Survey of Market Swine to Determine Prevalence of Trichinella Antibodies in Meat Juice Samples from Selected Abattoirs

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Abstract

Trichinella spp. have been associated with human zoonotic disease, predominately in temperate and polar regions. Humans and other mammals are incidentally involved after consumption of infected raw or inadequately cooked muscle tissues or from direct contact with infectious oocysts. Recent USA outbreaks have been mainly attributed to carnivorous wildlife (bears, mountain cats, and wild boar). This change has been aided by the steady decrease in swine Trichinella prevalence as USA swine herds moved toward confinement and limited feeding of uncooked garbage. For this study presence of Trichinella antibodies in meat-juice samples was tested by a commercial competitive ELISA test. A total of 138,682 samples from 12 abattoirs were collected and 74,500 selected based upon producer annual production. During the sampling period a total of 74,451 meat juice results were reported. Average seroprevalence was 0.05%. This project demonstrates the feasibility for a market-based survey to identify Trichinella status in market swine, and further demonstrates the need for an additional confirmation step following serologic evaluations to clarify status for serologically positive samples when establishing site Trichinella status.

Introduction

Trichinella spp. are widespread in the world and have been associated with human zoonotic disease since early times. All mammalian species are thought to be susceptible to Trichinella infestations. In temperate climates, swine, horses and bears are the most likely hosts, although other carnivores are also sources of infection. In more polar regions, bears and walruses are the identified natural hosts. In tropical climates, prevalence is lower than in the temperate or polar regions.1 Humans and other mammals are incidentally involved primarily after consumption of infected raw or inadequately cooked muscle tissues or directly by exposure to infectious oocysts in the environment. Swine have been incriminated for centuries as the primary host but recently in the USA has given way to assorted carnivorous wildlife (bears, mountain cats, and wild boar) as the primary source. Part of this change has resulted from the steady decrease in prevalence in swine as the USA swine herd has moved toward confinement and limited feeding of uncooked garbage. These practices enable the national swine herd to limit exposure to this parasite.

In the USA, from 1947 to 1951 Trichinella infections in humans averaged 393 cases annually with 57 deaths. From 1997 to 2001 the infection rate averaged 12 cases annually with no deaths. Also a shift toward wild game sources became more prominent.2 Correspondingly the swine population has reduced prevalence from 1.41% in 1980 to 0.125 in 1996-1970, and 0.013% in 1995.3 This decreasing trend has continued with 2000 and 2006 NAHMS survey data indicating 0 serologic positives in 14,061 and 6,238 samples market swine respectively.4 These prevalence reductions and the introduction of a USDA-APHIS-VS on-farm risk-based surveillance program prompted an attempt to estimate the prevalence of meat juice-based antibodies to Trichinella spp. in market swine harvested in 12 high-volume market swine abattoirs representing ~ 65% of the daily national harvest capacity.
Materials and methods

Trichinella antibodies were analyzed by groupings based upon estimated annual production, by harvest plant, and time of submission from November 2007 to March 2008 from lots collected concurrent to existing PRV surveillance. Twelve cooperating abattoirs supplied facilities and producer information for sampled lots. Five diaphragm muscles of approximately 75-100 grams were collected per lot as a convenience sample at the abattoir. Lots to be tested were selected from this larger population at the laboratory, based upon anticipated annual production sampling algorithms. Large suppliers were limited to 1 or 2 lots daily to reduce their over-representation in the data set. Smaller producers (< 10,000 head annual production) were sampled in every lot presented at the processing laboratory. A total of 138,682 samples were collected and 74,500 were selected using this formula. Production systems were divided by annual production estimates into the following groupings: 1-1000 head; 1001-2000; 2001-4000; 4001-9999; 10,000 – >1,500,000; and 10,000 – >1,500,000 (top 30 producers). These groups contained similar numbers of producers up to the 4001-9999 grouping then reduced rapidly, but total sample numbers increased.

Meat-juice samples were tested according to manufacturer’s instructions (ELISA OD ≥ 0.3 positive at a 1:10 dilution) by a commercial enzyme-linked immunosorbent assay (ELISA) (Safepath Laboratories, St, Paul, Minn. 55108, USA). Reported sensitivity was 100% (95% CL = 84.6 -100%); specificity was 100% (95% CL = 96.5 -100) when compared with a “gold standard” of tissue digestion. Each initial seropositive sample was retested using the same procedures to increase the possibility that only “true” positive samples were identified. Double positive samples were reported as “positive” and assessed accordingly.

Results

During the sampling period a total of 74,451 meat juice results were reported. Average seroprevalence was 0.05% for all samples tested double positive. Plant prevalence ranged from 0.00 – 0.12% for individual samples and 0.00 – 0.32% for lots (Table 1). Three plants had no positive lots and the range for the other 9 was 0.09 -0.82.

Table 1: Prevalence by Abattoir by Lot

<table>
<thead>
<tr>
<th>Abattoir Identifier</th>
<th>Prevalence</th>
</tr>
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<tbody>
<tr>
<td>8</td>
<td>0.02%</td>
</tr>
<tr>
<td>9</td>
<td>0.04%</td>
</tr>
<tr>
<td>12</td>
<td>0.06%</td>
</tr>
<tr>
<td>13</td>
<td>0.08%</td>
</tr>
<tr>
<td>14</td>
<td>0.10%</td>
</tr>
<tr>
<td>44</td>
<td>0.12%</td>
</tr>
<tr>
<td>68</td>
<td>0.14%</td>
</tr>
<tr>
<td>72</td>
<td>0.16%</td>
</tr>
<tr>
<td>88</td>
<td>0.18%</td>
</tr>
<tr>
<td>89</td>
<td>0.16%</td>
</tr>
<tr>
<td>717</td>
<td>0.18%</td>
</tr>
</tbody>
</table>

\( n = P < 0.05 \)
A monthly prevalence was not calculated because of the small numbers of positives and their variability -- November with 17 positive lots, December with 2, January with 9, February with 10 and March at zero (Table 2).

Table 2: Monthly prevalence of antibodies

![Graph showing monthly prevalence]

Month

A total of 2,538 producers were surveyed with 36 producers registering > 1 positive lots for the study period. Only two producers presented more than one positive lot in that period. These two lots for each producer were distributed as single reactions 1-2 months apart, rather than occurring in a single date. Annual production impacted the prevalence, as the smallest category was a significantly higher than the other five categories at 0.38% vs. the largest category at 0.02% (Table 3).

Table 3: Prevalence by Production Size

![Graph showing prevalence by production size]

Production System Size

a=P<0.05; b=P<0.05
Discussion

The sample population represents approximately 65% of the daily USA harvest capacity. This survey presents a picture of Trichinella prevalence in USA market swine that is different from the anticipated results based on the recent NAHMS results. Given the large number of producers with only one positive lot a concern arises about false positives being included in these tallies. Trichinella positive prevalence is recognized as being extremely low as production practices that limit exposure to wildlife, uncooked garbage, and environmental exposures have become more widely accepted. A deficiency of this study was that the meat samples residual to meat juice preparation were not used to retest positive samples to confirm Trichinella status for the individual sample with the muscle digestion “gold standard”. This confirmatory final step would be easily implemented in the surveillance system that was used in this study. Meat samples (diaphragm muscle) could be stored under refrigeration until the serologic results are reported. After positive samples are identified, the appropriate muscle samples could be digested and examined for the presence of muscle cysts. Failure to confirm a positive from digestion would indicate that the serologic test may have generated a “false” positive reaction.

With only 36 of 11,547 (0.31%) lots identified as positive this indicates that the majority of samples were properly allocated. The fact that only two of 2,538 (0.079%) producers had more than one positive and only four of 74,451 (0.0054%) of samples examined came from producers with more than one positive may be a key finding, and supports the NAHMS finding of extremely low antibody prevalence in the USA swine herd. With our PRV surveillance experiences, a producer that presented a positive lot one day was highly likely to present again the next time, if the herd was truly positive. With singleton reactors no evidence of infection could be found at the production site using case-finding methodologies. This relationship may be more tenuous because the low prevalence of Trichinella and the lack of inter-swine infectivity, short of within-herd cannibalism, but it provides additional epidemiologic information for consideration. These factors may explain the 1-2 month periods between positive lots for the only two producers identified as having multiple double positive samples. Experiences from an on-going surveillance program will help answer these questions, and provide additional confirmatory data to make serologic screening more practical.

Conclusions

This project demonstrated the feasibility for a market-based survey to identify epidemiologically important production practices across a wide spectrum of production sizes and systems for the certification of Trichinella status in market swine. It demonstrated tools for surveillance and monitoring of large populations at the plant level rather than requiring point of first sale or down-the-road sampling of these populations. Abattoirs, irrespective of size, can be used in these efforts to sample a range of sub-populations within the commercial compartment of the USA swine industry. It demonstrated a low prevalence for Trichinella antibodies generally and significantly different sub-populations based on annual production estimates. This process further demonstrated the need for an additional confirmatory step following serologic evaluations to clarify status for serologically positive samples. It also demonstrated how that step might be incorporated into an existing surveillance real-time system.

References