Trichinellosis in Switzerland


(1) Swine Clinic, University of Bern, Bremgartenstrasse 109a, POB 8466, 3001, Bern, Switzerland
(2) Institute for Parasitology, University of Bern, Länggass-Strasse 122, 3012 Bern, Switzerland
(3) SAFOSO, Bremgartenstrasse 109a, 3012 Bern, Switzerland
*corresponding author: friedericke.zeeh@knp.unibe.ch

Abstract

Human trichinellosis is a food-borne zoonosis exhibiting significant health and economical problems predominantly in countries with high pork consumption. During the past ten years the number of human outbreaks around the world has increased in certain areas. In Europe, more than 20,000 human cases have been detected between the year 1991 and 2000. Conversely, Trichinella infection has not been reported for many decades among Swiss domestic pigs. The last autochthonous cases of human trichinellosis in Switzerland date to a time period prior to the first half of the last century. Nevertheless, Trichinella occurs in a sylvatic cycle in Switzerland. Molecular and genotyping investigations on the taxonomy of present Swiss Trichinella isolates always yielded T. britovi as a species. In an earlier study, foxes had been tested using the artificial digestion method with a prevalence finding of 1.3%. Similar investigations in Swiss lynxes yielded a parasitological prevalence of 27%. Based upon this epidemiological background, a risk based surveillance project is presently running under the sponsorship of the Swiss Federal Veterinary Office, including a detailed parasitological and serological (E/S-ELISA and Westernblot) investigation of representative populations of domestic pigs, wild boars, foxes, lynxes and other wild carnivores. In order to obtain reference laboratory specimens for the standardization of parasitological and serological tests, experimental infections in pigs were carried out with T. spiralis, T. britovi and T. pseudospiralis. Seroconversion occurred on day 21 p.i. for T. spiralis and on day 28 p.i. for T. britovi and T. pseudospiralis. As expected, all sera of the three species cross-reacted with the T. spiralis E/S-antigen, thus indicating that this kind of antigen will be suitable to catch hold of all three species by serological means. All three Trichinella species yielded an appropriate muscle-stage larval infection intensity in order to provide positive reference muscle samples for Swiss diagnostic laboratories involved in the diagnosis of animal Trichinella infection.

Introduction

Trichinella sp. range among the porcine parasites which cause food-borne zoonosis in humans. These nematodes are the causative agents of trichinellosis in man, a disease that rarely affects animals. The transmission of the parasites occurs via oral intake of tissue containing infective larvae. Humans become infected by consumption of uncooked or improperly heated meat containing infectious larvae. During the past ten years the number of human outbreaks around the world has increased in certain areas. In countries with high pork consumption, health and economical problems are noteworthy. Human trichinellosis is characterised by abdominal problems, oedema in the head region, fever, muscle pain or skin reactions. In Europe, more than 20,000 human cases have been detected between the year 1991 and 2000. Conversely, Trichinella infection has not been reported for many decades among Swiss domestic pigs or wild boars (Gottstein et al., 1997). The last autochthonous cases of human trichinellosis in Switzerland date to a time period prior to the first half of the last century. Between 1935 and 1968 4 outbreaks occurred after the - in Switzerland uncommon - consumption of infected dog meat (Rehsteiner 1939; Kappeli, 1955; Hörmig, 1976). Nevertheless, Trichinella occurs in a sylvatic cycle in Switzerland. Wild carnivores as lynxes and foxes play an important role. Molecular and genotyping investigations on the taxonomy of present
Swiss *Trichinella* isolates always yielded *T. britovi* as a species (Gottstein et al., 1997; Gottstein et al., 2006; Müller et al., 2006). In an earlier study, foxes had been tested using the artificial digestion method with a prevalence finding of 1.3% (Jakob et al., 1994). Similar investigations in Swiss lynxes yielded a parasitological prevalence of 27% (B. Gottstein, pers. communication). With the pig keeping structures in Switzerland, where outdoor ranging or pasturing is becoming more common, contact with infected wild animals can not be excluded. Especially foxes are known to look for food near pig stables.

Additionally, in the course of the implementation of EU legislation regarding *Trichinella* monitoring in pork, the Swiss abattoirs have to test every slaughtered pig since 2007. In the sight of these requests and the assumed very low prevalence or freedom of *Trichinella* in Swiss domestic pigs, testing would lead to an enormous increase in costs and labour. Based upon the epidemiological and lawful background, a risk based surveillance project is presently running under the sponsorship of the Swiss Federal Veterinary Office, including investigation of representative populations of domestic pigs, wild boars, lynxes, foxes and other wild carnivores in Switzerland. Detailed parasitological and serological (E/S-ELISA and Westernblot) research will be undertaken. The aim of this project is the determination of the actual prevalence of *Trichinella* sp. in Switzerland, and based upon these findings a risk adapted control program for *Trichinella* sp. could be developed.

In order to obtain reference laboratory specimens to standardize parasitological and serological tests, experimental infections in pigs were carried out with *T. spiralis*, *T. britovi* and *T. pseudospiralis* at the Vetsuisse Faculty Bern.

**Materials and methods**

Three healthy fattening pigs weighing between 30 and 37 kg at day 0 of the trial were orally infected with infectious muscle-stage larvae 1 of three different species of *Trichinella*. Larvae were obtained by artificial digestion of experimentally infected mice. The three *Trichinella* species are thus routinely kept by serial passage in Balb/c-mice in the frame of the Swiss reference laboratory for trichinellosis. Pig no. 1 received 30,400 L1 of *T. spiralis*, pig No. 2 60,000 L1 of *T. pseudospiralis* and pig no. 3 60,000 L1 of *T. britovi*. Prior to inoculation, control blood and faeces were sampled for standard laboratory and parasitological investigations. Serum samples were tested for the demonstration of the absence of anti-*Trichinella* antibodies, and specimens underwent conventional parasitological examination (flotation and sedimentation technique). The three pigs were separately housed for 2 weeks. They were daily monitored upon conventional clinical examination and blood was collected at day 7, 14, 21, 28, 35, 42, 49, and 57 p.i. by puncturing the jugular vein. Clotted blood samples were sedimented for obtaining appropriate serum specimens. Sera were stored at -20°C. Serological investigation was carried out by a *Trichinella*-EIS ELISA as previously described by Gottstein et al. (1997). At day 61 of the trial, the three pigs were euthanised, and 2 litres blood and 25 to 35 kilograms muscle per animal were collected, respectively. Samples of 5 grams isolated from different striated muscles were quantitatively tested for the presence of *Trichinella* larvae by a standardized artificial digestion method, and sera were assessed for their anti-*Trichinella* antibody concentration by a standardized *T. spiralis* E/S-antigen-ELISA. Once laboratory testing had yielded the appropriate results, all meat and serum samples were aliquoted and stored frozen at -30°C for being further used as standard reference material in the frame of epidemiological surveys or of quality control trials for Swiss veterinary diagnostic laboratories.

**Results**

Prior to experimental infection, two of the three pigs were coproscopically positive for *Trichuris suis* and *Ascaris suum*. Consequently, all three animals were conventionally treated with flubendazole, treatment efficacy being demonstrated coproscopically. Subsequently, the three animals were orally infected with the *Trichinella* larvae. The pigs showed no signs of illness. Body temperature, digestion and muscular system were inconspicuous during the whole trial. All preinfection sera tested negative for *Trichinella* sp. by E/S-ELISA. Seroconversion occurred on day 21 p.i. for *T. spiralis* and on days 28 p.i. for *T. britovi* and *T. pseudospiralis*, respectively (Fig. 1). Thus, all sera of the three species significantly cross-reacted with the *T. spiralis* E/S-antigen.
Fig. 1: Time course of anti-Trichinella-antibody development in experimentally infected pigs. S8192: T. spiralis; S0952: T. pseudospiralis; S0946: T. britovi. The red arrow indicates the infection onset (22.06.2005).

The calculated densities of larvae recovered in the muscle samples are presented in Table 1.

Table 1: Infection doses and larvae burden at end of the trial in three experimentally infected pigs (average obtained from different samples).

<table>
<thead>
<tr>
<th>Pig infected with</th>
<th>Pig infected with</th>
<th>Pig infected with</th>
</tr>
</thead>
<tbody>
<tr>
<td>T. spiralis</td>
<td>T. pseudospiralis</td>
<td>T. britovi</td>
</tr>
<tr>
<td>Infection dose</td>
<td>30.400 larvae per os</td>
<td>60.000 larvae per os</td>
</tr>
<tr>
<td>Calculated larvae density in diaphragm</td>
<td>330 larvae/g</td>
<td>75 larvae/g</td>
</tr>
<tr>
<td>Calculated larvae density in the masseter</td>
<td>490 larvae/g</td>
<td>13 larvae/g</td>
</tr>
</tbody>
</table>

**Discussion**

*Trichinella* sp. are of importance in Switzerland. Beside legislative demands, the epidemiological background of the sylvatic circle and its possible connection with outdoor pigs require an appropriate monitoring. The assumed absence of *Trichinella* sp. in the Swiss domestic pigs population and the comparable low number of slaughtered pigs per year prompted Switzerland to look for alternatives instead of the testing each individual pig with direct detection methods. Serological testing such as the well described E/S-ELISA appear particularly suitable to carry out risk-based surveillance or monitoring programs. In order to provide standardized test procedures, sufficient amounts of positive and negative reference test materials (sera or meat juice) are required. Such sera and meat juice were now generated and obtained by experimental infection of three pigs with L1 of three *Trichinella* species. Testing the sera of the pig infected with *T. spiralis* larvae showed that seroconversion occurred after 21 days p.i.. In the two other pigs infected with *T. pseudospiralis* and *T. britovi*, respectively, first detectable antibodies became apparent 7 days later. The rise of the antibody concentration against *T. pseudospiralis* was relatively weak when compared to *T. britovi*. Nevertheless, the use of *T. spiralis* E/S-antigen proved
to be suitable to detect antibodies by ELISA against all three *Trichinella* species addressed in this study, thus a major cross-reactivity can be concluded from these findings.

With regard to the infection intensity determined by the quantitative artificial digestion method, the pig infected with *T. spiralis* yielded 330 larvae per gram diaphragm muscle and 490 L/g cheek muscle. A much lower infection intensity was anticipated and finally also demonstrated for *T. pseudospiralis* and especially *T. britovi*. This confirmed the much lower susceptibility of the domestic pig as a host for these latter two species, however without affecting the potential to develop an appropriate humoral immunity that sufficiently cross-reacts with *T. spiralis* E/S-antigen, thus allowing an adequate immunodiagnosis by the respective ELISA.

To summarise, *Trichinella* infection is a contemporary topic in Switzerland. Diagnosis of the parasitic nematodes, especially in the surroundings of safe pork production, requires efforts and adequate methods. Serology appears to suitably contribute to this approach.

**Conclusion**

The sera and meat juice as well as muscle specimens obtained in the present study can now be provided to Swiss veterinary diagnostic laboratories interested in the diagnosis of *Trichinella* infections.

**References**


