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Usefulness of sero-surveillance for *Trichinella* infections in animal populations

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ABSTRACT

In this paper we evaluate serology as a tool to monitor *Trichinella*-free pig herds. Indoor, industrial-raised fattening pigs in the Netherlands are practically *Trichinella*-free, and were used as a negative reference cohort. A positive cohort was not available but we used sera from an endemic region in Argentina to model a plausible distribution of serological responses (as OD levels) in positive sera, employing the difference between the endemic sera and the negative Dutch sera. We describe a method for correcting for variation among ELISA plates using on-plate reference sera, and demonstrate how to apply these corrections to a collection of test sera from pig farms. The positive and negative reference distributions can be used to estimate fractions true and false positives, necessary for defining appropriate cutoffs to be used for classifying positive and negative animals. Based on this analysis, the serological test was shown to lack the predictive power required for its large scale deployment. The properties of the serological test were also compared to the conventional digestion assay, which is highly specific but considerably less sensitive.

1. Introduction

In Western European countries the prevalence of *Trichinella* infections in swine populations has decreased significantly since the introduction of industrial swine production in closed housing systems and trichinae have been absent for many decades in most conventional swine production units (Pozio, 1998). The main risks of *Trichinella* infection for humans are from swine or swine products originating from small farms with outdoor access in endemic areas in Eastern and Central European countries where meat is used for local and regional consumption (Kapel, 2005). Since 2006, certification of *Trichinella*-free farms or swine from countries with negligible risk can be monitored by serology if approved by the community reference laboratory in Rome (EC SANCO/2075/2005).

In this paper we evaluate the use of serology as a tool to certify herds as free of *Trichinella* by first showing how sensitivity and specificity of the test can be estimated in the absence of a positive reference cohort. Secondly the correlation between serology and larval counts is investigated and we conclude by discussing the consequences of our findings for *Trichinella* surveillance.

2. Materials and methods

2.1. Serum sampling

Negative blood samples were taken from 96 swine, born and raised indoors under strictly hygienic conditions: rodent control, no cats allowed inside the facilities, and the

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animals fed with commercial feed. Pigs were bled by jugular venapuncture. Blood was centrifuged and stored at −20 °C until testing. An additional set of sera from 1240 pigs also reared indoors under strict hygienic conditions in the Netherlands was sampled for reference. For the endemic cohort, a total of 849 sera from swine from 11 different regions in 6 provinces in Argentina (Chubut, Neuquén, Rio Negro, Santa Fé, Santiago del Estero, and Tierra del Fuego) were sampled in the period between 2000 and 2006 (Döpfer et al., 2006). These animals were all from very small farms (herd size 1–10).

2.2. Serological assays

Swine sera were tested for antibodies to *Trichinella* by an ELISA assay as previously described (van der Giessen et al., 2007). Briefly, microtiter plates (flat-bottomed Polysorp, Nunc) were coated with *Trichinella spiralis* ES-antigen diluted in 0.1 M carbonate buffer (pH 9.6; Merck). After incubation (1 h, 37 °C), washing (4 times with 0.05% Tween 20 in PBS [PBS-T]), and blocking (1% BSA solution in PBS [BSA Grade 5, Sigma]), plates were incubated overnight at 4 °C. Duplicate serum samples, diluted 1:100, were incubated 1 h at 37 °C followed by washing four times with PBS-T. Then 100 μl/well of horseradish peroxidase-labeled rabbit anti-swine IgG conjugate (DakoCytomation) was added and incubated at 37 °C for 1 h. After washing, a tetramethylbenzidine substrate (KPL) was added and color development was stopped with 2 M H_2SO_4 (100 μl/well) after incubation for 10 min at room temperature. Optical densities (OD) were measured at 450 nm with a spectrophotometer (Bio-Tek Instruments) and corrected by subtraction of the average OD of blanks.

2.3. Statistical analysis

2.3.1. Analysis of serology

To characterize the properties of the ELISA, a positive and negative reference sample of sera was needed. The distribution of ODs in negative (uninfected) pigs was known from the negative cohort. A cohort of positive (infected) pigs was not available but the endemic cohort was a mixture of positive and negative pigs: the distribution of ODs in infected pigs can therefore be inferred from the difference between the endemic cohort and the negative cohort. This was achieved by using a statistical model for estimating both the shape (the parameters) of the distribution of ODs in positive (infected) pigs and their prevalence (the fractions positive and negative pigs in the endemic cohort). With given distributions of ODs in positive and negative pigs the rates of true and false positives and negatives can be derived for any cutoff value.

Plates used in this study contained negative control sera (4) and positive *Trichinella* control sera (2, in 8 dilutions), derived from experimental infected animals and blanks (no serum added, to measure background OD), and up to 32 test sera, all in duplicate After subtracting background OD (mean of duplicate blanks) all ODs were log transformed.

We used an additive model for the log transformed ODs: $x \sim N(\mu_{ser}, \tau_{repl})$ where $x = \log(\mathrm{OD})$, the natural logarithm, and $\mu_{ser} = \mu_{plate} + \mu_{0,ser}$. The plate average μ_{plate} is the experimental bias component, calculated as the mean of the controls on any plate, and $\mu_{0,ser}$ is the biological component contributing to the observed density. τ_{repl} is the precision of x (the inverse of its standard deviation), used to model variation between replicates, controls and test sera alike.

Controls were analyzed in a (Bayesian) hierarchical model: each control m had its own mean $\mu_{con} = \mu_{plate} + \mu_{0,con}$ so that $\mu_{0,con}$ represented the "biological" component of the log(OD) of controls. For $\mu_{0,con}$ we defined a prior $N(\mu_{1,con}, \tau_{1,con})$.

Sera can be positive or negative: we assumed different distributions for either category, both in mean and precision. Negative sera are $N(\mu_1, \tau_1)$ while positive sera were assumed $N(\mu_2, \tau_2)$. A Bernoulli variable decides between either component, effectively assigning observed OD values to positive or negative categories in a binary mixture (Spiegelhalter et al., 2004). By choosing the parameters of the component distributions as follows: $\mu_2 = \mu_1 + \theta\tau_2$ and $\tau_2 = \phi\tau_1$ (Gilks et al., 1996), identification problems were overcome. For μ_1 and θ normal priors were chosen, for τ_1 and ϕ we used gamma priors. For the Bernoulli mixing variable a bivariate dirichlet (beta) prior was used. The model was implemented in WinBUGS (Spiegelhalter et al., 2004); uninformed prior distributions were chosen for all parameters.

2.3.2. Relation between serology and detection of larvae

Use of a cutoff to classify sera into positives and negatives ignores most of the information that may be present in serological data. To test whether animals with high larval counts may have higher antibody titers, a statistical model describing the relation between larvae counts and serological data was formulated. Larvae counts were assumed random (Poisson distributed) with concentration proportional to the natural logarithm of antibody titer in serum from the same animal. Titers were also random: log titers were assumed normally distributed. The marginal joint distribution of the observed numbers of larvae (n) and antibody titers (x) can then be used to estimate the parameters describing the association between larvae concentration C and serum antibody level (optical density) log(OD).

3. Results

3.1.1. Frequency distributions of serum antibody levels

All optical densities were corrected for plate effects; model predictions of the distributions of corrected optical densities (antibody levels) in negative and positive sera are shown in Fig. 1a. These distributions can be used to calculate probabilities of true positives (true positive fractions, TPF) and false positives (false positive fractions, FPF) to construct a ROC curve, shown in Fig. 1b. Also shown in Fig. 1b are corresponding OD-levels. A list of the two errors (false positives and false negatives) is given in Table 1. With the cutoffs from Table 1 the reference cohort of 1240 negative animals showed very few positives (above cutoff), as shown in Table 2.