Evaluation of serological tests for Trichinella infections in pigs


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Abstract
The Dutch slaughter pig population is practically free of Trichinella spiralis. However, at slaughter every pig is tested for presence of larvae using the digestion method for export certification. A new 2006 EU directive concerning meat inspection for Trichinella spp. offers new opportunities to monitor Trichinella at herd level instead. Also serological methods are allowed when approved by the Community Reference Laboratory (CRL). To evaluate the usefulness of serological tests for monitoring a virtually free population for Trichinella, Bayesian methodology was used to estimate the diagnostic test parameters sensitivity and specificity, in the absence of a Gold Standard test.

Introduction
Trichinella are nematodes (round worms) which live as intracellular parasites. The diseases they cause are collectively referred to as trichinellosis. The most prevalent human infections are caused by T. spiralis. Domestic pigs are the dominant reservoir host for T. spiralis, which is now considered endemic in Japan and China. Trichinellae infect nearly all mammal species, making it one of the world’s most widely-distributed parasite groups. Infection occurs by ingesting contaminated raw or undercooked meat, which might cause severe symptoms, sometimes even death.

Trichinellosis is included in the EU white paper on food safety (EC Zoonosis Directive) and the costs for mandatory routine meat inspection of pigs, horses and game animals for Trichinella in the EU is estimated to be 570 million € annually. A new EU legislation concerning meat inspection for Trichinella spp., which came into force in 2006, offers new opportunities to monitor Trichinella-free herds using serological methods (EU 2075/2005). In the Netherlands, Trichinella is absent in industrialised pig farming, and the serological monitoring might replace individual carcass control for those herds fulfilling the criteria of Trichinella-free herds. In order to set up a surveillance system for population monitoring, information about the test parameters of available assays is necessary.

To evaluate the usefulness of serological tests applied to monitor a Trichinella free population, Bayesian methodology will be used to estimate the diagnostic test parameters: sensitivity and specificity, in the absence of a Gold Standard test. In the absence of positive Dutch serum samples for Trichinella, serum panels originating from regions with endemic Trichinellosis in Argentina and Croatia were used to estimate these test parameters.

The diagnostic test parameters from the imperfect serological tests under validation together with prior knowledge about the historically recorded infection status of farms will be used to set up a surveillance system in the future. The surveillance system has to guarantee freedom-of-infection to humans while using an imperfect test in a very low prevalence population.
Material and methods

Five serological assays were evaluated; 2 commercial ELISAs, 2 in-house ELISAs and a Biacore surface plasmon resonance (SPR) assay. All assays were based on ES-antigen. Cut-off values were used according to standard protocols.

One of the evaluated assays is based on the surface plasmon resonance phenomenon. Surface plasmon resonance (SPR) occurs at the surface of a gold film that is adhered to a glass plate (the sensor chip). Electrons on the surface of the gold film ripple around in waves (surface plasmons). When incident polarized light is directed to the gold film, energy is transferred to the surface plasmons and this causes a dip in intensity of the reflected light. A prerequisite for this to occur is that the incident light reaches the gold film at a certain angle (the resonance or SPR angle). The value of the reflected resonance angle changes when the refractive index at the sensor surface changes. Since the relation between the two is linear, the change in resonance angle can be used to measure changes in refractive index caused by a liquid (containing the analyte of interest) that is guided along the sensor chip surface. For instance, an antigen can be immobilized to the gold film of the sensor chip. A sample is injected into a flow channel that transports the sample to the sensor chip. The antigen captures the antibody (if present) from the liquid and this causes a mass change at the sensor chip surface resulting in a different refractive index at the sensor surface. In turn the value of the resonance angle of the reflected light is changed and measured. Events are displayed by a sensorgram on the screen of the computer that is interfaced to the Biacore apparatus. An advantage of this technology is that the surface can be regenerated and reused many times. Although Biacore technology is routinely used in many fields, it has not yet been used for the detection of antibodies directed against pathogenic microorganisms in animals in a routine setting. The serum sets included ~900 Dutch field sera from pigs that were digestion negative and sera from pigs infected with Salmonella enterica serotype Panama. In addition a total of 849 swine sera were collected during routine controls from 11 endemically infected regions in 6 provinces (Santiago del Estero, Santa Fé, 5 regions of Neuquén, 2 regions of Rio Negro, Chubut, and Tierra del Fuego) of Argentina on 18 different dates between 2000 and 2006. The pigs were older than 3 months of age and belonged to small subsidiary farmers. The animals were kept for local consumption in small groups of 1 to about 10 pigs in corrals with wooden sheds. Also a small set of field sera from Croatia was tested by all assays.

The test results from the Dutch, Croatian and Argentinean field sera were analysed employing Bayesian statistics. Prevalence (separately per region for the sera from Argentina and for the Croatian sera), sensitivity, and specificity were estimated, in the absence of a gold standard test, with a latent class model. The model accounted for possible (conditional) dependence between tests. Calculations were performed with Markov chain Monte Carlo (MCMC), employing the Gibbs Sampler, as implemented in WinBUGS (Spiegelhalter et al., 2000). For details about the model and the Bayesian inference we refer to Engel et al. (2006). Priors were set for prevalences to peak around 10% (Larrieu et al., 2004). Gamble et al. (2004) reported that the sensitivity of serological tests for Trichinella spp. was between 93.1 and 99.2%, while the specificity was between 90.6 and 99.4%. In this study, the priors for the sensitivity and the specificity were set to peak close to 100% with a large variance. Less informative priors were also employed and the impact of the priors on estimated values and Bayesian confidence intervals (credible intervals) was studied (and found to be small).

Results

The 5 evaluated assays did not show any positive responses in the tested Dutch field samples. Nor did any of the sera from animals infected with S. Panama, and which were positive in a Salmonella D-LPS ELISA, show up as positive. Those sera were tested because D serogroup Salmonella strains contain tyvelose in their LPS. Tyvelose is considered to be the most antigenic compound in ES-antigen. This possible cross-reactivity was investigated but has not been found. Within the Argentina serum set the exact same samples were found positive by all assays. Some differences between the assays were found when evaluating the Argentinean serum set, comprising of sera collected from 6 regions with different prevalences. The results of the Dutch, Croatian and Argentinean sera were used to estimate the test parameters and prevalences using Bayesian statistics. Predictably, the Dutch cohort had a marked impact on the specificity. The Argentinean results...
and Croatian cohorts primarily affected sensitivity, but specificity as well. The estimated sensitivity and specificity of the tests and the associated 95% credible intervals are shown in Table 1.

Table 1: The diagnostic test parameters and the 95% credible intervals (CI) for the 5 assays, estimated in absence of a Gold Standard, using 849 Argentinean field sera, 889 Dutch slaughter sera and 39 Croatian field sera

<table>
<thead>
<tr>
<th>Test</th>
<th>Estimated Sensitivity (credible interval)</th>
<th>Estimated Specificity (credible interval)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(post. median)</td>
<td>(post. median)</td>
</tr>
<tr>
<td>A</td>
<td>0.93 (0.78, 0.98)</td>
<td>0.997 (0.993, 0.999)</td>
</tr>
<tr>
<td>B</td>
<td>0.78 (0.65, 0.87)</td>
<td>0.997 (0.994, 0.999)</td>
</tr>
<tr>
<td>C</td>
<td>0.75 (0.62, 0.85)</td>
<td>0.998 (0.995, 0.999)</td>
</tr>
<tr>
<td>D</td>
<td>0.64 (0.53, 0.75)</td>
<td>0.981 (0.969, 0.991)</td>
</tr>
<tr>
<td>E</td>
<td>0.92 (0.83, 0.97)</td>
<td>0.985 (0.976, 0.994)</td>
</tr>
</tbody>
</table>

Conclusions
In this study, 5 serological Trichinella assays were compared including a SPR assay. Unique serum panels from endemic regions were used to estimate test parameters in the absence of a Gold Standard by Bayesian statistics. All Dutch sera that were negative in the digestion assay, were also found negative by the serological assays. The estimated test parameters can be helpful to calculate the sample sizes and frequency of a surveillance system for *Trichinella spiralis*. If necessary, sensitivity can be enhanced (lowering specificity) or vice versa to adjust to a different expectation of the monitoring system.

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