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Reliability of coprological diagnosis of *Paramphistomum* sp. infection in cows

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Abstract

A modified MacMaster method was tested to check its reliability for the diagnosis of bovine paramphistomosis in France. A total number of 148 fecal samples from cows examined post-mortem were analysed. Coprological results were in accordance with necropsic examinations. Bayesian techniques (Markov Chain Monte Carlo) were used to estimate the diagnostic parameters of each of these tests. Two scenarios were envisaged: one assuming a sensitivity of the necropsic examination equal to 1 and one assuming the specificity of the coprology equal to 1. Whatever the scenarios, each test presented good estimated parameters, always superior to 0.9. A significant relationship was clearly established between epg counts and parasites burden: more than 100 epg indicated the presence of more than 100 adult paramphistomes in rumen and/or reticulum.
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1. Introduction

Paramphistomosis in cattle has a wide geographic distribution. In subtropical and tropical areas, the infection leads to economic losses related to mortality and low productivity (Kilani et al., 2003; Spence et al., 1992, 1996). In Europe, the presence of adult paramphistomes was previously regarded to be relatively harmless in cattle. However, recent reports demonstrated that paramphistome species present in Europe (*Paramphistomum daubneyi* most frequently) can cause significant gastrointestinal disease, drop in production or even death (Dorchies et al., 2002). Moreover, a recent epidemiological survey in Central France (Mage et al., 2002) demonstrated that the prevalence of natural infections with *Paramphistomum* in cattle significantly increased from 5.2% (in 1990) to 44.7% (in 1999). The apparent spread of the paramphistomosis in France has been attributed to the efficacy of fasciolosis control that leave free the intermediate host (*Galba truncatula*). The development of extensive livestock farming or the propagation of metacercariae by mechanical vectors has also been incriminated (Dorchies, 1989). The clinical diagnosis of paramphistomosis remains difficult. Immunological methods and serum antibody detection are not conclusive (Horak, 1967, 1971; Alabay, 1981; Singh et al., 1983). As a consequence the diagnosis of paramphistomosis in live animals still depends on faecal

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detection of eggs. The present study was carried out in order to assess the reliability of the MacMaster coprological method for the detection of paramphistomes eggs.

2. Material and methods

A total number of 148 cows were examined post-mortem at two slaughterhouses in Eastern France (Champagne-Ardennes Region) to establish the presence of adult paramphistomes in their rumen and/or reticulum. Qualitative estimation of the infection level was done by visual observation: level "+" corresponded to less than 100 adult paramphistomes, level "++" to a parasitic burden comprised between 100 and 1000 and level "+++" to a parasitic burden higher than 1000. Faecal samples were collected from 60 infected animals (20 from each infection level) and from 88 from which no paramphistome could be detected. Twelve out 88 non-infected cows presented liver flukes infection (*Fasciola hepatica* or *Dicrocoelium lanceolatum*). All samples were sent to the laboratory of Parasitology of the veterinary College of Toulouse (ENVT). They were examined by the MacMaster method, modified by Raynaud (1970): 3 g of faeces were mixed with 42 mL of potassium iodomercurate ($d = 1.44$), homogenised and poured through a strainer. The MacMaster chambers were filled with a 1 mL sample (0.5 mL per chamber) and searched for eggs. The number of eggs counted inside the squared spaces of the two chambers was multiplied by 50 to obtain the value of mean eggs per gram (epg). The rest of the preparation was submitted to a total flotation: a tube was completely filled until obtaining a convex meniscus and recovered with a cover glass (22 mm × 22 mm); after 10 min, the cover glass was removed and put on a slide. If no egg was found in the MacMaster, this slide was searched for eggs. Whatever the number of eggs may have been, the result was conventionally 7 epg. For logistic reasons, coprological examinations could not be made independently from necropsic observations.

To assess a possible correspondence between epg counts and necropsic examinations, taken as a reference test, respective results were compared by the MacNemar chi 2 test and the evaluation of kappa coefficient (Kraemer et al., 2002). As none of the two tests can be considered as a gold standard, we could not use directly the results from one test to assess the parameters of the other one. Indeed, we cannot be certain that a lack of detection of rumen flukes is a true negative. To overpass this difficulty, we used the Bayesian approach presented by Joseph et al. (1995) for two diagnostic tests. We assumed Beta distribution for all the parameters of interest: prevalence and sensitivities and specificities of the two tests. The Gibbs sampler algorithm was performed in order to obtain posterior distributions of all the parameters. From these posterior distributions, we extracted median and 95% credible interval for each parameter of interest. The Gibbs sampler was run 25,000 cycles, the first 5000 to assess convergence and the last 20,000 for inference. For more details on the procedure, see Joseph et al. (1995). The model was run under two different scenarios: one with the sensitivity of the necropsic examination fixed to one and one with the specificity of the coproscopy fixed to one. Non-informative Beta distributions were affected to all unconstrained parameters (Beta (1.1)). Marginal prior distributions of the constrained parameters were chosen so that their probability mass equal to one on their constrained values: Beta (1.1×10^{-10}).

To assess whether a relation occurred between epg counts and real infection levels, we used an ANOVA test with the Bonferroni correction for multiple comparisons (Bland and Altman, 1995). The Box–Cox method (Box et al., 1978) was used to deal with the non-normality of the variable epg count, called y. Beforehand, the variable "epg" was rescaled with a shift parameter $c = 1$ so that it was strictly positive: $(y + 1)$.

To determine the optimal cut-off point of epg counts, which best separate the burden levels, a logistic regression was performed with the level as the dependent variable and the transformed epg count as the independent variable. The discriminatory ability of the logistic model was determined using the area under the receiver-operating characteristic curve (Greiner et al., 2000), with 1.0 and 0.5 meaning perfect and random discrimination, respectively. The probability cut point "s" was chosen so that the Youden index was maximised. For all tests, p-values less than 0.05 were considered to be statistically significant.

3. Results

No significant difference was revealed between results of post-mortem and coprological examinations ($p > 0.05$) (Table 1). The agreement between coprological and necropsic diagnosis of paramphistomosis was high ($\kappa = 0.915$).

Tests parameters for the two different scenarios are shown in Table 2. Whatever the scenario, each test presented good estimated parameters, always higher to 0.9. Constraining the sensitivity of the necropsic examination or the specificity of the coproscopy did not affect the value of the other parameters. Whatever

Table 1
Results of post-mortem and coprological diagnosis of paramphistomosis in 148 analysed cows

Detection of paramphistome eggs	Post-mortem diagnosis					Total
	Paramphistomes infection level			No paramphistomes		
	"+"	"++"	"+++"	Liver flukes	No liver flukes	
Positive	16	20	19	0	1	56
Negative	4	0	1	12	75	92
Total	20	20	20	12	76	148

Table 2
Prevalence, sensitivity and specificity of the different test procedures for the diagnosis of paramphistomosis in slaughtering cattle

Parameters	Scenario 1[a]		Scenario 2[b]	
	Median	Credible interval	Median	Credible interval
Prevalence	0.39	[0.32; 0.45]	0.40	[0.34; 0.46]
Post-mortem diagnostic				
Sensitivity	1.00	–	0.97	[0.91; 1.00]
Specificity	0.96	[0.91; 1.00]	0.96	[0.91; 1.00]
Coproscopy				
Sensitivity	0.94	[0.86; 1.00]	0.94	[0.85; 1.00]
Specificity	0.98	[0.94; 1.00]	1.00	–

[a] Scenario 1: value of sensitivity of the post-mortem diagnostic fixed to 1: marginal prior Beta distribution: Beta (1.1×10^{-10}).
[b] Scenario 2: value of specificity of the coproscopy fixed to 1: marginal prior Beta distribution: Beta (1.1×10^{-10}).

the scenario, the estimated prevalence of bovine paramphistomosis in the sample was about 40%. However, this value could not be extrapolated to the entire French bovine population as the sample was not representative.

Assuming the first scenario, we assessed the specific sensitivity of coproscopy for necropsic infection levels "+", "++" and "+++". The sensitivity was 86.02% (95% credible interval: [64.98; 99.33]%), 95.55% (95% credible interval: [84.36; 99.88]%) and 93.32% (95% credible interval: [79.36; 99.75]%), respectively, There was no significant difference between infection levels as the credible intervals overlapped each over.

The more appropriate power transformation of $(y+1)$, say $(y+1)^{\lambda}$, was found with $\lambda = 0.25$ (95% CI [0.17; 0.28]). The relation between $(y+1)^{0.25}$ and infection levels was significant (ANOVA, $p < 0.0001$). Bonferroni test revealed a significant difference between infection levels "+" and "++" ($p < 0.001$), and levels "+" and "+++" ($p < 0.001$) but not between infection levels "++" and "+++" ($p = 0.632$). For further analysis, levels "++" and "+++" were combined (level ">+").

A simple logistic regression was performed, the dependent variable being dichotomic ("level +" versus "level > +") and the independent variable being $(y+1)^{0.25}$. The class "level +" was chosen as reference. The regression coefficients are given in Table 3. The rescaled coefficient of determination of the model was $\tilde{R}^2 = 0.52$ which indicates a moderate fit of the model to the data. But the area under the receiver-operating characteristic curve was good: 0.889. The probability

Table 3
Logistic function to discriminate between burden levels "+" and "≥+" and its characteristics

	Estimates	
	m[a]	S.D.[b]
Discriminant coefficient		
Intercept	72.99	
	−4.46	1.38
$(y+1)^{0.25}$[c]	1.72	0.48
Model characteristics		
A-ROC[d]	0.89	
Sp[e]	0.90	
Se[f]	0.83	

[a] m: mean.
[b] S.D.: standard deviation.
[c] y: epg count.
[d] A-ROC: area under the receiver operating curve.
[e] Sp: specificity.
[f] Se: sensitivity.

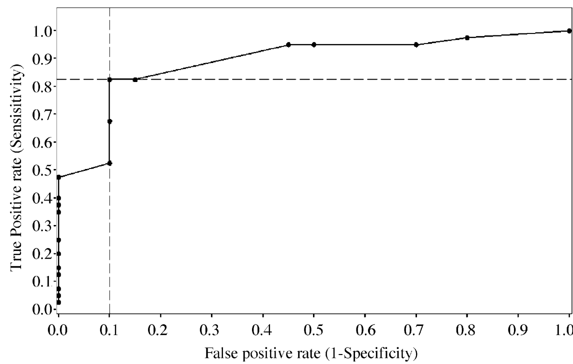

Fig. 1. ROC curve plotting the true positive rate versus the false positive rate according various cut-off value of eggs per gram for the prediction of the burden level of adult paramphistomes: less or equal to 100 ("+") and more than 100 (">+"). The inflection point of the curve corresponds to the best discriminant cut-off value; dashed lines give the corresponding true positive rate and false positive rate.

cut-point that maximised the Youden index was $s = 0.73$. So the discriminate logistic function was:

$$y \geq \exp\left[\frac{\mathrm{Ln}(\mathrm{Logit}(0.73) + 4.46/1.72)}{0.25}\right] - 1 \qquad (1)$$

with $\mathrm{Logit}(0.73) = \mathrm{Ln}(0.73/(1-0.73))$.

Then the cut-off value of epg counts which best discriminates the two burden level was 100. The ROC curve plotting the true positive rate (*sensitivity*) versus the false positive rate (*1 minus the specificity*) for various cut-off values of epg counts is presented in Fig. 1. The sensitivity and specificity associated with the cut-off value '100 epg' were 82.5% and 90.0%, respectively.

4. Discussion

In Europe, clinical signs of bovine paramphistomosis are usually limited and faecal examination remains the only way to demonstrate the presence of adult paramphistomes in live animals. The main disadvantage of the faecal egg count is the possible confusion of paramphistomes eggs with those of *F. hepatica*. However, the yellowish colour of *F. hepatica* eggs is a distinctive feature and mistakes are probably rare in veterinary analysis laboratories. We used Bayesian approach and run the model for different scenarios. Whatever the scenario, we could assume that coproscopy is a reliable test with good sensitivity and specificity, close to 1. This result is not in accordance with those reported in the literature as the sensitivity of the coproscopy is usually low. Rapsch et al. (2006) were able to show a high sensitivity for coproscopy to diagnose *Fasciola* infections in cattle if coproscopy was repeated using higher volumes of faeces. These good results should be confirmed performing the coproscopy independently from the necropsic observations.

As the size of the paramphistomes burden is an important factor determining the degree of rumen damage and potential economic losses, the lack of correlation between epg counts and the number of adult parasites would also be a major limit for coprological diagnosis. Mage and Dorchies (1998) were the first authors to study the relationship between the paramphistomes burden and epg counts in cattle. They examined 14 beef cattle who had been exposed to a natural infection by *P. daubneyi* in France and demonstrated that light infections (1–12 rumen flukes per animal) were associated with low epg counts (from 7 to 15 epg) whereas higher infections (29 to 628 digestive flukes per animals) led to higher epg counts (mean of 41.5 epg). In the present study, a larger number of animals was examined and the relationship between epg counts and the paramphistomes burden was established in a more valid statistical way. The relation between epg count and infection level was demonstrated but should be confirmed with a blind procedure. When more than 100 epg are detected with the MacMaster coprological method, it can be assumed, with a sensitivity of 82.5% and a specificity of 90.0%, that the animal is infected by more than 100 adult paramphistomes. This is a valuable information for the therapeutic decision as pathological effects of paramphistomosis are related to high worm burden. The lack of difference between epg counts for infections levels higher than 100 paramphistomes could be explained by the decrease of laying with the increase of worm burden (Horak, 1971).

In the present study, the MacMaster method was performed with potassium iodomercurate whose cost and toxicity are limiting factors for a routine use in veterinary practice. Considering environmental preoccupations, the use of potassium iodomercurate is now prohibited in several countries (including France since 2005). The difficulties with coprological methods call for the development of more sensitive methods to increase the knowledge of *Paramphistomum* infection in cattle and its actual economic impact. Coproantigen detection can be an interesting option to diagnose bovine paramphistomosis in the same way that some tapeworms are now diagnosed in humans and dogs (Allan et al., 1996; Deplazes et al., 1999) and *F. hepatica* in cattle with very high specificity (Abdel-Rahman et al., 1999).

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