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Diagnosis of bovine dictyocaulosis by bronchoalveolar lavage technique: A comparative study using a Bayesian approach

Thibaut Lurier[a,*], Marie Laure Delignette-Muller[a], Benoit Rannou[b], Christina Strube[c], Marie-Anne Arcangioli[d], Gilles Bourgoin[a]

[a] Université de Lyon, Université Lyon 1, CNRS, VetAgro Sup, UMR 5558, Laboratoire de Biométrie et Biologie Evolutive, F-69622 Villeurbanne, France
[b] Université de Lyon, VetAgro Sup, 1 Avenue Bourgelat, BP 83, F-69280 Marcy l'Etoile, France
[c] Institute for Parasitology, Centre for Infection Medicine, University of Veterinary Medicine Hannover, Buenteweg 17, D-30559 Hannover, Germany
[d] Université de Lyon, VetAgro Sup, Anses, UMR Mycoplasmes des Ruminants, F-69280 Marcy l'Etoile, France

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ABSTRACT

Bovine dictyocaulosis is a pulmonary parasitic disease present in temperate countries, with potential important clinical and economic impacts. The Baermann technique is routinely used despite its low sensitivity in adult cows. Recently developed serological tests seem to offer better sensitivity, but validations of these tests in field conditions are few. We aimed to study two non-previously evaluated diagnosis methods of dictyocaulosis based on bronchoalveolar lavage sampling (BAL), which allows finding lungworm stages in the lungs as well as determination of eosinophilia. We compared them to the Baermann technique and serological tests. As no gold standard was available, we performed a Bayesian analysis by the simultaneous use of latent class and mixture models. The study was carried out during the 2015 pasture season on 60 adult cows originating from 11 herds with clinical signs of dictyocaulosis, and 10 apparently healthy cows originating from the teaching herd of VetAgro Sup, in France. Prevalence of infection was highly variable among herds with clinical signs (10–90%). Despite a maximal specificity (100%), the sensitivity of parasitological methods was low (7.4% for the Baermann sedimentation and 24.7% for the examination of BAL fluids). Better results were observed with serology (Se = 74.9%, Sp = 85.5%) with an optimal cut-off value estimated at 0.397 for the optical density ratio. Even better results were obtained with the count of eosinophil in BAL (Se = 89.4%, Sp = 85.2%) with an optimal cut-off value estimated at 4.77% for the eosinophil proportion. The BAL is a relevant diagnostic method of dictyocaulosis for practitioners due to the opportunity to perform two analyses (direct parasitic research and the eosinophil count) and to its good sensitivity and specificity.

1. Introduction

Parasitic bronchitis caused by *Dictyocaulus viviparus* is a clinically and economically important parasitic disease in temperate countries. In Europe, recent studies in dairy cattle revealed the circulation of this parasite in at least 17.1–64.5% herds (Schunn et al., 2013; Bloemhoff et al., 2015) with an individual prevalence ranging from 6.0 to 13.75% in infected herds (Borgsteede et al., 2000; Chartier et al., 2013). The disease occurs during the pasture season and main clinical signs are coughing, nasal discharge and severe dyspnoea resulting in increased mortality in the herd and important economic losses for the farmer (Holzhauer et al., 2011). *Dictyocaulus viviparus* has a direct life cycle with cows being infected by swallowing third-stage larvae (L3) present on contaminated pastures. Historically, first grazing season animals were clinically the most affected by this parasite. However, since the 1990's, the epidemiology of dictyocaulosis changed in dairy herds with an increasing occurrence of outbreaks in older animals, mainly explained by weak contact with the parasite during previous years of life (Ploeger, 2002; van Dijk, 2004).

The routine diagnostic method used in veterinary clinics and diagnostic laboratories is the Baermann method, based on the isolation and morphological identification of larvae in the faeces (Baermann, 1917). This method has a high sensitivity (100%) for primary infection in young animals (Eysker, 1997), but it seems to be lower for older animals (Eysker, 1997; Ploeger et al., 2012). Different hypotheses have been proposed to explain this change of sensitivity with age, such as the higher dilution of larvae in the faeces of adults and the previously acquired immune response, limiting the development of *D. viviparus* to the adult stage and the egg-laying in the lungs (Eysker, 1997; Ploeger et al., 2012). The low sensitivity of this method to diagnose dictyocaulosis in

* Corresponding author.
E-mail address: thibaut.lurier@vetagro-sup.fr (T. Lurier).

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clinically affected adult dairy cattle reveals the need to develop more sensitive tools for practitioners.

Since 1993, two serological tests have been developed, both based on the detection of specific antibodies against adult lungworms by ELISA. In a first approach, a low molecular-weight fraction enriched for a 17 kDa protein, close to the major sperm protein (MSP), was extracted from adult lungworms and used as antigen (de Leeuw and Cornelissen, 1993). Later, an ELISA based on recombinant expressed MSP was developed (Von Holtum et al., 2008). These tests were promising due to their high sensitivity and specificity (> 99%) during patent infections as determined from experimental infections. However, recent studies under field conditions, using the Baermann technique as a gold standard, demonstrated lower sensitivity and specificity of the method developed by Von Holtum et al. (2008) with seasonal variations (Se = 58.7% and Sp = 94.9% in spring and Se = 80% and Sp = 83.7% in autumn; Ploeger et al., 2012, 2014). Considering the observed differences in the diagnostic abilities of these methods between experimental and field conditions and the potential biases associated with the use of the weakly sensitive Baermann test as a gold standard, further evaluations under field conditions are necessary.

Due to the pulmonary location of most developmental stages of *D. viviparus*, bronchoalveolar lavage (BAL) could be a promising method for the diagnosis of dictyocaulosis. The analysis of collected BAL fluids (BALF) may allow both direct and indirect diagnosis of dictyocaulosis: the observation of macroscopical lungworm stages (Hagberg et al., 2005; Holmgren et al., 2014) and the measurement of BAL's eosinophil proportion (EP). The latter measure points out the local immune response developed by the host when the parasite arrives into its lung (Schnieder and Daugschies, 1993; Hagberg et al., 2005; Holmgren et al., 2014). To our knowledge, BAL has only been used to characterize the immune pulmonary response of cattle to *D. viviparus* (Schnieder and Daugschies, 1993; Hagberg et al., 2005; Holmgren et al., 2014), but tests on its suitability to diagnose parasitic bronchitis are lacking yet. On healthy calves, eosinophil proportion represents less than 1% of the total pulmonary white cells (Trigo et al., 1984; Taylor et al., 1989; Hagberg et al., 2005), while it increases to 20–50% during primary infection and up to 70-80% during secondary infection (Schnieder and Daugschies, 1993; Hagberg et al., 2005; Holmgren et al., 2014). This eosinophilia appears as of 2 weeks after infection and remains for at least 12 weeks without re-infection (Schnieder and Daugschies, 1993; Hagberg et al., 2005; Holmgren et al., 2014). To our knowledge, there is no other disease which leads to pulmonary eosinophilia in cattle except when an acute hypersensitivity reaction occurs, *e.g.* after fungal infections with *Micropolyspora faeni* (Wilkie, 1978; Gershwin et al., 1990). Nevertheless, the epidemiology of these infections differs from dictyocaulosis as they are observed mostly in animals housed during wintertime.

None of the potential diagnostic methods could be considered as a gold standard as the true status of each animal remains uncertain, except when the parasite is detected by the Baermann method and/or in the BALF. In this context, we could use a latent class model to estimate the sensitivity and the specificity of each test by maximum likelihood (MLE) (Hui and Walter, 1980) or within a Bayesian framework (Enøe et al., 2000; Chaka et al., 2015; White et al., 2016). Serology and pulmonary eosinophilia supply quantitative results for which cut-off values need to be estimated during natural infection (previously estimated only for serology after experimental infections; Von Holtum et al., 2008). Estimating such cut-off values could be done using mixture models, as it has been widely suggested for serological diagnosis either by MLE (Greiner et al., 1995; Gay, 1996) or within a Bayesian framework (Nielsen et al., 2007; Opsteegh et al., 2010; Peel et al., 2013).

The present study aims to compare the four diagnostic methods (Baermann technique, serology, detection of the parasite and eosinophil proportion in the BALF) for detecting infected adult cows (clinical and subclinical dictyocaulosis) in herds with clinical signs of parasitic bronchitis. Bayesian approach is proposed to estimate the prevalence of infection in each herd, the cut-off values for the two quantitative methods (serology and pulmonary eosinophilia) and the characteristics of the four methods (sensitivity and specificity). This approach is based on a model simultaneously describing the results obtained with the four diagnostic methods using both latent class and mixture model principles.

2. Materials and methods

2.1. Study design and animals

The study is a prospective field study performed in France during the 2015 pasture season, with three veterinary practices near the veterinary school of Lyon (VetAgro Sup). The treating veterinarians, originated from five veterinary clinics near Lyon (France), reported pasturing dairy herds supporting presumption of dictyocaulosis with an enzootic coughing which led the farmers to consult a veterinarian (at least 30% of coughing cows). Eleven herds were reported and identified by a number from 1 to 11. Once we trapped all the cows from the herd at the feeding fence, five or six individuals were randomly sampled in each herd to get a representative sample of the herd (including clinically healthy and primiparous cows) and no more than 5 or 6 to ensure of the farmer approval. Ten additional cows from the teaching herd of VetAgro Sup, without clinical sign, were included in the study (the 12th herd). This last herd was included to have a sufficient diversity of herd prevalences in order to insure the model convergence. Even if this herd does not fit the inclusion criteria (herds with enzootic couching), we assume that cows originated from this herd are similar to non-clinical and non-infected cows originated from the other herds. The influence of the inclusion of this herd was evaluated by repeating the analysis without this herd. We collected blood samples, faeces and bronchoalveolar lavage fluids (BALF) on each sampled cow. The experimental protocol was approved by the ethical comity of VetAgro Sup (n° 1539-2015).

2.2. Bronchoalveolar lavage technique

BAL was performed on non-sedated animals. After cleaning the nostril (chlorhexidine solution diluted at 2%), a naso-tracheal tube (Cook®) was passed to the lungs. Two 60 ml aliquots of 0.9% sterile NaCl solution were introduced by the catheter and 30–40 mL of fluid was re-aspirated immediately. The collected fluid was then split into two 4 ml EDTA tubes for cytological analysis and the remaining fluid was placed in a sterile container for parasitological examination. Samples were stored 24–48 h at 4 °C before laboratory analyses.

2.3. Cytology

We pipetted 200 µl of homogenized BALF from one of the EDTA tubes to prepare one cytocentrifugated smear (10 min, 106 g) which was automatically stained (Aerospray 7150, Wescor®). We then assessed the eosinophil proportion (EP) by performing a differential count on at least 400 leucocytes on each cytospin.

2.4. Parasitological tests

Two parasitological tests were performed, (1) the Baermann sedimentation technique on faecal samples (BS test) and (2) the visual examination of the BALF for last stages of *D. viviparus* (BAL test). Faeces were sampled directly from the rectum of each selected cow and stored at 4 °C. Within 12 h after collection, we placed 50 g of faeces in a sieve (1 mm mesh size) with a standard gauze compress at the surface of the water on a funnel. After 24 h, we collected and centrifuged (5 min, 700 g) the first 12 ml of water at the bottom of the funnel, and performed microscopic examination (×25–40 magnification) of the

sediments. As it allows the isolation of *D. viviparus* larvae, the BS test could only diagnose patent infection with adult breeding parasites in the lungs. The two aliquots of BALF were directly examined macroscopically for the presence of lungworm stages. As this method could potentially detect any pulmonary stages of the parasite, we assume that it could diagnose both patent and pre-patent infection. Given that the identification of the larvae or adult *Dictyocaulus viviparus* is reliably feasible (Eysker, 1997), and as regard to the definition of the infection status (*i.e.* non infected, infected by *D.viviparus* and infected by breeding *D.viviparus*), we assumed the specificity of the two parasitological tests to be equal to one.

2.5. MSP Elisa

The ELISA analysis was performed at the Institute for Parasitology of the University of Veterinary Medicine Hannover (Germany) according to the protocol previously described by von Holtum et al. (2008). All sera were kept at −80 °C until analysis. This ELISA developed by von Holtum et al. (2008) uses the major sperm protein from adult male lungworms as recombinantly expressed antigen. Thus, it could only diagnose patent infection. Results were expressed as optical density ratios (ODRs).

2.6. Statistical analysis

We built a global model to describe all the available data for each animal of the twelve herds. The unknown infection status of the j th animal sampled in the i th herd, denoted $Sinf_{ij}$ is equal to 1 for infected and 0 for non-infected cows. It is supposed to have a Bernoulli distribution, with a Bernoulli parameter $Pinf_i$ being the prevalence in the herd. This prevalence is unknown but supposed to be dependent of the herd.

$$Sin\ f_{ij} \sim Bern(Pin\ f_i)$$

Given that the BS and the serology only diagnose patent infection, we also defined the unknown patent status of the j th animal sampled in the i th herd, denoted $Spatent_{ij}$. It is equal to 1 for patent cows and 0 for pre-patent and non-infected cows. It is supposed to have a Bernoulli distribution among infected animals, with a Bernoulli parameter $Ppatent$ being the overall proportion of patent animals among infected animals that can be defined as a function of the infection status:

$$\text{If } Sin\ f_{ij} = 0, \quad Spatent_{ij} = 0$$
$$\text{If } Sin\ f_{ij} = 1, \quad Spatent_{ij} \sim Bern(Ppatent)$$

Given that the identification of the larvae or adult of *Dictyocaulus viviparus* is reliably feasible, and as regard to the definition of this infection status (*i.e.* presence or absence of infection), the specificity of the two parasitological tests is assumed to be equal to one, and their respective sensitivities are denoted Se^{BS} and Se^{BAL}. Note that the model also includes $Seth^{BS}$ the theoretical sensitivity based on the diagnosis of patent infected animals and not all infected animals simply linked to the true sensitivity (Se^{BS}) by $Se^{BS} = Seth^{BS} \times Ppatent$. The results of those tests on each animal, denoted BS_{ij} and BAL_{ij} (1 if the test is positive, 0 in the other case), can simply be expressed as a stochastic function of sensitivities of the test and of the patent or infection status:

$$\mathrm{BS}_{ij} \sim \mathrm{Bern}(Seth^{BS} \times Spatent_{ij})$$
$$\mathrm{BAL}_{ij} \sim \mathrm{Bern}(Se^{BAL} \times Sinf_{ij})$$

Concerning the result of the MSP-ELISA, expressed as ODR and denoted ODR_{ij}, conditioned on the latent patent status $Spatent_{ij}$, the model assumes that the decimal logarithm of the ODR follows a normal distribution, where the mean μ_{ij} and standard deviation σ_{ij} are controlled only by the patent status:

$$\log 10(ODR_{ij}) \sim N(\mu_{ij}, \sigma_{ij})$$

The mean of the normal distribution for patent animals was supposed to be greater than that of non-patent animals (μ_{non_patent}), and parameterized by θ, the positive difference between these two means ($\mu_{patent} = \mu_{non\_patent} + \theta$). This parameterization was chosen to prevent the classical un-identifiability of any mixture model (is group 1 the patent or the non-patent group?). Standard deviations for patent (σ_{patent}) and non-patent (σ_{non_patent}) groups were not supposed equal.

The results of the eosinophil count were also modelled in close way by a Gaussian mixture with two components representing infected and

Table 1
Model parameters with corresponding prior distributions and posterior estimates.

	Parameter	Prior distribution	Posterior estimate Median [95% credible interval]
Test sensitivities	Se^{BS}	Unif(0, 1)	0.074 [0.017; 0.194]
	Se^{BAL}	Unif(0, 1)	0.247 [0.119; 0.429]
Herd prevalences	P_1	Unif(0, 1)	0.395 [0.035; 0.843]
	P_2	Unif(0, 1)	0.331 [0.0147; 0.856]
	P_3	Unif(0, 1)	0.797 [0.28; 0.991]
	P_4	Unif(0, 1)	0.665 [0.153; 0.982]
	P_5	Unif(0, 1)	0.888 [0.536; 0.996]
	P_6	Unif(0, 1)	0.727 [0.337; 0.957]
	P_7	Unif(0, 1)	0.102 [0.004; 0.439]
	P_8	Unif(0, 1)	0.903 [0.589; 0.996]
	P_9	Unif(0, 1)	0.596 [0.172; 0.928]
	P_{10}	Unif(0, 1)	0.392 [0.081; 0.77]
	P_{11}	Unif(0, 1)	0.311 [0.048; 0.7981]
	P_{12}	Unif(0, 1)	0.06 [0.002; 0.29]
Proportion of patent animals among infected ones	$Ppatent$	Unif(0, 1)	0.749 [0.479; 0.958]
ODR mixture parameters	μ_{non_inf}	Unif(-10, 10)	−0.587 [−0.640; −0.526]
	θ	Unif(0, 10)	0.400 [0.316; 0.471]
	$\sigma_{non\_\mathrm{inf}}$	Unif(0, 5)	0.162 [0.128; 0.213]
	σ_{inf}	Unif(0, 5)	0.123 [0.087; 0.195]
EP mixture parameters	μ'_{non_inf}	Unif(-10, 10)	−4.87 [−5.81; −4.01]
	θ'	Unif(0, 10)	3.23 [2.33; 4.12]
	$\sigma'_{non\_\mathrm{inf}}$	Unif(0, 5)	1.76 [0.94; 2.54]
	σ'_{inf}	Unif(0, 5)	1.07 [0.83; 1.45]

non-infected animals, characterized as for the previous mixture by parameters μ'_{non_inf}, θ', σ'_{inf} and σ'_{non_inf}. This mixture Gaussian distribution was used to describe the logit distribution of the eosinophil proportion (EP_{ij}) in the pulmonary leucocytes. This former variable is not directly measured but estimated on each animal from the count of EC_{ij} eosinophils among a total count of TC_{ij} leucocytes (the total count is indexed by i and j and considered as a covariate in the model as it was not exactly equal to 400 for each animal). The count of eosinophils for each animal EC_{ij} is thus modelled by a binomial distribution of size parameter TC_{ij} and probability parameter the latent variable EP_{ij}, the logit of which is defined by the Gaussian mixture:

$$EC_{ij} \sim \text{Binom}(TC_{ij}, EP_{ij})$$
$$\text{logit}(EP_{ij}) \sim N(\mu'_{ij}, \sigma'_{ij})$$

Vaguely informative uniform priors were assigned to each parameter allowing variation within a realistic range and forcing probability parameters ($Seth^{BS}$, Se^{BAL}, $Pinf_i$ and $Ppatent$) to be between 0 and 1 and θ and θ' to be positive (Table 1). Monte Carlo Markov-Chain (MCMC) techniques were used to estimate the full joint posterior distribution of parameters from prior distributions and data. Computations were performed using the JAGS software *via* the R package rjags (Plummer et al., 2016) (a corresponding R script is provided in Supplementary material both in Appendix A and in a runnable script with corresponding data). Three independent MCMC chains were run in parallel. For each chain 110,000 samples were produced. We discarded the first 10,000 as burn-in. To avoid autocorrelation, we thinned the remaining 100,000 samples by selecting one out of 20 samples, thus keeping 5000 samples per chain. We checked the convergence again by displaying MCMC chain traces and autocorrelation plots and by computing the Gelman and Rubin's statistics as modified by Brooks and Gelman (1998). For each parameter, its point estimate was defined as the median of its marginal posterior distribution, and the 95% credible interval was defined from the 2.5 and 97.5 percentiles of this distribution.

For both Gaussian mixtures, ROC curves accompanied with their 95% credibility bands were built from the joint posterior distribution using the 15,000 MCMC samples obtained by pooling the 5000 samples of each chain (Details of the computation are given in Appendix B. Supplementary material). For each of the 15,000 sampled ROC curves, an optimal cut-off was estimated by minimizing the distance to the upper left corner corresponding to sensitivity and specificity equal to one (Perkins and Schisterman, 2006). For each test the final optimal cut-off was estimated using the median of the 15,000 sampled optimal cut-off values. We then estimated the sensibility and specificity of each test at this optimal cut-off value using once again the joint posterior distribution. We also computed the posterior probabilities of each animal to be infected and to be patent using the 15,000 MCMC samples of the latent variables $Sinf_{ij}$ and $Spatent_{ij}$ in the model.

For representation of continuous observed data from serology and cytology, we used transformed data on which we assumed a mixture Gaussian distribution: we represented the optical density ratios (ODR_{ij}) in decimal logarithm and the eosinophil proportions (EP_{ij}) in logit. As some eosinophil proportions were estimated to 0, for representing data we fixed the corresponding values to an arbitrary value of -7. In fact, these values are censored and only estimated to be below the detection limit equal to 1/400 (roughly -6 in logit). This censoring was not a problem for modelling as the model directly describes the raw data (eosinophil count EC_{ij}).

We performed a posterior predictive check with the fitted model by comparing predicted and observed values of the numbers of animals in each combination of the four tests, predicted and observed cumulative distribution functions of both quantitative measures (ODR and EP values), and predicted and observed numbers of individuals above and below the chosen cut-off values. In order to check the constant accuracy of tests across population, we also repeat the analysis with the exclusion of each herd of the study one a time (Mahmmod et al., 2013). This study has been reported following the guidelines of the STARD BLMC (Kostoulas et al., 2017).

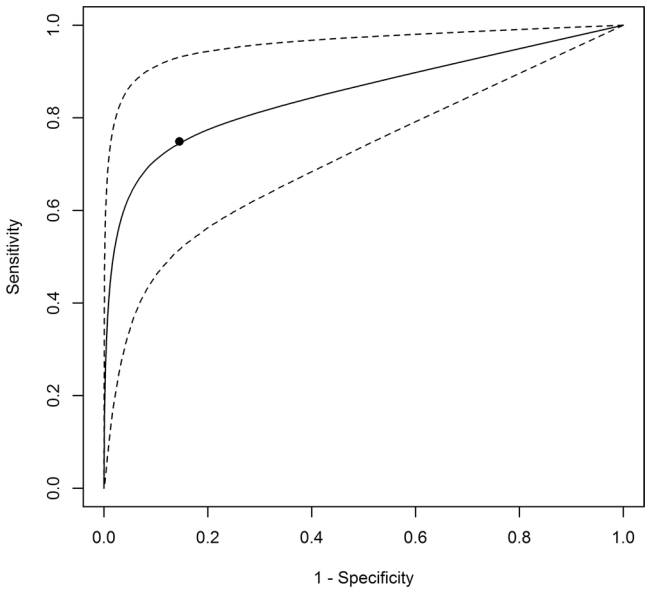

Fig. 1. ROC curve for the serology. Dotted lines represent a 95% credible interval around the ROC curve and the point on the ROC curve corresponds to the estimated cut-off of 0.397 for the optical density ratio (ODR; -0.415 in decimal logarithm).

3. Results

Prior distributions and descriptive statistics of posterior distributions (medians and 95% credible intervals) of all model parameters are summarized in Table 1 and representation of both Gaussian mixtures are shown in Fig. 3. Posterior distributions of parameters characterizing the two mixture models and the test sensitivities were highly shrunk using data in comparison to prior distribution, as it is generally expected in such an inference. The fitted model seems to present a high

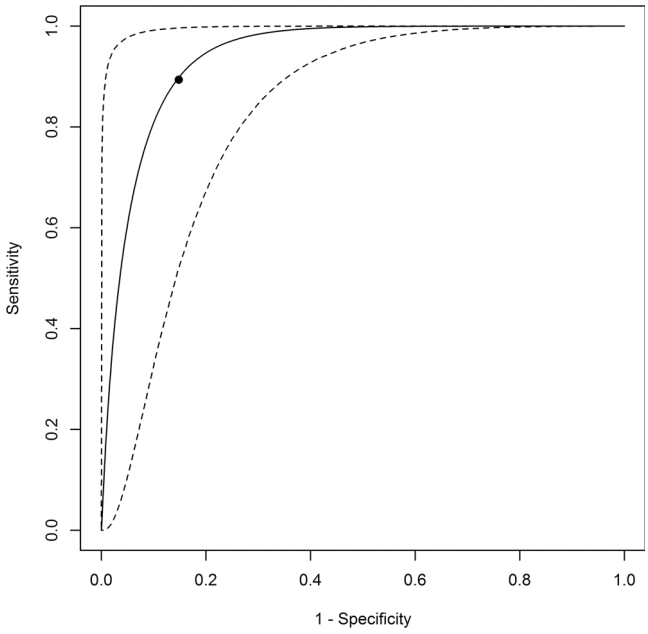

Fig. 2. ROC curve for the cytology. Dotted lines represent a 95% credible interval around the ROC curve and the point on the ROC curve corresponds to the estimated cut-off of 4.77% for the eosinophil proportion (-2.99 in logit scale).

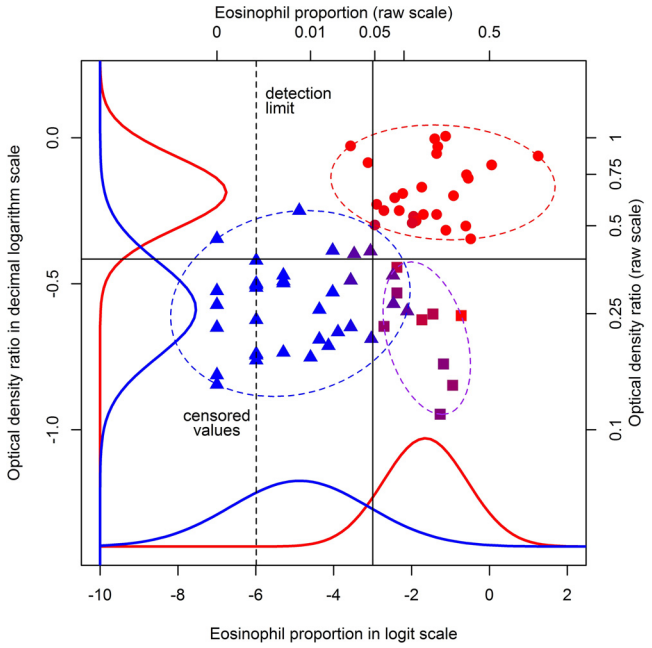

Fig. 3. Scatter plot of observed data from the serology and the cytology tests, with points coloured according the posterior probability of infection of each animal calculated from the model (colour gradient from blue for 0 to red for 1). Circular points represent animals with a posterior probability of being patent superior to 0.5, triangular points the ones with a probability of infection inferior to 0.5 and square points the ones with a probability of infection superior to 0.5 but a probability of being patent inferior 0.5. These three clusters are surrounded by ellipses respectively coloured in red, blue and purple. Red and blue curves represent the estimated density of the two components of the Gaussian mixture for each test (the red ones for positive animals and the blue ones for negative animals). Plain vertical and horizontal lines indicate optimal cut-off estimates respectively for the eosinophil proportion and the optimal density ratio. The vertical dotted line represents the detection limit for the eosinophil proportion (one eosinophil out of 400 total cells counted) (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

overall goodness of fit regarding the results of the posterior predictive check (Table C1 and –Figs. C2 and C3 in Appendix C, Supplementary material). Table C1 shows that 12 out of the 16 observed numbers of animals in each test pattern are equal to their posterior median, and none are outside of the 95% credibility interval. Similarly, the observed numbers of animals above and below the chosen cut-off values for both continuous tests match their predicted posterior modes (Fig. C1). Furthermore, Figs. C2 and C3 also point out that the two modelled Gaussian mixtures well describe the observed cumulative distribution of ODR and EP values.

Infection prevalences of herds were sometimes difficult to estimate, with 95% credible intervals (95% CI) not far from the maximal interval [0, 1] (herds 1, 2, 4, 9), while the others were more precisely estimated. The overall proportion of patent animals among infected ones was estimated at 0.75 (95% CI [0.48; 0.96]). These patent animals are those with relative high EP and ODR values (red circle points in Fig. 3). We estimated the sensitivities of parasitological tests to relatively low values: 7.4% (95% CI [1.7; 19.4]) for the BS test and 24.7% (95% CI [11.9; 42.9]) for the BAL test. Note that we detected only one or two immature lungworms (less than 2 cm) in each bronchoalveolar fluid of the eight BAL positive cows. For the serology and cytology tests, the sensitivity (Se) and specificity (Sp) depend on the chosen cut-off value. For each of these two continuous variables (log(ODR) for the serology and logit(EP) for the cytology), Se and Sp were plotted as a function of the cut-off within a classical ROC curve analysis (Figs. 1 and 2). The optimal cut-off value for serology was estimated at 0.397 ODR, corresponding to a sensitivity of 74.9% (95% CI [62.7; 79.7]) and a specificity of 85.5% (95% CI [72.1; 93.8]). We also estimated the theoretical sensitivity of the serology at the optimal cut-off, if used to diagnose only patent animals and not all the infected animals. This theoretical sensitivity was estimated at 96.9% (95% CI [80.3; 99.8]).

The optimal cut-off value for the cytology was estimated at 4.77% corresponding to a sensitivity of 89.4% (95% CI [76.9; 96.7]) and a specificity of 85.2% (95% CI [68.8; 99.4]). As shown in Fig. 3, the detection limit of the cytology (−5.99 in logit) was far lower than the optimal cut-off value (−2.85 in logit). Thus, counting 400 cells during the cytology test seemed sufficient to prevent an impact of censored values on the diagnosis. As the bronchoalveolar lavage combines two diagnostic methods, the parasite identification in the BALF and the pulmonary eosinophilia, we estimated the sensitivity and specificity of a global BAL test combining these two methods. We considered an animal positive if at least one of the two tests was positive, and negative if both tests were negative. Thus, the sensitivity of the global BAL test was estimated at 91.4% (95% CI [80.7; 97.4]) and the specificity at 85.2% (95% CI [68.8; 99.5]).

At the herd level, for the case where these methods would be applied to confirm a dictyocaulosis presumption (*i.e.* to find at least one positive animals in the herd), we calculated the number of samples required to reach a herd sensitivity of 95%. Veterinarians would have to perform a minimum of either 40 Baermann sedimentation tests (95% CI [14; 170]), 11 BAL parasitological tests (95% CI [6; 24]), 3 serology tests (95% CI [2; 4]) or 2 eosinophil proportion counts (95% CI [1; 3]). For the two last methods, the herd specificity corresponding to the probability to find at least one positive animal in an uninfected herd would drop to 62.2% (95% CI [37.3; 83.0]) and 72.7% (95% CI [47.7; 98.9]) respectively.

4. Discussion

This study evaluated the performance of four diagnostic methods for dictyocaulosis in adult cattle under field conditions and in the absence of a known gold standard test. The sensitivity and specificity of each test, as well as cut-off values for the two quantitative methods, were estimated by Bayesian inference, fitting a global model describing all the raw data collected in the experiment. One of the main benefits of such an approach is the global consideration of the four test results to estimate the individual status of each cow. Likewise, the two mixture models are fitted simultaneously but not independently, allowing a better consideration of discordant results and limiting the animal misclassification for both tests. Furthermore, the censoring of low values of eosinophil proportions is naturally included in the Bayesian approach by directly modelling the count of eosinophils, without any need of specific methods dedicated to censored data.

In a Bayesian framework, the posterior estimation depends upon both prior information and observed data. In our study, prior information was particularly poor because none of the four diagnostic methods has been characterized in the field with adult cows. Ploeger et al. (2014) determined in a field study the sensitivity and the specificity of the serology with the faecal larvae excretion (*i.e.* the Baermann technique) as reference test, while it has a poor sensitivity in adults. Similarly, there are few data about the BALF cytology in cows. Even if none of the previous publications we have had access to mentions healthy cows with an eosinophil rate at over one percent, we cannot be sure that every healthy cow has an eosinophil rate less than one percent. Therefore, all prior information was chosen only vaguely informative.

One of the assumption of latent class analysis is that all diagnostic methods measure the same infection status which is not the case in our study because Baermann sedimentation and serology require mature worms (patent infection) to be positive whereas pulmonary eosinophilia and BAL test only require presence of parasites in the lung whether they are fully developed or not (pre-patent and patent

infection). By modelling the three latent statuses (non-infected, pre-patent and patent) the latent class model remains valid. The use of multiple latent disease status is also a method allowing the consideration of conditional dependence between test (Dendukuri et al., 2009). Indeed, in our case, BS and serology could not have been considered as independent conditionally to the infection status because they both detect only patent infection. However, conditionally to the three latent disease status, the four diagnostic methods could be considered as independent because they are based on different biological principles.

The model assumes that the overall proportion of patent animals among infected animals is constant in all herds. It is an important constraint that allows an easy consideration of pre-patent and patent infections. However, this proportion might vary from one herd to another especially if the outbreak occurs at different times before the sampling or if cows are well immune against *Dictyocaulus viviparus*. The model could be improved by taking into account this proportion variability for instance using a hierarchical model but the simple proposed model already provides a good fit to the data as shown by the results of the posterior predictive check (see Table C1 and Figs. C1–C3 in the Supplementary material).

The Bayesian latent class model requires herds with different prevalences to ensure a proper convergence of the algorithm. It led us to include a clinically healthy herd with no history of dictyocaulosis (the 12th herd). Then, the assumption of constant accuracy of tests across population might not have been appropriate. We checked this assumption by repeating the analysis with the exclusion of each herd of the study one at a time and results are presented in the table D in the supplementary material. The exclusion of each herd led to slightly different point estimate of cutoff, sensitivity and specificity of each test but every credibility interval stay very close to the original estimations. In particular, the exclusion of the 12th led to quite similar point estimate but wider credibility intervals. Then the assumption of constant accuracy of test across population could be considered as reasonable.

Parasitological results are quite different compared to previous studies (Ploeger et al., 2012). Only 3.3% of cows originating from clinically positive herds were positive with the BS test, with a low level of larval excretion (less than 1 larvae per 10 g of faeces; data not shown), whereas it was 20.1% in the quoted study. The climatic conditions could partly explain these differences, as spring and summer 2015 were very dry. These particular climatic conditions may have lowered larvae survival in the environment and consequently reduced the number of larvae ingested by the cows, resulting in rather low infection intensities. Furthermore, acquired immunity during the present or previous pasture season may have prevented invasion and maturation of a large number of ingested parasites, again resulting in reduced larval excretion by infected cows. We might have found perhaps more larvae and positive cows by using the McKenna modified method of sedimentation instead of the Baermann method (McKenna, 1999), even if it could have been compensated by the use of a higher amount of faeces (50 g *vs* 30 g). As the sensitivity of faecal examination may vary according to the technique and the amount of faeces used, the estimated sensitivity of the Baermann sedimentation in this study may perhaps not be generalised to other faecal examination techniques. The sensitivity of the BS test was evaluated at 7.4% (95% IC [1.7; 19.4]) in adult cows. Identification of *D. viviparus* in the BALF (BAL test) seems to be more sensitive (Se = 24.7% (95% IC [11.9; 42.9]) than the BS test. Despite a relatively high proportion of patent animals (0.75; 95% CI [0.48; 0.96]) which should host fully developed worms, we only recovered immature *D.viviparus* in the BALF of BAL positives cows. It seems not to be due to the diameter of the lung catheter as we already recovered adult parasites (5–6 cm) with the same device during preliminary tests (*unpublished data*), but rather to the immunity of adult cows, which may slow down the *D.viviparus* development in the lungs. Then, they may not be able to reach the mature stage, which could explain the low recovery of adult worms in the BALF and also the low sensitivity of the Baermann sedimentation.

The lung cytology and serology seem to be better-suited diagnostic tools for dictyocaulosis than parasitological tests. The optimal cut-off value of the serology proposed by the model (*i.e.* 0.397 ODR) is lower than the recommended cut-off (*i.e.* 0.500 ODR) by Von Holtum et al. (2008). This model-proposed cut-off value of 0.397 ODR is probably adapted to adult cows but it seems too low for reliable differentiation between infected and non-infected calves, as in the study by Von Holtum et al. (2008) the sera from helminth-negative calves reached values up to 0.490 ODR. Otherwise, according to our model, at the cut-off indicated by Von Holtum et al. (2008), the sensitivity would be 61.2% (95% CI [40.8; 82.6]) and the specificity 96.1% (95% CI [87.8; 99.2]). As expected, these values were lower than those estimated from controlled experimental infections (Se and Sp > 99%; (Von Holtum et al., 2008)), especially for the sensitivity. Most of the differences observed on the serology characteristics between field and experimental studies must be due to the presence of pre-patent infections which reduce the observed sensitivity of the serology. A seasonal variation of the proportion of pre-patent infected animals could also explain the observed seasonal variation of the sensitivity and specificity of the serology (Ploeger et al., 2012, 2014).

To our knowledge, this study is the first to provide a cut-off (-2.99 in logit or 4.77% eosinophils in cellular proportion of BAL, designed by the model) as well as sensitivity and specificity values (89.4% with 95% CI [76.9; 96.7] and 85.2% with 95% CI [68.8; 99.4], respectively) for bronchoalveolar cytological measurement for the diagnosis of dictyocaulosis in adult cows. The proposed cut-off (4.77%) is relatively high compared to previously reported eosinophil proportion values in healthy calves (all < 1%, n = 15; Hagberg et al., 2005). The combined bronchoalveolar lavage diagnostic method (the parasite identification in the BALF and the pulmonary eosinophilia) is as specific as the pulmonary eosinophilia alone but slightly more sensitive (91.4% with 95% CI [80.7; 97.4]). This difference comes from few cows with EP values under the cut-off value despite we found parasites in the BALF.

At the herd level, we calculated the number of infected animals to sample to reach a herd sensitivity of 95%. Unfortunately, as we did not monitor the clinical status of cows, we could not predict if clinical cows were more likely to be positive than sub-clinical ones. It could be interesting to refine our analysis based on cows with known clinical status. The two parasitological methods seem to be limited, because they had to be performed on a large number of infected animals to reach 95% sensitivity. The individual serology seems to be more suitable than the parasitological methods to diagnose dictyocaulosis infection at the herd level because it only requires 3 samples to reach a herd sensitivity of 95% even if it has a low herd specificity. Collecting BAL fluid is more complicated than collecting blood or faeces, but it can be a relevant technique as it may allow diagnosing dictyocaulosis in a herd with only two samples. Moreover, the bronchoalveolar lavage combines two diagnostic methods, increasing the opportunities to diagnose dictyocaulosis. If BAL is performed on two animals suffering from dictyocaulosis in a herd, the probability of isolating *D. viviparus* in the BALF is 43% (95% CI [22; 68]) and if negative, cytological examination could be performed with a 95% probability to diagnose dictyocaulosis with an eosinophil proportion superior to 4.77%.

Performing analyses on pooled samples could be an interesting alternative to screen more animals, with limited time and financial costs, but this might be at the expense of the sensitivity. Bulk tank milk ELISA (Fiedor et al., 2009; Schunn et al., 2012) or ELISA on pooled serums (Chartier et al., 2015) could also be interesting methods of herd diagnosis. Serology is also easier than the BAL to carry out on the farm and is more suitable for large epidemiological studies. Furthermore, the serology only detects patent infections whereas BAL detects both patent and pre-patent infections. Thus, at the individual level, BAL should be preferred to confirm a dictyocaulosis presumption.

5. Conclusion

This study provides for the first time a combined estimation of the characteristics of different commonly used methods to diagnose dictyocaulosis, including the first evaluation of the BAL technique. Overall, we confirmed the rather low sensitivity of the Baermann technique in adult cows (7.4%). Furthermore, bronchoalveolar lavage proved to be a suitable technique to confirm a dictyocaulosis suspicion at the individual level in dairy cows. This technique is interesting as it combines two complementary and relevant diagnostic methods: first, the BAL parasitic test which can potentially provide a quick result on the farm, and second, the eosinophil proportion, a highly sensitive diagnostic measure of dictyocaulosis at the veterinary office. Despite a lower sensitivity due to pre-patent infections, the serology remains a suitable method to confirm a hypothesis of dictyocaulosis in adult cow herds and it is more practicable for large epidemiologic studies.

Conflict of interest

No conflict of interest to be declared.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.prevetmed.2018.03.017.

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