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Estimating the true prevalence of *Fasciola hepatica* in cattle slaughtered in Switzerland in the absence of an absolute diagnostic test

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Abstract

A survey of 1,331 cattle presented for slaughter at two abattoirs in Switzerland was used to estimate the true prevalence of *Fasciola hepatica* infection and the diagnostic parameters of visual meat inspection, coproscopy after sedimentation technique, a commercial ELISA test for specific antibody detection in serum and the post mortem microscopic detection of eggs in bile. Faeces, blood and the gall bladder were taken from most cattle presented for slaughter. In addition, livers that were rejected by the meat inspectors were also dissected to examine for the presence of liver fluke. Bayesian techniques (Markov Chain–Monte Carlo) were used to estimate the diagnostic parameters of each of these procedures and the true prevalence of bovine fasciolosis. The true prevalence of *F. hepatica* infection was estimated at 18.0% (95% credible intervals 15.9–20.3%). The diagnostic sensitivity of coproscopy, bile examination, antibody ELISA and meat inspection were estimated at 69.0% (57.3–79.7%), 93.4% (88.0–97.5%), 91.7% (87.2–95.2%) and 63.2% (55.6–70.6%), respectively. The diagnostic specificity of the ELISA test was estimated at 93.7% (91.7–95.2%). These results demonstrate that the prevalence of bovine fasciolosis is higher than previously thought due to the low sensitivity of meat inspection. They also demonstrate that traditional coproscopy can be very efficient if there is repeated sampling, resulting in sensitivity of approximately 92%.

Keywords: *Fasciola hepatica*; Diagnosis; Bayesian analysis; Markov Chain–Monte Carlo; Latent class models

1. Introduction

Fasciola hepatica infects cattle and other mammalian species and is endemic in many parts of the world (Torgerson and Claxton, 1999). Previous abbatoir studies have suggested that the prevalence of bovine fasciolosis in Switzerland is approximately 10% (Ducommun and Pfister, 1991; Eckert et al., 1975; Schweizer et al., 2003). This widespread infection of the cattle population results in considerable economic losses (Schweizer et al., 2005). Crude prevalence rates are often obtained from the numbers of livers condemned which are based on visual evaluation in abattoirs. In live animals, diagnosis has traditionally relied on faecal egg counts (Happich and Boray, 1969). More recently, serological techniques that detect circulating antibody (e.g., Cornelissen et al., 2001; Reichel, 2002), circulating antigen (Leclipteux et al., 1998), coproantigen (Mezo et al., 2004) or eggs from bile samples taken under ultrasonic guidance (Braun et al., 1995) have been utilised. A commercial serological test with a sensitivity and specificity greater than 98% has been reported (Molloy et al., 2005). However, this test

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was evaluated on two separate populations of animals and the accuracy may be subject to error, as the sensitivity and specificity of tests will vary according to the population on which they are tested (Lachs et al., 1992; Whiting et al., 2004; Leeflang and Bossuyt, 2005). Furthermore, no tests currently available can be considered as having both 100% sensitivity and 100% specificity.

To overcome these problems, it is possible to perform multiple tests on populations of animals and use latent class models, maximum likelihood or Bayesian techniques to evaluate the true prevalence and diagnostic characteristics of the tests used (Enoe et al., 2000). It is feasible to estimate the true prevalence and diagnostic performance of tests if at least three diagnostic tests are used on the same population if no prior information is available (Toft et al., 2005). More tests may be required if the sensitivity and/or specificity of two or more tests are not conditionally independent. Fewer tests may be used if there is prior knowledge of some of the test characteristics.

A survey of 1,331 cattle presented for slaughter at two abattoirs was used to estimate the prevalence of *F. hepatica* infection and the diagnostic parameters of four different diagnostic strategies: meat inspection, coproscopy, a commercial ELISA and examination of bile taken from gall bladders of examined livers. A Bayesian approach was utilised to calculate the unknown prevalence rate and the unknown diagnostic parameters of the test procedures.

2. Materials and methods

2.1. Animals

From May 2004 to August 2005, two abattoirs in the Swiss cantons of Zurich and St. Gallen were visited on several occasions. From a total of 1,331 cattle presented for slaughter, faeces, blood and/or the gall bladder were taken (Table 1). Samples were taken from cattle of all age ranges (median age 4.5 years, range 4 months to 18 years). In addition, 122 livers, rejected by the meat inspectors for suspected liver fluke infection, were carefully dissected to examine for the presence of liver fluke. This was from a total of 1,087 inspected visually. The remaining 965 livers were judged to be negative for liver fluke. The animals were of various breeds and included dairy and beef cattle.

Table 1
Summary of the diagnostic procedures applied to 1,331 cattle at two abattoirs

Diagnostic procedure	Number of animals
Meat inspection, serology, bile and coproscopy	122[a]
Meat inspection, serology and bile	624
Meat inspection, serology and coproscopy	137[a]
Meat inspection, bile and coproscopy	7
Meat inspection and serology	143
Meat inspection and bile	49
Meat inspection and coproscopy	2
Meat inspection only	3
Serology, bile and coproscopy	192[a]
Serology and bile	0
Serology and coproscopy	15[a]
Serology only	36
Bile and coproscopy	1[a]
Bile only	0
Coproscopy only	0

[a] A total of 467 faecal samples were taken, of which 203 were examined on a single occasion (10 g), eight were examined twice (2 × 10 g) and 256 were examined three times (3 × 10 g). Of the 1,331 cattle, 122 were examined by all four diagnostic procedures, 960 by three procedures, 210 by two procedures and 39 by one procedure.

2.2. Samples

Faecal samples were taken and stored (for up to 1 month) without fixatives at 4 °C until they were analysed by coproscopy. A standard sedimentation technique was performed (Eckert et al., 2005) using 10 g rather than 6 g of faeces for the detection of *Fasciola* eggs. For many of these faecal samples the coproscopy examination was repeated three times in an attempt to improve the sensitivity of the test.

Gall bladders were taken and stored (for up to 2 weeks) at 4 °C until further investigation. Two 10 ml samples were then taken with a needle and syringe from the previously stirred contents, and after washing and sedimentation, examined for eggs of *F. hepatica* using the same sedimentation procedure as the faecal samples.

Blood samples were centrifuged 10 min at 3,500*g* within a few hours of being collected and serum was stored at −20 °C before further testing using a commercial ELISA (Institut Pourquier, Montpellier, F) according to the manufacturer's instructions.

A total of 122 condemned livers were collected and carefully dissected within 2 days. Major bile ducts were opened with a pair of scissors and any *F. hepatica* collected. Then the liver was cut into slices of about 5 cm. Smaller bile ducts were cut open and all flukes were collected. Any remaining *F. hepatica* were expressed from the tissue (Clery et al., 1996). Flukes collected were then counted.

2.3. Statistics

Bayesian techniques (Markov Chain-Monte Carlo) were used to estimate the diagnostic parameters of each of these procedures and the true prevalence of *Fasciola* infections in cattle. For each diagnostic test, it was assumed that the sensitivity was unknown and, hence, uniform non-informative prior distributions of between 0 and 1 were used in the analysis. Also, prior specificities of the serological and coprological tests were assumed to be unknown. Livers which had been rejected by the meat inspectors, dissected and found to be infected were assigned as infected, whilst livers not rejected or rejected but not harbouring flukes were assigned as not infected. Thus, liver inspection was assigned a specificity of 1 because flukes were recovered from livers. The specificity of bile examination was assigned a prior specificity of 1 on the assumption that eggs