals with low and high parasitemia.

Groups	----------X-ray film digestion test----------		----------Gelatin hydrolysis test----------	
	Positive n (%)	Negative n (%)	Positive n (%)	Negative n (%)
NP	46 (95.8%)	2 (4.2%)a	45 (93.7%)	3 (6.3%)a
Low PL	15 (93.7%)	1 (6.3%)ac	12 (75.0%)	4 (25.0%)ab
High PL	20 (36.3%)	35 (63.7%)bc	26 (47.2%)	29 (52.8%)b

Different lower-case letters in the column indicate statistical difference ($P < 0.001$) by chi-square test.
Legend: Parasitic load (PL) and non-parasitic (NP).

depends on motor and secretory functions and, in herbivores, on the microflora activity in the pre-stomach compartments of ruminants (RADOSTITS et al., 2002) which may compensate, to some degree, the compromised secretory digestion.

SAKAMOTO et al. (1980) reported normal values for the activity of the enzyme amylase, similar to GASTE (1991), who evaluated 26 bovines with a minimum load of 700 *Eurytrema coelomaticum*. BURGGRAAF (1933) investigated the pancreatic function of cattle infected by *Eurytrema* sp. and demonstrated that, in cases of severe injuries, there is a decrease in or absence of trypsin and amylase in the pancreatic juice.

With the present study, it was possible to prove the association between the parasitic load and the alteration of the amylase and lipase enzymes. Other previous studies have failed to identify an increase in pancreatic enzymes because they did not associate it with the number of parasites (SAKAMOTO et al., 1980; GASTE, 1991).

For the evaluation of the endocrine pancreas, glucose and fructosamine values were evaluated, but the latter did not show any difference between the groups studied. Regarding glucose, all groups had mean values above the reference for the species. This hyperglycemia can be explained by the release of adrenaline due to pre-slaughter stress (STOCKHAM & SCOTT, 2011). Considering eurytrematosis, BURGGRAAF (1933) described an increase in the glucose values only in cases of very severe injuries, when there is a decrease in the number of islets of Langerhans.

Serum cholesterol and triglyceride concentrations were also analyzed to evaluate hyperlipidemic disorders that accompany worm-induced pancreatitis, but there was no difference between the groups studied ($P > 0.05$). GASTE (1991) also reported not finding significant differences in cattle parasitized by *Eurytrema coelomaticum* regarding total lipids, cholesterol, and triglycerides.

Spearman's correlation of the biochemical tests was calculated and only amylase and total protein correlated with the number of parasites observed in the pancreas, with values of $R = 0.238$ ($P = 0.008$) and $R = 0.190$ ($P = 0.038$), respectively. In order to complement this analysis, logistic regression was performed with the calculation of the odds ratio (OR), as seen in table 2.

The OR of approximately 0.4 indicated that there is a 40% lower chance of the amylase enzyme being altered in animals with high parasitic load of *Eurytrema* sp. than in the control group (non-parasitized). That is, in 60% of cases, when there is high parasitemia, there is a greater possibility of hyperamylasemia. Whereas, in the group with low parasitic load, the OR of 0.18 means that there is an 18% lower chance of the amylase enzyme being altered and, therefore, the animals have an 82% chance of having hyperamylasemia when compared to those non-parasitized. When assessing high and low parasitic loads, the confidence interval (0.811 to 5.957) included 1, which means that the association is not statistically significant, and the results may be due to chance.

Regarding the lipase enzyme, animals with low parasitemia are 3.646 times more likely to

Table 2 - Odds Ratio and Wald confidence intervals.

Biochemicals	Groups	Estimation	-------95% Confidence Limit------	
Amylase	Low PL x High PL	2.199	0.811	5.957
	High PL x NP	**0.397**	**0.200**	**0.788**
	Low PL x NP	**0.181**	**0.064**	**0.512**
Lipase	Low PL x High PL	**3.646**	**1.322**	**10.058**
	High PL x NP	**0.438**	**0.221**	**0.865**
	Low PL x NP	**0.120**	**0.041**	**0.347**
Total Protein	Low PL x High PL	0.776	0.289	2.083
	High PL x NP	**0.361**	**0.182**	**0.719**
	Low PL x NP	0.466	0.169	1.281
Globulins	Low PL x High PL	0.947	0.353	2.542
	High PL x NP	**0.437**	**0.221**	**0.863**
	Low PL x NP	0.461	0.168	1.268

Numbers in bold are statistically significant ($P < 0.05$).
Legend: Parasitic load (PL) and non-parasitic (NP).

have hyperlipasemia than those with a high parasitic load. Since the 95% confidence level value (1.32 to 10.05) did not include 1, we can concluded that this OR is statistically significant. Moreover, when the lipase value of highly parasitized animals was compared with that of non-parasitized, the latter are approximately 56% more likely to have an increase in the lipase enzyme, and when that value is compared to the group with low parasitemia, the odds ratio changes to 0.12, which indicated that there is an 88% chance that these animals have hyperlipasemia.

An OR of 0.36 indicated that the chance of total serum protein being altered in animals with a high parasitic load of *Eurytrema* sp. is 36% lower than the chances of finding this change in the control group (non-parasitized). Thus, in 64% of cases, when there is high parasitemia, there is the possibility of hyperproteinemia. Furthermore, this increase is more likely to occur due to hyperglobulinemia because, with an OR of 0.43 and a confidence interval of 0.221 to 0.863, this indicates that there is a 57% chance of an increase in globulins.

BELÉM et al. (1994) quantified the degree of injury to the bovine pancreas and the average parasitic load and classified them from I to IV. By analyzing their findings, it was possible to better correlate the changes detected in this study. Grades I and II mean that the lesions are still preliminary, but already apparent, with a slight change in the pancreatic enzymes (amylase and lipase). When the lesions reach grade III or IV, the large number of parasites promotes fibrosis, and the enzymatic changes disappear. Moreover, changes in pancreatic digestive enzymes in the stool appear only when there are severe changes in the pancreatic parenchyma.

CONCLUSION

When evaluating Eurytrematosis in the Concórdia region, Santa Catarina, Brazil, there was an absence of pancreatic digestive enzymes in the feces of highly or moderately parasitized animals. In addition, elevated serum levels of lipase and amylase were seen in animals with low burden, which also indicated the disease is compromising pancreatic exocrine functions.

SOURCES AND MANUFACTURES

Prietest TOUCH®: ROBONIK, Navi Mumbai, India.

EPIDAT 4.2 (Dirección Xeral de Saúde Pública, Xunta de Galicia, Spain).

BIOETHICS AND BIOSECURITY COMMITTEE APPROVAL

The Ethics Committee on Animal Use (CEUA) of the IFC campus Concórdia (Santa Catarina, Brazil) approved the methodology of this study under protocol No. 01/2020.

ACKNOWLEDGEMENTS

Authors are grateful to Frigorífico São Clemente, as well as IFC for funding this work.

DECLARATION OF CONFLICT OF INTEREST

We have no conflict of interest to declare.

AUTHORS' CONTRIBUTIONS

All authors contributed equally for the conception and writing of the manuscript. All authors critically revised the manuscript and approved of the final version.

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