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**DEVELOPMENT OF A RISK-BASED SURVEILLANCE PROGRAM
FOR *TRICHINELLA* SPP. IN DOMESTIC SWINE AND WILDLIFE IN
SWITZERLAND**

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Whenever you are asked if you can do a job, tell 'em, „Certainly I can!“
Then get busy and find out how to do it.

Theodore Roosevelt (1858-1919)

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Executive summary

Trichinella spp. is the causative agent of trichinellosis in humans, and can occur in many different animal species. Infection between hosts is transmitted via oral intake of tissue containing infective larvae. In line with European regulations, Switzerland implemented a meat inspection program for domestic pigs to protect public health. However, data indicated that *Trichinella* infections did not occur in pigs in Switzerland, and therefore an alternative, equally effective but more efficient surveillance approach should be considered. The goal of this dissertation was to develop a risk-based surveillance system for *Trichinella* spp. in Switzerland.

Serology was considered as a diagnostic tool for a risk-based surveillance system. Available serological techniques, in particular ELISA, had the disadvantage of having a specificity of less than 100%, thus leading to false-positive results. A Western Blot was therefore evaluated as a serological confirmatory method. In a first study, the sensitivity and specificity of a Western Blot assay based on somatic *Trichinella spiralis* muscle stage (L1) antigen was evaluated using Bayesian modeling techniques. A total of 295 meat juice and serum samples from pigs negative for *Trichinella* larvae by artificial digestion, including 74 potentially cross-reactive sera of pigs with other nematode infections, and 93 meat juice samples from pigs infected with *Trichinella* larvae were included in the study. The diagnostic sensitivity and specificity of the Western Blot ranged from 95.8 to 96.0% and from 99.5 to 99.6%, respectively. A sensitivity analysis showed that the model outcomes were hardly influenced by changes in the prior distributions, providing a high confidence in the outcomes of the models. This validation study demonstrated that the Western Blot is a suitable method to confirm samples that reacted positively in an initial ELISA.

Then, the test characteristics of an in-house and a commercial ELISA for *Trichinella* diagnostics were assessed. Neither of the tests could be considered a true gold standard, and Bayesian techniques were used to evaluate tests in the absence of a gold standard. A total of 875 *Trichinella* larvae-negative samples of pigs and 93 *Trichinella* larvae-positive samples of both naturally and experimentally infected pigs were included in the study. Bayesian modeling techniques were used to correct for the absence of a perfect reference test. The sensitivity and specificity of the in-house ELISA were 97.0-97.4% and 99.4-99.6%, respectively. The sensitivity and specificity of the commercial ELISA was 97.1-97.8% and 99.5-99.8%, respectively. Sensitivity analysis demonstrated a high stability of the models.

In order to assess the prevalence of *Trichinella* infections in wildlife, samples from 1,298 foxes, 55 lynxes and 1,458 wild boar were tested. The fox and lynx samples were tested by artificial digestion only and the wild boar samples were tested both by artificial digestion and serology. Any recovered larvae were tested by a multiplex PCR to determine the species and/or genotype. *Trichinella* spp. was found in 21 foxes (1.6%) and in 15 lynxes (27.3%). Multiplex-PCR performed on recovered larvae yielded *T. britovi* as infecting species in all cases. No larvae were recovered from wild boar, but 3 wild boar were seropositive ((seroprevalence: 0.2% (95% CI 0.07%-0.60%)).

A study was conducted to demonstrate the absence of infection in Swiss domestic pigs. An ELISA was used as the initial screening test, and sera reacting in ELISA were further investigated using both a Western Blot for serology and an artificial digestion test with 20 grams of diaphragm tissue for larval detection. A total of 7,412 adult pigs, 9,973 finishing pigs and 2,779 free-ranging pigs were tested. Samples from 17 (0.23%) adult pigs, 16 (0.16%) finishing pigs and 9 (0.32%) free-ranging pigs were ELISA-positive, but all of these sera were subsequently negative by Western Blot and by the artificial digestion method. Based on these findings, an absence of *Trichinella* infections in adult pigs (target prevalence 0.04%) and finishing pigs (target prevalence 0.03%) can be concluded. The results also demonstrated that the prevalence of *Trichinella* infections does not exceed 0.11% in free-ranging pigs, the group with the highest risk of exposure.

In order to evaluate the probability of human exposure to *Trichinella* spp. in Switzerland, a qualitative risk assessment was conducted. The risk assessment was conducted according to the standards of the World Organisation for Animal Health. Various relevant meat sources were considered, including game meat, pork and imported meat. Inactivation steps for raw meat were not included in the assessment. The risk assessment demonstrated, the probability of human exposure through wild boar meat used for private consumption and through pork from free-range pigs was very low. No data were available to assess the importance of privately imported game meat. To reduce the overall probability of human exposure to *Trichinella* spp., monitoring and meat inspection activities should be targeted at free-range pigs. Also, awareness of hunters and travellers should be increased regarding the risks related to the consumption of game meat that was not tested for *Trichinella* spp. and possibilities for risk reduction.

Finally, an epidemiological model was designed to evaluate the results from the current meat inspection program for domestic pigs and to compare those with the results of a possible future risk-based surveillance in pigs. First, the results from artificial digestion tests in

Switzerland were evaluated over a time period of 15 years to determine by when freedom from infection based on these data could be confirmed. Freedom was defined as a 95% probability that the prevalence of infection was below 0.0001%. Freedom was demonstrated after 12 years at the latest. A new risk-based surveillance approach was then developed based on serology. Risk-based surveillance was also assessed over 15 years, starting in 2010. It was shown that by using this design, the sample size could be reduced by at least a factor of four when compared with the traditional testing regime, without lowering the level of confidence in the *Trichinella*-free status of the pig population.

This dissertation demonstrated that it is possible to design a serological, risk-based surveillance system for domestic pigs in Switzerland without lowering the standards for public health protection, even though infected wildlife is known to be present. Within the pig population, free-range pigs have the highest probability of exposure to and infection with *Trichinella* spp., therefore surveillance activities should be focused on this risk group.

Zusammenfassung

Humane Trichinellose wird von dem Parasiten *Trichinella* spp. verursacht, einem Parasiten, der viele verschiedene Tierarten befallen kann. Die Ansteckung findet über die orale Aufnahme von trichinellenhaltigem Gewebe statt. In der Schweiz wurde zum Schutz der öffentlichen Gesundheit ein Fleischuntersuchungsprogramm gegen Trichinellose in Hausschweinen umgesetzt, das den Europäischen Richtlinien entspricht. Verfügbare Daten weisen allerdings darauf hin, dass Trichinelleninfektionen bei Hausschweinen in der Schweiz nicht vorkommen, und aus diesem Grund soll die Möglichkeit für eine alternative Trichinellenüberwachung geprüft werden. Dieser neue Ansatz soll eine gleichwertige Effektivität aber eine höhere Effizienz als die heutige Fleischuntersuchung aufweisen. Ziel dieser Dissertation war es, ein risikobasiertes Überwachungssystem für *Trichinella* spp. in der Schweiz zu entwickeln.

Als diagnostische Methode für eine risikobasierte Überwachung wurde die Serologie in Betracht gezogen. Die zur Verfügung stehenden serologischen Tests, insbesondere ELISA, wiesen alle eine Spezifität von weniger als 100% auf, was zu unerwünschten falsch-positiven Testergebnissen führen kann. Um das zu verhindern, wurde ein Western Blot als serologischer Bestätigungstest evaluiert und validiert, der auf der Verwendung von somatischem Antigen der *Trichinella spiralis* Muskelphase (L1) Larven beruht. Die Sensitivität und Spezifität dieses Western Blots wurden evaluiert und die statistische Auswertung der Ergebnisse erfolgte mittels bayesischer Modellierung. Es wurden 295 Fleischsaft- und Serumproben von Schweinen getestet, die vorgängig mittels der künstlichen Verdauungstechnik als *Trichinella*-negativ eingestuft worden waren. Darunter waren 74 Serumproben von Schweinen, die andere Nematodeninfektionen hatten, die zu serologischen Kreuzreaktionen führen könnten. Zusätzlich wurden 93 Fleischsaftproben von *Trichinella*-positiven Schweinen getestet. Die diagnostische Sensitivität und Spezifität des Western Blots lagen zwischen 95.8% und 96.0%, beziehungsweise zwischen 99.5% und 99.6%. Eine Sensitivitätsanalyse zeigte, dass die Modellierungsergebnisse kaum von Änderungen in den *a priori* Verteilungen beeinflusst wurden, was zu einer hohen statistischen Genauigkeit in die Ergebnisse führte. Diese Validierung zeigte, dass der Western Blot für Proben, die vorgängig in einem ELISA-test positiv getestet wurden, eine geeignete Bestätigungsmethode ist.

In einem nächsten Schritt wurden die Sensitivität und Spezifität eines nicht-kommerziellen sowie eines kommerziellen ELISAs evaluiert. Keiner dieser Tests konnte als Goldstandard

betrachtet werden, und aus diesem Grund wurden auch hier bayesische Modellierungstechniken verwendet. Insgesamt wurden 875 Proben von *Trichinella*-negativen Schweinen und 93 Proben von natürlich wie auch von künstlich infizierten Schweinen untersucht. Die Ergebnisse zeigten, dass der *in-house* ELISA eine Sensitivität von 97.0% bis 97.4% und eine Spezifität von 99.4% bis 99.6% aufwies. Die Sensitivität des kommerziellen ELISAs lag zwischen 97.1% und 97.8% und dessen Spezifität zwischen 99.5% und 99.8%. Auch hier zeigte die Sensitivitätsanalyse eine hohe statistische Stabilität der Modelle.

Um die Prävalenz der Trichinelleninfektion in der Schweizer Wildtierpopulation zu ermitteln, wurden Proben bei 1'298 Füchsen, 55 Luchsen und 1'458 Wildschweinen entnommen und getestet. Die Proben der Füchse und Luchse wurden nur mittels der künstlichen Verdauungsmethode untersucht, die Proben der Wildschweine wurden zusätzlich serologisch untersucht. Isolierte Larven wurden mittels multiplex PCR untersucht, um die genaue Spezies und/oder den genauen Genotyp zu bestimmen. Eine Trichinelleninfektion wurde bei 21 Füchsen (1.6%) und 15 Luchsen (27.3%) festgestellt. Alle isolierte Larven waren Larven der Spezies *T. britovi*. Aus keiner der Wildschweinproben wurden Trichinellenlarven isoliert, dennoch waren 3 Wildschweine serologisch positiv (Seroprävalenz 0.2% (95% Konfidenzintervall 0.07%-0.60%)).

Es wurde eine weitere Studie durchgeführt, um die Abwesenheit von Trichinelleninfektionen in Schweizer Hausschweinen zu belegen. Ein ELISA wurde als *screening* Test eingesetzt, und ELISA-positive Proben wurden anschliessend mit einem Western Blot bestätigt. Zusätzlich wurde von ELISA-positiven Proben 20 Gramm Zwerchfellgewebe mit der künstlichen Verdauungsmethode untersucht, um einen Direktnachweis der Larven zu ermöglichen. Es wurden 7'412 Zuchtsauen und -eber, 9'973 Mastschweine und 2'779 Schweine aus Freilandhaltung untersucht. Proben von 17 (0.23%) Zuchtsauen und -ebnern, von 16 (0.16%) Mastschweinen und von 9 (0.32%) Schweinen aus Freilandhaltung waren ELISA-positiv, aber keine dieser Proben war positiv im anschliessenden Western Blot oder im künstlichen Verdauungstest. Aufgrund dieser Ergebnisse wurde die Abwesenheit von Trichinelleninfektionen in Zuchtsauen und -ebnern (Zielprävalenz 0.04%) und in Mastschweinen (Zielprävalenz 0.03%) belegt. Die Ergebnisse zeigten zudem, dass die Trichinellenprävalenz in Schweinen aus Freilandhaltung, die Gruppe mit dem höchsten Infektionsrisiko, nicht über 0.11% lag.

Um die Wahrscheinlichkeit einer Trichinellenexposition für den Menschen in der Schweiz zu ermitteln, wurde eine qualitative Risikoabschätzung durchgeführt. Die Risikoabschätzung wurde gemäss der Richtlinien der Welttiergesundheitsorganisation durchgeführt.

Verschiedene relevante Fleischsorten wurden berücksichtigt, darunter einheimisches Wildfleisch und Schweinefleisch, sowie importiertes Fleisch. Schritte in der Fleischproduktion und -zubereitung, die zu einer Inaktivierung oder Tötung der Trichinellenlarven führen könnten, wurden in dieser Risikoabschätzung nicht berücksichtigt. Die Risikoabschätzung zeigte, dass die Wahrscheinlichkeit einer Exposition für den Menschen durch Wildschweinefleisch, das für den privaten Konsum benutzt wird, sowie durch Fleisch von Schweinen aus Freilandhaltung sehr gering war. Die Rolle von privat importiertem Wildfleisch konnte mangels Daten nicht evaluiert werden. Damit die Wahrscheinlichkeit einer Exposition gesenkt werden kann, sollten die Überwachungsaktivitäten für Schweine aus Freilandhaltung verstärkt werden. Zudem soll das Bewusstsein von Jägern und Reisenden erhöht werden, zu welchen Risiken der Konsum von nicht auf Trichinellen untersuchtem Wildfleisch führen kann, und welche Massnahmen getroffen werden können, um dieses Risiko zu senken.

Schliesslich wurde ein epidemiologisches Modell entwickelt, um die Ergebnisse der heutigen Fleischuntersuchung für Hausschweine zu evaluieren, und sie mit den Ergebnissen eines möglichen zukünftigen risikobasierten Überwachungsprogramms zu vergleichen. Zuerst wurden die Ergebnisse der künstlichen Verdauungstests über einen Zeitraum von 15 Jahren beurteilt, um festzustellen ab wann die Infektionsfreiheit in der Hausschweinepopulation aufgrund dieser Daten belegt werden konnte. Infektionsfreiheit wurde als Wahrscheinlichkeit definiert, dass die Prävalenz unter 0.0001% lag. Diese Freiheit konnte spätestens nach 12 Jahren belegt werden. Zweitens wurde ein risikobasiertes Modell entwickelt, das auf Serologie beruht. Die risikobasierte Überwachung wurde wiederum über einen Zeitraum von 15 Jahren beurteilt, mit Start im 2010. Es wurde gezeigt, dass die benötigte Probenzahl um mindestens den Faktor 4 im Vergleich zum heutigen Überwachungsmodell gesenkt werden konnte, ohne dass das Vertrauen in der Status der *Trichinella*-Freiheit der Hausschweinpopulation abnehmen würde.

Diese Dissertation hat gezeigt, dass ein serologisches, risikobasiertes Überwachungsprogramm für Trichinellen bei Hausschweinen in der Schweiz möglich ist, ohne dass der Schutz der öffentlichen Gesundheit gesenkt werden muss, auch wenn *Trichinella* spp. in der Wildtierpopulation vorkommt. Vor allem Schweine aus Freilandhaltung haben eine erhöhte Wahrscheinlichkeit für eine Exposition und eine allfällige Infektion mit *Trichinella* spp., und das zukünftige Überwachungsprogramm sollte dieses erhöhte Risiko berücksichtigen.

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Introduction

***Trichinella* spp.**

The nematode *Trichinella* spp. was first discovered in the 19th century, and it was this discovery that led to the implementation of official inspection for *Trichinella* at pig slaughter once it was realized that the presence of *Trichinella* spp. in pork was the cause of clinical trichinellosis in humans (70). Since then, twelve different species and genotypes have been discovered. *Trichinella* spp. does not only occur in pigs, but has been found in many omnivorous and carnivorous animal species, both domestic and wildlife. The parasite is present around the globe, although each species or genotype has a specific geographical distribution. Four genotypes are present in Europe: *T. spiralis*, *T. nativa*, *T. britovi* and *T. pseudospiralis*; however, *T. nativa* is only present naturally in arctic regions (25, 33, 56, 57).

Trichinella spp. is transmitted orally through intake of raw or undercooked meat (25, 55, 56, 70), therefore many predating and scavenging animal species can be affected. Following oral intake, the L1 stage larvae that are embedded in muscle tissue are released from their muscle nurse cells in the stomach. They migrate to the intestines, where they go through four molting stages until they reach the adult stage. They replicate, and subsequently the newborn larvae migrate to muscle tissue through the blood and lymph system. Once in the muscle tissue, they embed in a muscle nurse cell, and encapsulating *Trichinella* species encapsulate. Then the larvae wait for ingestion by a new host to continue their lifecycle (25, 30). One distinct difference between animal and human infections is that infections in animals do not lead to clinical disease whereas infections in humans can lead to disease with mild to severe clinical signs and possibly even death (25, 30, 32, 53).

***Trichinella* in livestock systems**

The presence of *Trichinella* spp. in domestic pig production is primarily a result of management practices such as poor hygiene and low biosecurity in the husbandry system. Domestic pigs can become infected when being fed with kitchen waste containing contaminated meat or with slaughter waste from infected pigs or wildlife, or when they can scavenge on carcasses of infected pigs, rodents or other wildlife (22, 44, 55, 56, 58). This is confirmed by the fact that *Trichinella* spp. is virtually absent from high-biosecurity pig husbandry systems, but still occurs regularly in pigs kept under poor hygiene and biosecurity conditions. A study in Argentina showed that *Trichinella* infections did not occur in large-scale, indoor pig farms, but infections were present in free-ranging pigs that were feeding on

garbage (66). Following the collapse of the Soviet Union and the subsequent collapse of the veterinary systems in the Soviet states and protectorates, the prevalence of *Trichinella* infections in pigs and humans increased dramatically. A similar pattern was seen in the area of former Yugoslavia, as a result of the different wars. Following re-establishment of preventive measures, the prevalence in humans and animals is decreasing again (11, 54). In China, the prevalence of *Trichinella* infections in pigs is very high, with a prevalence of more than 30% in certain counties (36), but also infected dogs play an important role in the epidemiology of human trichinellosis in China (36, 82). A lack of sufficient hygiene practices in livestock production and a weak implementation of *Trichinella* inspection at slaughter are responsible for this situation (36, 82). In Europe, *Trichinella* is almost completely absent from pigs, as can be seen from the results of meat inspection (17). In Germany, between 1999 and 2003, 1 infected pig was detected among 212 million pigs that were tested (45). In 2006, an outbreak affecting 17 people was detected, which was traced back to a single backyard pig that had been slaughtered in a local butchery (67). In the Netherlands, a seroprevalence of 0.24% was detected among pigs from organic farms, but in the same study no seropositive pigs were found among pigs from free-range or intensive husbandry systems. It was noted that the seropositive result could have been due to a false-positive test result (78). In Denmark, more than 99% of the pigs are produced indoor, and between 1990 and 2005 more than 300 million pigs were tested without any positive finding (1).

The presence of *Trichinella* infections in wildlife poses a potential source of infection for domestic pigs (22, 44, 55, 56), but the importance of this source must be evaluated carefully because it is directly linked with hygiene and biosecurity practices in pig production (58). For example, in Germany the prevalence in raccoon dogs was estimated up to 5% (45), and infections are also regularly detected in wild boar and foxes (45, 48). In Denmark, the prevalence in foxes was found to be 0.1% (14). In Ireland, infected foxes were found after a period of more than 30 years in which no infections had been detected in any local animal species (65). In all of these countries, *Trichinella* in domestic pigs is absent or extremely rare (1, 17, 45, 65).

Wildlife infections are also of importance for humans, in particular for hunters and other people consuming game. Many outbreaks have been reported from hunters of wild boar, bears, warthogs, etc (24, 54, 62-64). Such outbreaks are the result of not cooking meat thoroughly, because *Trichinella* larvae are killed when meat is cooked to at least 72°C (20, 22).

Human *Trichinella* infections caused by consumption of horse meat have been a bit of an enigma for a long time, because horses are considered to be herbivorous and thus not to ingest meat. However, several large human outbreaks caused by horse meat were reported, in particular from France (2, 35). Subsequently, a study conducted in Serbia revealed that it was common practice among farmers raising horses for meat to supplement them with meat to increase weight gain. It was also shown that horses voluntarily eat meat patties when offered (43). Thus, horse meat should also be considered as a possible source of infection for humans, however also here the importance of this source is clearly linked with the quality of the husbandry systems.

Diagnostic techniques

There are two main diagnostic strategies to detect infected animals: direct detection of the parasite or detection of antibodies against the parasite (20, 83). Currently, direct detection is the method of choice for meat inspection purposes. Both trichinoscopy and the artificial digestion technique can be used, but the artificial digestion technique is clearly preferred because of a better sensitivity and a higher throughput capacity (20, 83). With trichinoscopy, small pieces of tissue of a total of 1 gram – diaphragm tissue in the case of domestic pigs – are pressed between two glass plates and studied carefully by microscope to detect any *Trichinella* larvae. It is assumed that the tissue should contain at least 3 larvae per gram (LPG) for a reliable detection (80). However, non-encapsulated *Trichinella* species such as *T. pseudospiralis* are very difficult to detect by this method. Therefore, this method is not recommended for routine inspections (20, 83). With the artificial digestion technique, tissue samples from several animals can be pooled and analysed simultaneously. In the case of domestic pigs, 1 gram diaphragm tissue samples from up to 100 pigs can be analysed at the same time. The samples are subjected to a process to artificially digest the diaphragm tissue and release the *Trichinella* larvae from their capsules. Subsequently, the fluid is studied under a microscope to detect the larvae (20, 83). In this approach, it is officially considered that the artificial digestion technique can reliably detect samples containing 1 LPG when using 1-gram samples (83), however it was shown that reliable detection (100% diagnostic sensitivity) was only achieved when such samples contained at least 3-5 LPG. The diagnostic sensitivity for 1-gram samples containing 0.1-0.9 LPG was 40% (18). Therefore, reliable detection is now only considered to be guaranteed when a 1-gram sample contains at least 3 LPG (68). Nevertheless, the artificial digestion technique is considered sufficiently sensitive to prevent human clinical disease and is therefore the method of choice for routine meat inspection (15, 20).

A different diagnostic approach is serology, detecting antibodies against *Trichinella* spp. It was shown that the antibodies show a high level of cross-reaction between different *Trichinella* species (23, 31, 49), which facilitates serological screening tests when it is not known which *Trichinella* species may be present. Several serological techniques have been developed, but the ELISA is most popular because of its low costs and high throughput capacity (20, 23, 47). Using ELISA, antibodies can be detected against infections with larval densities as low as 0.01 LPG (21, 50). The diagnostic sensitivity of ELISAs was evaluated in many studies, with outcomes ranging from 72.7% to 99.2% (42, 46, 52, 79). The specificity of the ELISA was estimated between 90.6% and 99.6% (42, 46, 52, 79). Serology can be done both with serum and with meat juice, whereas the dilution for meat juice is lower than for serum (46). Clearly, a drawback of serology is the possibility of ELISA-positive results where infection could not be confirmed by a direct detection technique, because uncertainty remains about whether this was a false-positive ELISA-result or not. A second disadvantage of serology is that the time of seroconversion is correlated with the number of infective larvae that were ingested and with the *Trichinella* species involved. The earliest seroconversion was reported 17 days after infection, with reports of up to 60 days (21, 31, 40, 43, 49, 73, 74, 81), leaving the possibility that an infection would already be detectable by direct detection but not by serology. Many studies looked at the time of seroconversion (21, 31, 40, 43, 49, 73, 74, 81), however there have been no studies looking at the time of seroconversion and the first moment for direct detection simultaneously. Therefore, this possibility can neither be confirmed nor rejected.

Legislation for *Trichinella*

European Union (EU)

Since 2006, EU Regulation 2075/2005 is in force. This regulation prescribes that all pigs, horses and other *Trichinella*-susceptible animals must be tested for *Trichinella* at slaughter or at the game handling plant. The prescribed test is the artificial digestion technique. The regulation allows for the possibility of so-called *Trichinella*-free pig holdings or negligible risk regions in which the full-scale testing of pigs could be reduced to a risk-based surveillance (15). It is not further specified how this risk-based surveillance should be designed. *Trichinella*-free pig holdings must at least comply with the requirements for “controlled housing systems” as described in the regulation. Among others, these systems must be completely indoor with an exception for piglets before weaning when the *Trichinella* prevalence in wildlife is favorable. For a negligible risk region, it must be demonstrated that the prevalence in pigs does not exceed 0.0001% and the *Trichinella* prevalence in wildlife must be below 0.5%. Until today, only Denmark has been able to achieve the status of negligible risk for *Trichinella*, due to a long history of *Trichinella* testing of pigs without any

positive finding, a pig production system which is almost completely based on indoor housing and a very low prevalence in wildlife (1, 16). However, despite enormous testing efforts, also in other countries of the EU very few cases of *Trichinella*-infected pigs are found. Only in particular Romania still has a very high prevalence of *Trichinella* in domestic pigs (17). In table 1 the results of the *Trichinella* testing program are presented.

Table 1. Results of the *Trichinella* testing program in the EU

	2004	2005	2006	2007	2008
	EU25	EU25	EU25	EU27*	EU27*
Domestic pigs					
Tested	140,405,180	131,456,607	137,976,058	220,680,358	167,499,799 (164,125,931)
Positive	69	43	42	NA**	1,179 (162)
% positive	0.00005	0.00003	0.00003	<0.1	0.0007 (0.0001)
Horses					
Tested	156,025	NA	NA	NA	NA
Positive	0	0	NA	NA	NA
% positive	0	0	NA	NA	NA
Wild boar					
Tested	523,579	751,714	405,043	450,505	459,260
Positive	470	555	598	NA	892
% positive	0.09	0.07	0.1	0.1	0.2
Other wildlife					
Tested	10,695	7,439	7,523	103,660	132,780
Positive	306	253	298	NA	437
% positive	2.9	3.4	4.0	0.3	0.3
Humans					
Positive	270	86	176	779 (303)	670 (100)
Incidence per 100,000	0.06	<0.1	0.04	0.2	0.1

* Figures in brackets represent results excluding data from Bulgaria and Romania

** Not available

Source: EFSA, Community Summary Reports on Trends and Sources of Zoonoses and Zoonotic Agents in the European Union 2004-2008. www.efsa.europa.eu

World Organisation for Animal Health (OIE)

The OIE has developed recommendations for *Trichinella* spp. in pork. Included in these recommendations are guidelines to declare a country as free from *Trichinella* in domestic pigs (84). To achieve freedom a country can conduct a serological survey of the slaughter sow population to demonstrate that the prevalence does not exceed 0.02% and a survey of the finishing pigs to demonstrate that the prevalence does not exceed 0.01%. Following this, a serological survey of the slaughter sow population must be conducted every three years to demonstrate freedom at a target prevalence of 0.2% and a tri-annual survey of the finishing pigs must demonstrate freedom at a target prevalence of 0.5%. Here it must be noticed that the OIE requirements for obtaining freedom from *Trichinella* are less strict than the EU requirements.

The OIE also proposes two concepts to facilitate obtaining a favorable disease status for selected areas or products from a country (85). First, the OIE used the concept of zoning. Zoning means that a clearly defined area with a clearly defined animal population within a country can achieve a different (usually higher) animal health status with regard to a specific disease, when the required surveillance, control and biosecurity measures have been implemented (85). The possibility of obtaining a negligible risk status according to EU regulation 2075/2005 can be interpreted as a possibility for zoning. Second, compartmentalization means that a clearly defined animal population in one or more establishments with a common, appropriate biosecurity system and appropriate surveillance and control measures can achieve a different (higher) animal health status with regard to a specific disease than animals outside of these defined establishments (85, 72). The concept of *Trichinella*-free holdings in EU regulation 2075/2005 is an example of compartmentalization.

Switzerland

Prior to 2007, testing of pigs for *Trichinella* spp. was not compulsory, because *Trichinella* was not considered to be a problem in domestic pigs (29). Nevertheless, tests were conducted in several export-approved slaughterhouses and also these results indicated that *Trichinella* was not present in the domestic pig population (7, 8). Since 2007, Switzerland has implemented EU Regulation 2075/2005 and initiated routine *Trichinella* testing at slaughter, as part of the bilateral agreements with the EU about equivalence of the animal health situation (3). Clearly, this led to an increased use of human and financial resources for testing of pigs, without an obvious improvement of public health, and therefore the Swiss Federal Veterinary Office was interested in exploring possibilities for implementing an alternative *Trichinella* surveillance system.

Concepts for surveillance

Conventional surveillance

When discussing surveillance, first the difference between monitoring and surveillance must be established, because these definitions have often been used interchangeably and thus caused confusion. Monitoring was defined as “a continuous, dynamic process of collecting data about health and disease and their determinants in a given population over a defined period of time”, whereas surveillance was defined as “a specific extension of monitoring where obtained information is used and measures are taken if certain threshold levels related to disease status have been passed” (12).

In a conventional monitoring or surveillance program, a specific disease agent or disease is selected and samples are collected from the population under observation to observe the occurrence of this disease or disease agent. The purpose of the program may be to determine the prevalence or incidence or to demonstrate absence of disease, and the required sample sizes are calculated accordingly adjusted for the size of population under observation (12, 51, 69). Samples are then collected from randomly selected animals.

Though conventional monitoring and surveillance programs have been very useful, they have a few important disadvantages. First, the results of the program may not reflect the true situation in clearly defined subgroups of the total population (4, 13). Certain subgroups may have a much higher or lower probability of being infected or diseased than the overall population. For example, the probability of detecting bovine spongiform encephalopathy (BSE) infected cows was increased significantly when the surveillance was targeted on defined risk categories (6, 13). Detailed information about disease occurrence in specific subgroups is important to tailor risk management measures to those groups where measures are needed and so increase their efficiency. Second, when dealing with rare diseases or with diseases that are considered to be absent from the population, the required sample sizes for the overall population are extremely large (12). Monitoring and surveillance programs of such volumes require large amounts of human, financial and logistical resources. Therefore, methods have been developed to increase the efficiency of traditional monitoring and surveillance approaches.

Risk-based surveillance

Risk-based surveillance was defined as “a surveillance program in the design of which exposure and risk assessment methods have been applied together with traditional design approaches in order to assure appropriate and cost-effective data collection” (75).

Risk-based tools for surveillance can be applied at a strategic level, by applying risk assessment techniques to determine which disease agents or hazards should be included in the national disease surveillance program, for example by prioritizing hazards (75). In Belgium, 51 foodborne zoonotic agents were prioritized using a semiquantitative approach based on criteria for their impact on public health, animal health and food. Four agents were identified that were considered to be of high importance and that should be included in surveillance activities (10). Prioritizations of foodborne zoonotic agents in meat were also conducted in several other studies (76, 19, 39). Prioritization of animal diseases was done less frequently, but for example New Zealand conducted a qualitative risk assessment for the importation of freshwater prawns from Hawaii to evaluate possible health hazards for these

prawns and to propose risk management measures including guidelines for surveillance where necessary (5). Currently, application of risk-based tools at a strategic level is not yet very common.

Risk-based tools can also be applied at an operational level, meaning within a surveillance program for a selected disease or disease agent (75). This application is more common than at the strategic level. Probably the most well-established surveillance program using risk-based tools is surveillance for BSE. In the earlier studies, groups of cattle with a higher probability of being infected were determined and these groups were targeted for surveillance (6, 13), later a model was developed in which points were given for each tested cow. The number of points depended on the probability that a cow would be infected and infection would be detectable (59, 60). However, risk-based surveillance systems were also developed for tetracycline residues in calves (61), viral hemorrhagic septicemia in freshwater fish (26) and paratuberculosis in cattle (34), among others.

The goal of risk-based surveillance is to increase efficiency of the surveillance activities, while maintaining or increasing the efficacy (75). However, to demonstrate this, tools are needed that allow comparing results from different surveillance strategies. A scenario tree approach was proposed to compare the sensitivity of different surveillance systems and thus the capacity of surveillance systems to detect infection or disease when it would be present (38), and this approach was already applied to different case studies of contagious diseases (28, 37, 41). But also surveillance efforts for non-contagious hazards can be compared, as was demonstrated using a different technique for tetracycline residues in slaughtered calves in Switzerland (61).

When several surveys are conducted consecutively, information about disease occurrence or absence of disease that was obtained from the first survey could be used in the design and analysis of the next survey (9). This technique can be used twofold. First, the sample size in the second survey can be reduced, while maintaining the same level of confidence as if two independent, consecutive surveys with the same sample sizes would have been conducted. This technique was used to design surveys to document freedom from non-highly contagious animal diseases in Switzerland, and it was demonstrated that the sample sizes in the second survey could be reduced by 60-80% (27). Later, this technique was further developed to take into account more than two consecutive surveys (71). Second, the sample size in the consecutive surveys can be kept constant, but the confidence in the outcome of the survey (for example freedom from disease) increases with every survey. This approach was demonstrated using the example of classical swine fever in Denmark (37). This approach

may also be of great value for small animal populations or for surveillance for rare or absent disease agents, because in these two situations the required sample size may sometimes be larger than the available number of animals. With such an approach discounting for time, freedom from disease does not need to be demonstrated in one single survey, but can be demonstrated using several consecutive surveys.

Another important component of any, including risk-based surveillance system is the choice of the diagnostic test. Depending on the design of the system, it can be decided to test for the disease agent directly or indirectly via antibodies. The efficacy of the selected diagnostic test may vary over time depending on the infection status of the selected animal (77). For example, as stated earlier, seroconversion in pigs following a *Trichinella* infection occurs after 17-60 days, depending on the number of larvae ingested and the *Trichinella* species involved (21, 31, 40, 43, 49, 73, 74, 81). This means that serological tests are not suited to detect infected pigs during the early stages of infection. However, once seroconversion occurred, ELISAs are able to detect infected pigs even when they harbor very low larval densities (21, 50). The artificial digestion technique can detect infected pigs once larvae are embedded and encapsulated in muscle tissue, however, infected pigs can only be detected reliably using 1-gram diaphragm samples when they harbor more than 3-5 LPG (18, 68). This sensitivity is considered sufficient to prevent human clinical disease (15, 20), but since 15-20% of naturally infected pigs harbor larval densities of less than 1 LPG (58), many infected pigs may not be detected using this technique. The selection of the appropriate diagnostic techniques must therefore be seen in conjunction with the goal of the surveillance activities.

Goal and overview of this thesis

Goal

The Swiss Federal Veterinary Office considered the Swiss domestic pig population to be free from *Trichinella* spp., and therefore considered that the implementation of routine *Trichinella* testing at slaughter lead to an increased use of resources without leading to a significant higher level of public health protection. The Swiss Federal Veterinary Office therefore wanted to consider alternative surveillance approaches for *Trichinella* spp.

The overall goal of this thesis was to develop a risk-based surveillance system for *Trichinella* spp. in Switzerland. To achieve this, four intermediate goals were defined:

1. To determine the prevalence of *Trichinella* spp. in Swiss wildlife;
2. To demonstrate freedom from *Trichinella* infection in the domestic pig population;
3. To assess the probability of human exposure to *Trichinella* spp. in Switzerland, and to determine the most probable routes of human exposure;
4. To design a risk-based surveillance model for domestic pigs that can demonstrate freedom from *Trichinella* infection with at least an equivalent level of confidence as the current meat inspection procedures.

Overview

A system to demonstrate freedom from infection should make use of the most appropriate test for this purpose. For reasons laid out above, serology appears as the most appropriate technique for *Trichinella* surveillance in pigs.

First, we addressed the problem that current ELISAs have imperfect specificity, and that false-positive results will therefore occur. Positive ELISA results may be confirmed by applying the artificial digestion test to a sample of diaphragm tissue. However, the amount of required tissue is not standardized, the larval density in the tissue may be too low for reliable detection or it may be impractical to collect tissue samples in the framework of a serological surveillance program. Therefore, a confirmatory test based on serology would be a very useful tool for a serological surveillance program for *Trichinella* spp. We considered to use a Western Blot as a confirmatory test. The results of this study are presented in the chapter **“Validation of a Western Blot for the detection of anti-*Trichinella* spp. antibodies in domestic pigs”**.

Second, we assessed the test characteristics of two ELISAs for *Trichinella* diagnostics. Neither one could be considered a true gold standard, and we used Bayesian techniques to evaluate tests in the absence of a gold standard. The results of this study are presented in the chapter **“Evaluation of a new commercial enzyme-linked immunosorbent assay for the detection of porcine antibodies against *Trichinella* spp.”**.

The presence of *Trichinella* spp. in wildlife is not only of importance for the domestic pig production, but also for hunters and other consumers of game meat. The *Trichinella* species present in wildlife and the prevalence level are two factors that determine the probability that infection will spread from wildlife to domestic pigs or consumers. We conducted surveys in foxes, lynxes and wild boar in Switzerland. The results of these surveys are presented in the chapters **“Assessment of the prevalence of *Trichinella* spp. in red foxes and Eurasian**

lynxes from Switzerland” and **“Occurrence of *Trichinella* spp. in wild boar in Switzerland”**.

Risk-based surveillance of domestic pigs can only be considered if it is first demonstrated that *Trichinella* infections are not present in pigs. The results of the meat inspection are a first indicator that *Trichinella* is indeed absent, but low-grade infections may not be detected using the routine artificial digestion technique. For this purpose, serological techniques are needed. The design of a survey should ensure that all risk groups within the pig population are properly represented. In the chapter **“A study to demonstrate freedom from *Trichinella* spp. in domestic pigs in Switzerland”** we present the results of a serological survey of 20,000 finishing pigs, breeding sows and free-range pigs.

Domestic pigs are not the only possible source for human exposure to *Trichinella* spp. Other animal species, including wildlife and horses, must also be considered. In order to protect public health, risk management measures should therefore be focused on those animal species that are the most likely source of human exposure. We conducted a risk assessment to evaluate through which routes consumers in Switzerland could be exposed to *Trichinella* spp. and to evaluate the probability of each of these routes. The results of this assessment are presented in the chapter **“Qualitative assessment of the probability of human exposure to *Trichinella* spp.”**.

Finally, we had all required elements to design a risk-based surveillance model for *Trichinella* spp. in domestic pigs: appropriate diagnostic techniques, knowledge about the infection pressure in wildlife, knowledge about the high-risk categories within the pig population and information that infection was not present in the pig population. The risk-based surveillance program should provide at least an equivalent level of confidence that *Trichinella* infection is truly absent from the pig population as the current meat inspection program. We therefore designed a model for a risk-based surveillance based on serology and we compared the outcomes of this model with the outcomes of the meat inspection as it is currently conducted. The results are presented in the chapter **“Comparing the demonstration of freedom from *Trichinella* infection of domestic pigs by traditional and risk-based surveillance”**.

In the last chapter **“Discussion”** we discuss the implications of this research for Switzerland and we put these results in the context of recent developments in the EU.

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Approaches to investigate *Trichinella*-infections in domestic and wild animals

Based on:

Methoden zur Untersuchung von *Trichinella*-Infektionen bei Haus- und Wildtieren

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Summary

Trichinellosis is an important parasitic zoonosis that is caused by the intracellular nematode *Trichinella* spp. Infection of humans occurs through consumption of raw (or undercooked) meat containing infectious larvae. In Europe, meat from pork, horse, and wild boar have been identified as most important sources of *Trichinella* infections in humans. In Switzerland, both the domestic pig and wild boar population are considered free of *Trichinella*. Conversely, Swiss foxes, lynxes and recently a wolf were found to be infected; the species identified in these animals was always referred to as *Trichinella britovi*. Although this species rarely infects pork and, compared to *Trichinella spiralis*, only causes reduced pathogenic effects in humans, the basic presence of *Trichinella* in Switzerland cannot be neglected. This fact has gained increasing importance since the responsible authorities in the European Union (EU) are preparing regulations for the official *Trichinella*-control in meat in order to improve food safety for consumers. These regulations will be implemented as a consequence of the recent association of east European countries with the EU. This new legislation particularly takes into account that, in the past, most cases of human trichinellosis in the EU were due to consumption of imported eastern European meat. Within the framework of the bilateral agreements of Switzerland with the EU, the Swiss veterinary public health authorities will have to comply with the foreseen EU regulations. Although diagnostic methods for the direct demonstration of *Trichinella* in pork meat are already routine practice in several Swiss abattoirs, the implementation of a meat control program for *Trichinella* for the entire slaughter pig population of the country would lead to an enormous increase in costs for the administration and would require an increased infrastructure in veterinary services. In order to find a reduced testing format for monitoring *Trichinella* infections in Swiss pork, a risk-oriented survey strategy is currently being evaluated. In the present article, this minimized survey strategy is discussed regarding its compatibility with the EU regulations for the official control of meat for *Trichinella*.

Introduction

Trichinellosis is worldwide considered to be one of the major parasitic zoonotic diseases that can cause serious clinical disease in humans (1). Even though the natural reservoirs for infection are mainly found in wildlife, domestic pigs can also become infected, and on a global scale, domestic pigs are the most important source of infection for humans. Therefore, human infections are most prevalent in countries with a nonexistent or weak meat inspection system. In many countries, compulsory meat inspection of susceptible animal species has therefore been implemented to prevent human infections. Despite these efforts at least in certain areas where *Trichinella* infections in animals are endemic, human infections still occur regularly. For example, in many Eastern European countries the prevalence of *Trichinella* infections in both humans and animals suddenly increased in relatively recent times. Following the accession of Eastern European countries, these developments posed serious problems for the European Union (EU) for the internal meat market of the EU as well as the exportation of pork to non-EU countries. Therefore, the relevant EU regulations were adapted to take into account the higher number of *Trichinella* infections in the new member states (10, 16). Efforts were also made to define *Trichinella*-free regions in the EU. However, current efforts focus more on the use of a certification system to identify *Trichinella*-free pig farms (3, EU-Regulation 2075/2005).

Distribution and presence in Europe and Switzerland

On a global scale, domestic pigs are the most important source of infection in a domestic cycle with *Trichinella spiralis* (5). Human infections are caused by the oral intake of meat containing larvae that was insufficiently cooked. Various EU member states have reported cases of human trichinellosis over the last 25 years (13), with Spain, France and Italy being the most frequently affected. The newer, Eastern European EU member states have also reported several outbreaks of human trichinellosis. Pork was often identified as the source of infection, but infected horsemeat has also been implicated in several human outbreaks (13). Other outbreaks were traced back to consumption of game meat. Table 1 presents an overview of selected outbreaks in Europe.

The sylvatic cycle is maintained by carnivorous wildlife predating on other carnivorous wildlife species. In Switzerland and its neighbouring countries, foxes and lynxes are mainly affected by *Trichinella britovi* (7, 9). Carnivorous birds may play a role in the transmission of *Trichinella pseudospiralis*, but this *Trichinella* species can also occur in the domestic cycle,

as was recently demonstrated in the Slovak Republic (8). Muscle stage *Trichinella* larvae can survive in dead animals and decaying tissue for weeks or months, and can therefore easily infect scavenging animals.

The presence of *Trichinella* spp. in domestic pigs varies significantly between countries. In the USA, between 0.1-4% of the pigs are infected (14), and as a result, approximately 40% of the human trichinellosis cases can be traced back to pork. Around 50% of the human cases in the USA are related to the consumption of bear meat (<http://www.cdc.gov/mmwr/preview/mmwrhtml/ss5206a1.htm>). At the other end of the scale is Germany, where only one infected pig was found among 210 million tested pigs between 1999 and 2003 (K. Nöckler, pers. comm.). Relatively high numbers of infected pigs are found in Eastern Europe. The International Commission on Trichinellosis (ICT) reported a prevalence of 0.0008% in Poland for the period 1995-1997. In Russia, it is estimated that around 3-4 pigs per 1 million pigs are infected. In Southeastern European countries the prevalence was ranging from 0.16% (former Yugoslavia) to 5% of the domestic pig population.

In Switzerland, infected domestic pigs have not been found for over more than a century, and the domestic pig population can therefore be considered free from infection. More recent research studies have not revealed any infected pigs, and meat inspection of pig carcasses has also yielded no indications of *Trichinella* infections (7, 9).

Wildlife species such as wild boar, fox, wolf, marten, badger and lynx can be infected with *Trichinella* spp. In Germany, approximately 0.003% of wild boar are infected, in France 0.03%, in Italy up to 0.06%, in Spain between 0.08% and 0.48%, and in Finland 1.3% of the wild boar are infected (K. Nöckler, pers. comm.). In Switzerland, *Trichinella* infections in wild boar have not been found for many years. However, infection is known to occur in the lynx, fox and wolf populations. In all three of these species, only *T. britovi* has been found. Switzerland can therefore be considered free from *T. spiralis*, but not from *T. britovi*.

Infection cycle and disease symptoms

Today, 8 different species and 3 different genotypes of *Trichinella* are recognized (figure 1, table 2). Some species and genotypes have overlapping geographical distributions or host animals (13).

The entire development of *Trichinella* spp. occurs in the same host. A new host ingests larvae by oral intake of uncooked, or insufficiently cooked meat after which the larvae develop into their adult stage in the small intestines. Following replication, newborn larvae migrate to the muscle tissue via the blood vessel system. Once they have entered muscle tissue, the larvae develop into their muscle stage (figure 2). *Trichinella* larvae are mainly found in muscle tissues that are well supplied by blood, such as tongue, diaphragm and masseter. Within a month, the new muscle stage larvae have become infectious.

The disease symptoms and the course of disease of the host can vary depending on the infecting *Trichinella* species and the infectious dose that was ingested. Infected animals normally do not show any disease symptoms. An infectious dose of 70 to 750 *T. spiralis* larvae can already lead to clinical disease in humans (3), whereas a mouse does not show any clinical signs after infection with 1000 larvae. Typical symptoms in humans include eosinophilia, fever, oedema, myalgia, and muscle stiffness, and a heavy infection can even lead to a patient's death. Symptoms may be less or even absent following infections with *T. nativa*, *T. britovi* and *T. pseudospiralis*, the three other *Trichinella* species present in Europe. Also, the infectious dose for these three species that leads to clinical disease in humans is higher than for *T. spiralis*.

Diagnostic methods

Basically, there are three internationally recognized diagnostic techniques for the detection of *Trichinella* infections in animals, two of which are direct detection techniques. Trichinoscopy is a microscopic investigation of muscle tissue compressed between two glass plates. For pigs, mostly diaphragm tissue is used. This method has a rather low sensitivity, a detection limit of three larvae per gram of muscle tissue, and it is not suitable for a pooled sample procedure. A further disadvantage is that this method is not suitable for the detection of non-encapsulating *Trichinella* species, such as *T. pseudospiralis*. Direct detection of the parasite is therefore more commonly done using the artificial digestion technique. The alternative to direct detection is indirect detection using serological techniques, such as ELISA and/or Western Blot (6, 11).

In the artificial digestion technique meat samples are digested artificially using pepsin. This treatment releases the *Trichinella* larvae from the muscle tissue and subsequently they can be detected microscopically. In larger slaughterhouses, the artificial digestion technique is usually automated to allow the processing of larger sample volumes. When single samples of

20 grams are tested, the sensitivity of this method is 0.05 larva per gram of muscle tissue. Using pooled samples with pools of up to 100 samples of 1-5 gram each, the sensitivity is currently estimated at around 3-5 larvae per gram (4). Earlier studies have demonstrated the detection of heavily infected pigs using the artificial digestion method around 17 days post infection. Detailed European guidelines have been established for the direct detection of *Trichinella* spp. in pigs (EU Regulation 2075/2005).

Serology can be used to detect antibodies against *Trichinella* spp. in serum, body fluid or meat juice. The most important serological test is an ELISA based on the excretory/secretory antigen of muscle stage larvae. This test is considered to have a very low limit of detection, because antibodies have been detected in pigs with larval densities of 0.1-0.01 larvae per gram muscle tissue. The specificity of ELISA is reasonable. The results of serological tests can nowadays even be further improved by the additional use of a Western Blot. Following a moderate to heavy infection of pigs, antibodies are detectable from around 14 days post infection. After a mild infection, seroconversion only takes place around 3-5 weeks post infection. In pigs, following seroconversion, antibodies are detectable during their entire lifetime.

The species-identification of isolated *Trichinella*-larvae is done using a multiplex-PCR and a subsequent gel electrophoresis, in which different *Trichinella*-species can be identified by their different banding patterns (figure 3). This procedure is currently only performed at the Swiss Reference Laboratory for Trichinellosis.

Disease control

Although trichinellosis has a worldwide distribution, infections are hardly found in the domestic livestock cycle in the EU. In 2003, around 140 million pigs were tested according to the results of an EU-wide monitoring program. Spain reported the highest number of infected pigs, namely 24 of 34.6 million tested pigs. Finland (2 pigs) and Germany (1 pig) were the only other 2 member states to report infected pigs in the same year.

In most EU-countries trichinellosis was already classified as a disease that had to be monitored. Suspect cases had to be reported to the competent authorities, but there was no obligation for further measures. Now, the European Commission has issued a new regulation on the monitoring of *Trichinella* spp. in meat (Regulation 2075/2005) to further improve food safety for European consumers. This regulation requires that all pigs, horses, wild boar and

other susceptible animals that are intended for human consumption must be tested for *Trichinella* spp. However, *Trichinella*-free pig holdings and categories of pig holdings can be recognized that can be subjected to a reduced testing program. *Trichinella*-free pig holdings would need to fulfill certain prerequisites, such as hygiene measures, traceability and absence of outdoor access for finishing pigs. In order to establish a category of *Trichinella*-free pig holdings, it must additionally be demonstrated with a 95% confidence that the prevalence of infection has not exceeded 0.0001% during the last 10 years. The new regulation also foresees the possibility of the recognition of regions with a negligible *Trichinella* risk. However, it does not specify details for the monitoring programs in such regions. The option of *Trichinella*-free countries has not been included because experts considered that it was impossible to determine regional freedom when the spread of infection in the wildlife cycle cannot be controlled. Therefore, it cannot be excluded entirely that infection can spill over from the wildlife cycle to the domestic cycle (http://www.efsa.eu.int/science/biohaz/biohaz_opinions/1281/biohaz_op_ej277_trichinella_fa_en1.pdf).

In contrast to the EU, the World Organisation for Animal Health (OIE) has provisions for the recognition of zones or countries free from *Trichinella* spp. in domestic pigs, even when infections occur in wildlife. To demonstrate freedom at the determined target prevalence levels, a monitoring and surveillance program must be implemented for finishing pigs and breeding sows. The OIE explicitly requires the use of serological techniques for the monitoring of breeding sows (12).

Outlook for Switzerland

Switzerland will implement the new EU regulations for the monitoring of *Trichinella* spp. in meat. However, recognition of *Trichinella*-free pig holdings or categories of pig holdings will not be possible in Switzerland, due to the structure of the Swiss pig production system with its many small pig farms, the common presence of outdoor access for finishing pigs, and the presence of *Trichinella* infections in wildlife. The implementation of routine testing for *Trichinella* spp. in all Swiss slaughterhouses will lead to an enormous increased use of financial, human and infrastructural resources. The associated benefits of such a full-scale program of routine testing, namely a reduction of human trichinellosis, are expected to be very low.

Given this imbalance between increased use of resources and lack of associated benefits, the possibilities must be explored to design an alternative, risk-based surveillance system for *Trichinella* spp. in Switzerland. A first step towards such a risk-based surveillance system should be to demonstrate freedom from infection in the domestic pig population. This freedom should be demonstrated using serological techniques because this approach would allow documenting freedom by demonstrating the absence of antibodies against *Trichinella* infections. Once freedom from infection in domestic pigs has been documented, targeted sampling of pigs with an increased risk of infection may reduce the size of the surveillance program.

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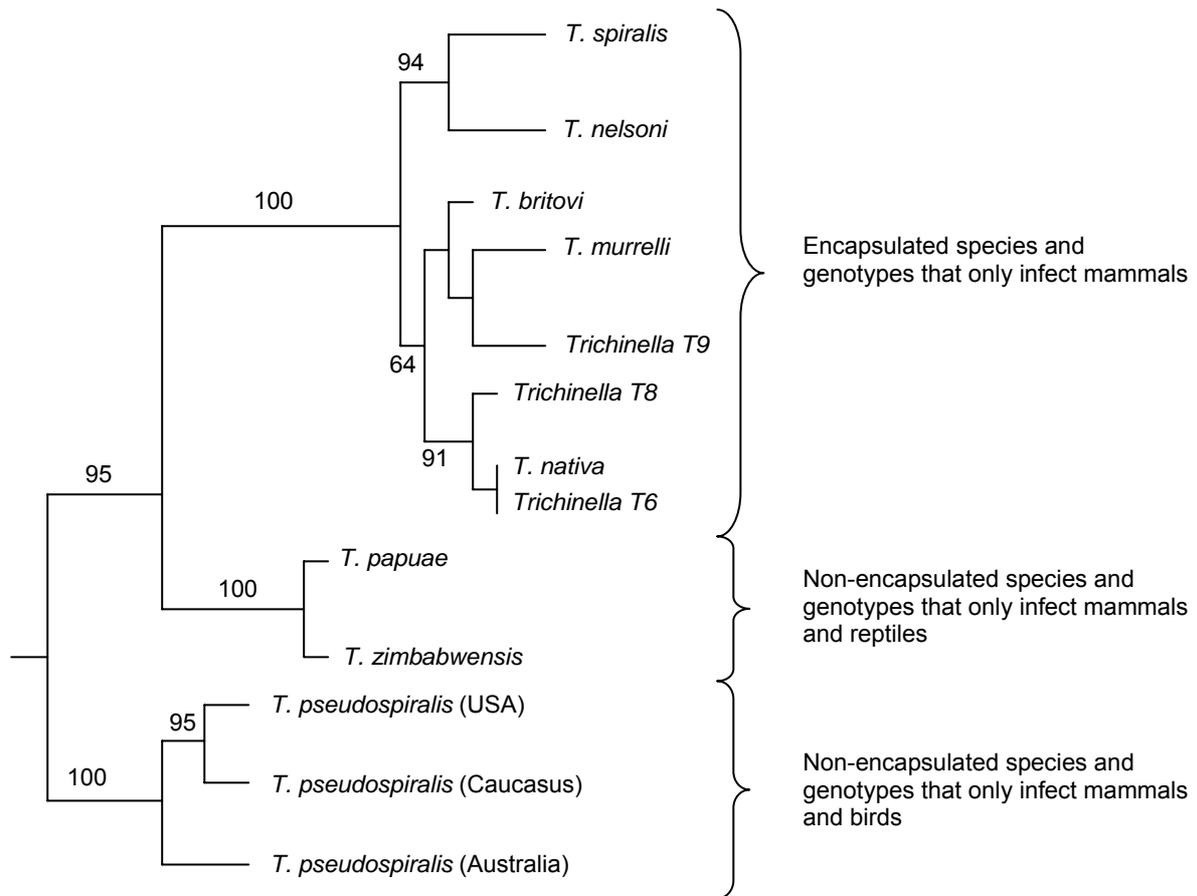


Figure 1. Phylogenetic tree of the currently recognized species and genotypes of *Trichinella* spp. (from [13])



Figure 2. *T. britovi* larvae that were isolated by artificial digestion, one is released from its capsule, the other is still in its nurse cell

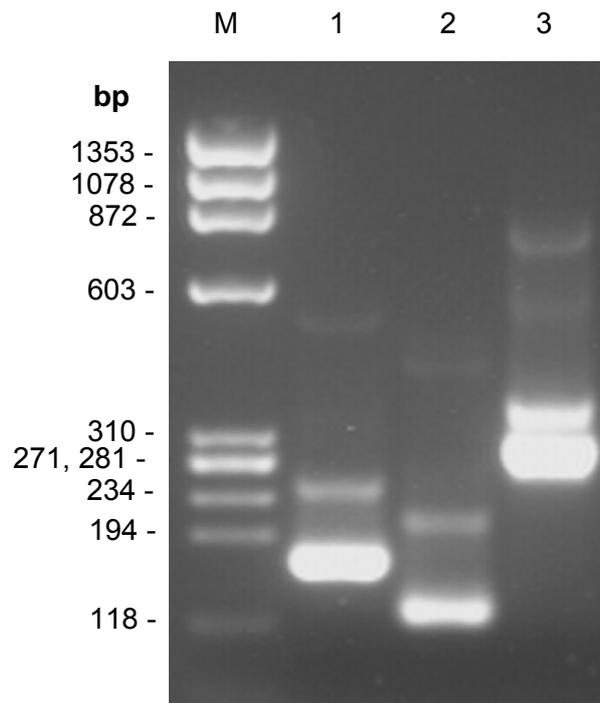


Figure 3. Agarose gel electrophoresis analysis of PCR products to identify *T. spiralis* (lane 1), *T. britovi* (lane 2) and *T. pseudospiralis* (lane 3). Indicated on the left is the size of the DNA fragments in base pairs. The main PCR products are 173 (*T. spiralis*), 127 (*T. britovi*) and 310 (*T. pseudospiralis*) base pairs.

Table 1. Selected outbreaks of trichinellosis in Europe, which were reported to the ICT* or PubMed between 1985-2006

Country	Number of outbreaks	Number of infected people		<i>Trichinella</i> -species	Source of infection (when known)
Germany	2	55	People	<i>T. spiralis</i>	Wild boar (Romania), ? ¹
France	7	239	People	<i>T. spiralis</i>	Horse (Poland, USA, Mexico, Serbia, Yugoslavia)
		9			
	1	4	People	<i>T. pseudospiralis</i>	Wild boar (France)
Italy	5	306	People	<i>T. spiralis</i>	Horse (Poland, ?), sausages (Eastern Europe)
	1	20	People	<i>T. britovi</i>	Wild boar (?)
	2	16	People	<i>T. britovi</i>	Pork (Sardinia)
Spain	6	114	People	<i>T. spiralis</i>	Wild boar (Spain), ?
Slovak Republic	1	336	People	<i>T. britovi</i>	Dog (Slovak Republic)
Poland	4	765	People	<i>T. spiralis</i>	Pork (Poland), wild boar (Poland)
Croatia	2	24	People	<i>T. spiralis</i>	Pork (Croatia)
Serbia	3	756	People	<i>T. spiralis</i>	Pork (Serbia), sausages (Serbia), ?
Romania	1	202	People	?	Pork (Romania)
		7			
Ukraine	10	136	People	?	Pork (Ukraine)
Former Sovjet rep.	10	669	People	?	Pork, bear, dog, badger
Lithuania		Ø 17-719 cases per year		?	Pork, wild boar (Lithuania)
Latvia		Ø 15-81 cases per year		?	Pork (Latvia)
Russia		Ø 470 cases per year		?	?

* International Commission on Trichinellosis

1) Source of infection could not be identified

Table 2. Known *Trichinella* species and genotypes (-) (from [11])

Trichinella-species	Genotype	Most important hosts	Geographic distribution
<i>T. spiralis</i>	T1	Pig, carnivores, rat, human	Cosmopolitan
<i>T. nativa</i>	T2	Carnivores (including bear), walrus, human	Arctic, holarctic
<i>T. britovi</i>	T3	Carnivores (Switzerland: fox, lynx), human	Temperate zones of the palearctic
<i>T. pseudospiralis</i>	T4	Mammals (incl human) and birds	Cosmopolitan
<i>T. murrelli</i>	T5	Carnivores, human	Temperate neoarctic North America
-	T6	Various mammals	Arctic and subarctic of America
<i>T. nelsoni</i>	T7	Carnivores (hyena, lion), suidae (warthog), human	Tropical Africa (east and south)
-	T8	Various mammals	South Africa and Namibia
-	T9	Various mammals	Japan
<i>T. papuae</i>	T10	Pig, human, reptiles	Papua New-Guinea
<i>T. zimbabwensis</i>	T11	Various mammals, crocodile	Subsaharan Africa

Validation of a Western Blot for the detection of anti-*Trichinella* spp. antibodies in domestic pigs

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Abstract

Trichinellosis is a zoonotic disease in humans caused by *Trichinella* spp. According to international regulations and guidelines, serological surveillance can be used to demonstrate the absence of *Trichinella* spp. in a defined domestic pig population. Most ELISA tests presently available do not yield 100% specificity, and therefore a complementary test is needed to confirm the diagnosis of any initial ELISA-seropositivity. The goal of the present study was to evaluate the sensitivity and specificity of a Western Blot assay based on somatic *Trichinella spiralis* muscle stage (L1) antigen using Bayesian modeling techniques. A total of 295 meat juice and serum samples from pigs negative for *Trichinella* larvae by artificial digestion, including 74 potentially cross-reactive sera of pigs with other nematode infections, and 93 meat juice samples from pigs infected with *Trichinella* larvae were included in the study. The diagnostic sensitivity and specificity of the Western Blot were ranged from 95.8 to 96.0% and from 99.5 to 99.6%, respectively. A sensitivity analysis showed that the model outcomes were hardly influenced by changes in the prior distributions, providing a high confidence in the outcomes of the models. This validation study demonstrated that the Western Blot is a suitable method to confirm samples that reacted positively in an initial ELISA.

Introduction

The nematode *Trichinella* spp. is the etiological agent of trichinellosis, a zoonotic disease (7). Many carnivorous and omnivorous animal species may become infected with *Trichinella* spp., including domestic pigs. There is a general agreement that animals do not get sick following infection. However, the course of infection in humans includes disease that can range from subclinical to fatal (7, 21, 29). In order to prevent disease in humans, susceptible animal species destined for human consumption must be tested for the presence of *Trichinella* spp. at slaughter or at game handling plants in the European Union (EU) and Switzerland (1, 8).

The method prescribed for routine testing of pigs is the artificial digestion method (8), which is typically applied by pooling up to 100 samples of at least 1 gram of diaphragm muscle tissue. The artificial digestion method is frequently considered to be the reference technique for detection of infected pigs. However, this test has limitations in terms of diagnostic and analytical sensitivity. Analytical sensitivity is defined as the smallest detectable amount of the specimen (34). The theoretical limit of detection for a 1 gram sample is considered to be 1 larvae per gram (LPG) (24). However, the diagnostic sensitivity, i.e. the proportion of known infected reference animals that are tested positive (34) by artificial digestion, is below 100% when samples of less than 5 gram are used (9).

The EU regulation as well as guidelines of the World Organisation for Animal Health (OIE) foresee the possibility of serological surveillance to demonstrate the absence of *Trichinella* spp. in a defined domestic pig population (8, 35). Several serological techniques were developed so far for detection of antibodies against *Trichinella* spp. Especially the enzyme-linked immunosorbent assay (ELISA) was regularly used for research purposes to evaluate the presence of anti-*Trichinella* antibodies in pig populations (10, 11, 25, 26). The diagnostic sensitivity and diagnostic specificity of such ELISAs do not yield 100% (11), however an advantage of ELISA tests is their higher analytical sensitivity in comparison to the routine artificial digestion method. ELISAs are able to detect antibodies in pigs with larval densities at least as low as 0.01 LPG (10, 34).

If the ELISA is considered to be used for routine serological surveillance purposes, a protocol should be developed to deal with samples reacting positively in the ELISA. ELISA-positive samples may be truly positive, when the sample originated from a truly infected pig.

However, due to imperfect specificity of the ELISA, a seropositive reaction in ELISA should be subjected to a confirmatory test before a final decision is made.

Confirmation could be done by artificial digestion of a larger muscle sample. Artificial digestion of a larger muscle sample is currently considered the reference method not only for routine testing, but also for confirmatory *Trichinella* testing of seropositive individuals. The OIE even recommends to use at least 100 gram of tissue to confirm seropositive findings (34). However, collection and storage of such large samples may prove impractical under the conditions of routine serological surveillance. Confirmation of ELISA results by an appropriate second serological method may therefore be of high practical value.

Western Blot assays are already used for diagnostic investigations in other parasitic diseases like echinococcosis and cysticercosis in humans (13, 23) or infections in cattle with *Neospora caninum* (33) and *Besnoitia besnoiti* (5). For serological testing in humans for *Trichinella* spp., commercialized Western Blot assays are routinely used as a confirmatory test to distinguish between patients with *Trichinella* infections and other helminth infections (21, 31, 37). Western Blots have also been used for the detection of anti-*Trichinella* antibodies in animals, mostly in pigs (22, 26, 27) and horses (30, 32, 36). The specificity of the Western Blot is considered to be very high (31, 37), but little is known regarding the sensitivity of this method.

Before the Western Blot can be used as a confirmatory method in routine serological surveillance, it must be validated. As laid out above, no “gold standard” serological test is available for such a validation, and also the routine artificial digestion test cannot be considered to be a gold standard test. In a situation of validation without a true gold standard, appropriate statistical methods should be used to correct the estimates of sensitivity and specificity of the new test for the imperfect reference test (34). Sensitivity and specificity of both tests can be estimated reliably if both tests are applied in two populations with a different prevalence (18, 19). The underlying assumption of this approach is that the sensitivity and specificity of the diagnostic tests are the same in both populations. By applying Bayesian techniques in the test validation, prior information about the sensitivity and specificity of the diagnostic test can be used to improve their posterior estimates (3). Most models assume that the results of the different tests are independent from each other, conditional on the infection status (18, 19). This assumption is valid when the Western Blot assay is compared with the artificial digestion technique, because the first test is based on antibody detection, whereas the second is based on detection of the infecting agent. However, when the tests are conditionally dependent, the outcomes of the validation may be

biased if the model is not corrected for this conditional dependence (3, 14). This could be the case when the Western Blot assay is compared with the ELISA, because both tests are based on antibody detection.

The goal of this study was to validate a Western Blot assay for the detection of anti-*Trichinella* antibodies in domestic pigs. Bayesian modeling techniques were used to account for the absence of a gold standard test, as well as to correct for conditional dependence between serological tests.

Materials and methods

Test samples

The minimum required sample size was calculated using standard epidemiological techniques as 70 samples for each of the groups finishing pigs, free ranging pigs, adult pigs, potentially cross-reactive samples, and *Trichinella*-positive pigs, based on an assumed sensitivity and specificity of the Western Blot of 99.9%. This would allow determination of sensitivity and specificity with a precision of 1% and a 99%-confidence interval. No quantitative prior estimates for the sensitivity and specificity of the Western Blot were available, therefore the assumption was based on personal judgement of the authors.

A total of 295 *Trichinella*-larvae negative (221 meat juice and 74 serum samples) and 93 *Trichinella*-larvae positive meat juice samples were included in the evaluation study (Table 1). In the period of January-March 2007, a total of 221 diaphragm tissue samples of 20 gram each were collected at Swiss slaughterhouses from finishing pigs, free ranging pigs and adult breeding pigs. The infection status of the pigs was determined at the slaughterhouse by routine pooled artificial digestion of 1 gram (finishing pigs) or 2 gram diaphragm tissue (adult pigs). All samples were negative for *Trichinella* larvae. Meat juice samples from these 221 pigs were used for the serological analysis. Additionally, 74 serum samples from *Trichinella*-negative pigs which were known to be infected with other nematodes were used. These samples had been collected within the framework of a study (porc'99) designed to monitor the health status of the Swiss pig population (4, 16, 17). The coprological detection of intestinal helminth infections had been carried out with a conventional sedimentation and flotation technique (2).

Seventy-nine meat juice samples from naturally infected pigs from Italy and Croatia were included in the study. Larvae could be isolated from 60 naturally infected pigs and had been

identified as *T. spiralis* (58 samples) and *T. britovi* (2 samples) using a multiplex PCR (38). Finally, 14 meat juice samples from experimentally infected pigs were included in the validation study. Pigs had been inoculated with *T. spiralis* (12 pigs), *T. pseudospiralis* (1 pig) or *T. britovi* (1 pig). Additional details on the meat juice and serum samples used for this study are provided in Table 1.

ELISA

For the in-house ELISA, E/S antigen coated plates and positive and negative control sera of experimentally infected pigs were provided from BfR, Berlin, Germany (10, 12, 26). Serum samples and controls were diluted 1:100 in phosphate-buffered saline containing 0.05% Tween 20 (PBS-T), pH 7.2. Meat juice samples were diluted 1:10 in PBS-T. Of each of the samples and controls, 50 µl were added per well. The plate was incubated for 30 minutes at 37 °C and then washed three times with PBS-T. 50 µl of peroxidase-labelled IgG-conjugate (Sigma Cat No: A5670) (diluted 1:4000 in PBS-T) were added to each well, followed by incubation for 30 min at 37°C. After incubation, microtiter plates were washed three times with PBS-T. Then, 50 µl of freshly prepared developing solution was added, prepared by dissolving one ABTS (2,2'-azino-di (3-ethylbenzthiazoline-6-sulfonate di-ammonium) tablet (Roche, Switzerland) into 50 ml of ABTS buffer (Roche, Switzerland). After an incubation of 15 min at room temperature (RT), the absorbance values were read at 405 nm. The results were calculated as percentage positivity (PP) based on the A_{405nm} of the positive control on the plate:

$$\frac{A_{405nm}^{Sample}}{A_{405nm}^{PositiveControl}} * 100\% = X\% PP$$

The cut-off was set at 23 % PP after ROC analysis (data not shown). Results obtained above or equal the cut-off of 23% PP were considered positive. Results obtained below the cut-off of 23% PP were negative.

Western Blot

In order to generate somatic antigens for the Western Blot, muscle larvae of *T. spiralis* were recovered by HCl-pepsin digestion from experimentally infected mice. These mice had been orally infected with 500 *T. spiralis* L1 at least 3 months before their euthanasia. The larvae were washed in PBS, supplemented with 0.2 mM of proteinase inhibitor phenyl methyl sulfonyl fluoride (PMSF), freeze-thawed three times and then sonicated (2 x 30 sec, 70 W, 0°C). After centrifugation (1000 x g, 30 min, 4°C), the supernatant was separated on a 10% SDS-PAGE gel under reducing conditions (33) and subsequently electrophoretically blotted

on nitrocellulose membranes (pore size 0.45 µm; Whatman, Cat No: 10401196). The membranes were cut into 17 strips each. Positive and negative control sera of experimentally infected pigs were obtained from BfR, Berlin, Germany. Meat juice samples were diluted 1:50 in PBS with 0.3% Tween 20 and 2% fish gelatine (PBS-T-G), serum samples were diluted 1:50 in PBS-T-G and 5% milk powder. Strips were incubated with 500 µl of the diluted samples for 2 hours at RT, before the sample liquid was removed and the strips were washed five times with PBS-T pre-warmed to 45°C. Protein A-conjugate (Calbiochem, Switzerland Cat No: 539253) was diluted 1:10'000 in PBS-T, added to each strip and incubated for 1 hour at RT. Afterwards, strips were washed four times with PBS-T and two times with PBS. Subsequently, the substrate was added (1 tablet of 4-chloro-1-naphtol (Sigma Cat No: C6788) diluted in 10 ml methanol, 30 ml PBS and 40 µl 30% H₂O₂ and incubated for 10 minutes at RT. The strips were washed with deionised water, dried with filter papers and the banding pattern was interpreted in comparison with the positive and negative controls. A Western Blot was considered positive when the following banding pattern became apparent: within a pattern of bands localizing between 35 and 65 kD, specific bands localized at 47 kD, 49 kD and 52 kD and two specific bands at 60 and 63 kD had to appear qualitatively, independent of the banding staining intensity (see figure 1) (6, 28, 31).

Confirmatory artificial digestion

Individual artificial digestion tests were performed with at least 20 grams of diaphragm per animal to be tested. The artificial digestion (magnetic stirrer) method was carried out according to the EU regulation (8).

Sequence of diagnostic tests

First, the infection status of all samples had been determined using artificial digestion. This infection status was later used in Bayesian modelling to divide the samples into a non-infected population (no larvae detected by artificial digestion) and an infected population (larvae detected by artificial digestion) (see also section below). Subsequently, all samples were tested in parallel by Western Blot and ELISA. Finally, an additional confirmatory artificial digestion was conducted for samples that were negative in routine artificial digestion of 1 or 2 gram, but positive in at least one serological test.

Statistical analysis

Data were analysed using NCSS 2007 (NCSS Statistical Software, Kaysville, Utah, USA), and the freeware WinEpiscope 2.0 (available through www.vetschools.co.uk/EpiVetNet/Epidemiological_analysis_software.htm) and WinBUGS 1.4

(available through www.epi.ucdavis.edu/diagnostictests/software.html). Beta distributions were established with the freeware BetaBuster.

In an initial step, the sensitivity and specificity of the Western Blot were calculated in NCSS 2007 using deterministic techniques based on the assumption that the artificial digestion technique acted as gold standard with a perfect sensitivity and specificity. In a second step the sensitivity and specificity of the Western Blot were modelled against the artificial digestion test using Bayesian techniques in WinBUGS as described by Branscum et al. (3). Here, the model for “2 independent tests - 2 populations - no gold standard” was selected, because it was considered that the artificial digestion test and the Western Blot were conditionally independent from each other. The parameterization for this model is given in Branscum et al. (3). The appropriate programming code for this and other models can be found on the website given above.

In this model, the specificity of the artificial digestion method was still considered to be perfect, but the model allowed the sensitivity of the artificial digestion test to vary. The model was run with 100,000 iterations including a burn-in phase of 2,000 iterations. The prior distribution for the sensitivity and specificity of the Western Blot had mode=0.90 with a 10th percentile=0.60, resulting in a Beta distribution with $\alpha=6.05$ and $\beta=1.56$. The same prior distribution was selected for the sensitivity of the artificial digestion test. These prior distributions were selected, because it was considered reasonable to assume that the sensitivity and specificity of the diagnostic tests would be well above 0.50, but the model still had enough freedom to vary. The prior distribution for the prevalence in the *Trichinella*-negative pig population had mode=0.02 with a 95th percentile=0.1 (Beta(1.84, 42.11)). The prior distribution for the prevalence in the *Trichinella*-positive pig population had mode=0.98 with a 5th percentile=0.90 (Beta(42.11, 1.84)). These prior distributions were selected to stress that infection was considered completely absent from the first population, and present in the second.

The sensitivity and specificity of the Western Blot were also modelled against the ELISA test. Here, a “2 dependent tests - 2 populations - no gold standard” model was selected (3). The model was also run with 100,000 iterations including a burn-in phase of 2,000 iterations. The prior distributions for the sensitivity and specificity of the ELISA had mode=0.90 with a 10th percentile=0.60 (Beta(6.05, 1.56)). The prior distribution for the prevalence in the *Trichinella*-negative pig population had mode=0.02 with a 95th percentile=0.1 (Beta(1.84, 42.11)). The prior distribution for the prevalence in the *Trichinella*-positive pig population had mode=0.98

with a 5th percentile=0.90 (Beta(42.11, 1.84)). Prior distributions for the correlation parameters (λ , γ) had mode=0.90 with a 5th percentile=0.10 (Beta(1.32, 1.04)).

Alternative models were developed to assess the sensitivity of the two models regarding changes in the prior distributions. Prior distributions for the sensitivity and specificity of the diagnostic tests were narrowed, and the prior distributions for the prevalence were widened. Also, the models were adapted to specifically allow a zero-prevalence in the *Trichinella*-negative population ($\tau < 1$). Finally, a “3 tests - 1 population - no gold standard” model was selected (3), in order to analyse the data as a single data set with three different diagnostic techniques. Prior distributions for the sensitivity and specificity of the diagnostic tests were as described above. The prior distribution for the prevalence had mode=0.25 with a 90th percentile=0.70 (Beta(1.45, 2.34)).

Results

The results of the Western Blot and ELISA tests are presented in Table 2. In the Western Blot, positive samples were characterized by a pattern of specific bands localizing between 35 and 65 kD (figure 1). If the artificial digestion test was assumed to have a perfect sensitivity and specificity (deterministic model, Table 3), the sensitivity of the Western Blot was 96.8% and its specificity was 100.0%. Three digestion-positive samples in which no anti-*Trichinella* antibody was detected by the Western Blot originated from pigs naturally infected with *T. spiralis* with larval densities of 0.4, 1 and 32 LPG, respectively. The Western Blot did not detect anti-*Trichinella* antibodies in any of the samples from pigs with other nematode infections. Based on the same assumption of a perfect artificial digestion test (deterministic model, Table 3), the in-house ELISA test had a sensitivity of 97.8% and a specificity of 98.6%. Two digestion-positive samples in which no anti-*Trichinella* antibody was detected by the in-house ELISA originated from pigs naturally infected with *T. spiralis* having a larval burden of 0.025 and 0.4 LPG, respectively. The sample containing 0.4 LPG had not been detected by Western Blot either. The ELISA reacted positively with four digestion-negative samples originating from breeding sows. For three of these samples, additional diaphragm tissue was available for a confirmatory artificial digestion test. In none of the samples, larvae were detected. The ELISA test classified all samples from pigs with other nematode infections as negative for anti-*Trichinella* antibodies.

Subsequently, the sensitivity and specificity of the Western Blot were modeled while allowing the sensitivity of the artificial digestion test to vary. Results are presented in Table 3. Various

models were run to evaluate the sensitivity of the model to the selected parameters. The results showed that the sensitivity and specificity of the Western Blot were slightly overestimated under the assumption of a perfect artificial digestion test. The results also showed that the diagnostic sensitivity of the routine artificial digestion test was below 100%, even when the larval density of the samples exceeded the limit of detection, implying that routine artificial digestion does not detect all infected pigs. Under the assumption of imperfect sensitivity of the artificial digestion test, the sensitivity and specificity of the Western Blot were 95.8% and 99.6% respectively, and the sensitivity of the artificial digestion test was 98.8%.

The level of agreement between the Western Blot and the ELISA was good; the Kappa value was 0.95 (CI: 0.85-1.00).

The sensitivity and specificity of the Western Blot were modeled against the results of the ELISA, assuming conditional dependence of the two tests (Table 4). The sensitivity and specificity of the Western Blot were 96.0% and 99.5%, respectively, the sensitivity and specificity of the ELISA were 97.4% and 98.3%.

The results of the “3 tests - 1 population - no gold standard” model were very similar to the results presented above (data not shown).

Discussion

The Western Blot validated in this study was methodically based on using antigens derived from mature whole muscle larvae of *T. spiralis* (crude worm extract/CWE). Several studies demonstrated that CWE is useful for Western blot analysis for anti-*Trichinella* antibodies (20, 22, 26, 30). Although CWE may present a more complex banding pattern than E/S-antigen, and may thus be more difficult for determining the exact molecular mass of all bands that react specifically with anti-*Trichinella* antibodies, there are several arguments in favor of CWE. First, the dominant antigenic E/S components are part of CWE, as shown by Pozio et al. (30). Gruden-Movsesijan et al. (15) demonstrated three bands (45, 49 and 53 kDa) to range among the dominant E/S antigens, all three appear also in CWE. A similar banding profile was shown by Denkers et al. (6) in a mouse model, and by Özkoç et al. (28) with human trichinellosis patients. The pattern of the major bands we demonstrated in the present paper matched also that revealed by Robert et al. (31), Denkers et al. (6) and Özkoç et al. (28). Robert et al. (31) were able to specifically discriminate between a true anti-*T. spiralis*

humoral immune response in humans from potential cross-reactive sera due to autoimmune or other parasitic diseases based upon immunoreactivity to a 47-55 kDa banding pattern. Similar to our findings with pig sera, Robert et al. (31) revealed some minor bands that cross-reacted with antibodies from sera originating from patients with other helminthic diseases. Thus, unspecific or cross-reactive banding activity can occur (26, 30), but can be distinguished upon the overall specific banding pattern characteristic for trichinellosis. E/S antigen that is also widely used for Western blot (26, 30) is, however, more time consuming, laborious and also more expensive in production when compared to CWE.

Three samples that were *Trichinella*-positive in the artificial digestion test, were subsequently seronegative in Western Blot analysis. One of these samples was also negative in the ELISA. These three samples were obtained from pigs originating from various places, and all three specimens had been stored for an extended period of time prior to the present investigation. This is in contrast to the samples of negative pigs, which were all freshly collected in Swiss slaughterhouses and directly thereafter properly stored for a short time period prior to laboratory testing. Due to the extended storage period and multiple freezing and thawing processes prior to our laboratory testing, the quality of the *Trichinella* positive serum samples may have degraded over time. The aspect of storage of diagnostic samples may require further respective investigations, as it may be an important parameter to standardization of serology, especially if samples are collected under rough field conditions such as slaughterhouses.

The estimated sensitivity and specificity of the Western Blot did not vary much between the deterministic and Bayesian approaches. However, the results of the Bayesian modeling provided a higher confidence in the results. If a new test is validated against an existing test that is erroneously assumed to be a gold standard, the estimated sensitivity and specificity of the new test will be biased (18). Our Bayesian models were run under the assumption that the reference test (artificial digestion test or ELISA) was not a gold standard. The estimated sensitivity and specificity of the Western Blot were corrected for the imperfect sensitivity and specificity of the reference test and therefore provided a more accurate estimation.

The sensitivity analysis also showed only minor changes between the basic and alternative models. Adaptations in the prior distributions of the parameters did not cause large changes in the model outcomes. This demonstrated that the outcomes of the models were largely influenced by the data itself, i.e. by the results of the testing, and not by the selected prior distributions for the parameters. This also increased the confidence in the results of the model.

The Bayesian models demonstrated that the sensitivity of the artificial digestion test was below 100%, and also the 95% probability interval did not include 100%. It is biologically plausible that the sensitivity of the routine artificial digestion test is not 100%, as is the case with most diagnostic tests. *Trichinella* larvae may not be distributed equally throughout the tissues (24), and a certain probability exists that the sample selected from an infected pig does not contain larvae. Assuming that larvae are randomly distributed following a Poisson distribution and considering a larval density of 1 LPG, the Poisson distribution demonstrates that there is a 0.7% probability that a 5 gram sample does not contain any larvae at all. The maximum achievable diagnostic sensitivity of the artificial digestion using a 5 gram sample would therefore be 99.3%. Using 1 gram samples, even 36.8% of the samples would not contain any larvae. The maximum achievable diagnostic sensitivity will be further reduced when larvae are not randomly distributed. Furthermore, larvae may unwittingly be lost during the artificial digestion procedure, e.g. by not carefully rinsing the blender in the digestion beaker or by not allowing enough time for the sedimentation process.

It may be argued that the sensitivity of the artificial digestion test was similar to that of the Western Blot. However, it must be kept in mind that these estimates relate to the diagnostic sensitivity. In the case of the artificial digestion test this is closely related to sample size, as discussed above. Also, the analytical sensitivity of the artificial digestion test is lower than that of serological tests, as demonstrated by the different limits of detection (10, 24, 34). ELISA tests can detect antibodies in pigs with larval densities of at least 0.01 LPG (10, 34). This is a clear advantage if serological surveillance is used to demonstrate freedom from infection in a domestic pig population.

In a surveillance program for *Trichinella* spp. in domestic pigs, the test protocol that is used should provide sufficient guarantees that the number of false-positive and false-negative results is as low as possible. In an animal population that is considered to be free of infection, false-positive results may have consequences for the status of this animal population. False-negative results may have consequences for public health. For a serological surveillance of *Trichinella* infections in domestic pigs, the ELISA may be used as a screening test to investigate large numbers of samples. Samples that yield positive in the ELISA should be re-tested by Western Blot for confirmation of this result. Using the estimates from the basic model in Table 4, the sensitivity of such a test combination is 94.2% (95% probability interval/PI 88.3-97.8) and the specificity 99.8% (95% PI 98.9-100.0%). In a country like Switzerland, where *Trichinella* spp. is considered to be absent from the pig population, the negative predictive value (the probability that a negative test result is truly

negative) will be extremely close to 1, whereas the positive predictive value will be very low. Sero-positive results should therefore be followed-up by epidemiological investigations.

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Table 1. Description of the meat juice and serum samples from pigs used in this study

Infection status	Explanation	Number of samples	Origin of sample	Larval density in larvae per gram (number of samples)	Infective species (number of samples)
Trichinella-negative	Finishing pigs	72	meat juice	-	-
	Free ranging pigs	73		-	-
	Adult pigs	76		-	-
Trichinella-negative, other nematode infections	<i>Hyostrogylus</i>	21	serum	-	-
	<i>Ascaris</i>	8		-	-
	<i>Trichuris</i>	32		-	-
	<i>Strongyloides</i>	1		-	-
	Multiple infection	12		-	-
Trichinella-positive	Naturally infected from Croatia	77	meat juice	0.025-350 (72), unknown (5)	<i>T. spiralis</i> (58), unknown (19)
	Naturally infection from Italy	2		4-34 (2)	<i>T. britovi</i> (2)
	Experimentally infected	14		1.65-454 (14)	<i>T. spiralis</i> (12), <i>T. britovi</i> (1), <i>T. pseudospiralis</i> (1)

Table 2. Results of the ELISA and Western Blot testing in reference to the artificial digestion method

Artificial digestion (%)		In-house ELISA (%)		Western blot (%)	
Negative	295 (100)	Negative	291 (98.6)	Negative	291 (98.6)
		Positive	4 (1.4)	Positive	0 (0)
Positive	93 (100)	Negative	2 (2.2)	Negative	4 (1.4)
		Positive	91 (97.8)	Positive	0 (0)
		Negative		Negative	1 (1.1)
		Positive		Positive	1 (1.1)
				Negative	2 (2.1)
				Positive	89 (95.7)

Table 3. Median (95% probability interval) for the sensitivity and specificity of the Western Blot and artificial digestion

	Western Blot				Artificial digestion			
	Sensitivity		Specificity		Sensitivity		Specificity	
Deterministic model	96.8	(90.9-98.9)	100.0	(98.7-100.0)	100.0	-	100.0	
Basic model	95.8	(90.7-98.6)	99.6	(98.4-100.0)	98.8	(95.3-99.9)	100.0	
Alternative model 1¹	95.8	(90.6-98.6)	99.6	(98.4-100.0)	97.4	(94.3-99.2)	100.0	
Alternative model 2²	95.6	(91.8-98.1)	98.9	(97.4-99.6)	98.8	(95.3-99.9)	100.0	
Alternative model 3³	95.6	(91.8-98.0)	98.9	(97.4-99.6)	97.4	(94.3-99.1)	100.0	
Alternative model 4⁴	95.8	(90.7-98.6)	99.6	(98.4-100.0)	98.8	(95.3-99.9)	100.0	
Alternative model 5⁵	95.8	(90.7-98.6)	99.6	(98.4-100.0)	98.8	(95.3-99.9)	100.0	

1. Prior distribution for the sensitivity of the artificial digestion test: mode= 0.95, 10th percentile=0.90
2. Prior distribution for the sensitivity and specificity of the Western Blot: mode=0.95, 10th percentile=0.90
3. Prior distribution for the sensitivity and specificity of the Western Blot, and for the sensitivity of the artificial digestion test: mode= 0.95, 10th percentile=0.90
4. Prior distribution for the prevalence in the negative (positive) population: mode=0.50, 95th (5th) percentile=0.95 (0.05)
5. Probability that infection occurs in negative population (τ)=0.10

Table 4. Median (95% probability interval) for the sensitivity and specificity of the Western Blot and ELISA

	Western Blot				ELISA			
	Sensitivity		Specificity		Sensitivity		Specificity	
Deterministic model	96.8	(90.9-98.9)	100.0	(98.7-100.0)	97.8	(92.5-99.4)	98.6	(96.6-99.5)
Basic model	96.0	(90.8-98.8)	99.5	(98.2-99.9)	97.4	(92.9-99.5)	98.3	(96.4-99.4)
Alternative model 1¹	95.7	(90.6-98.7)	99.4	(98.1-99.9)	96.6	(93.0-98.7)	97.8	(95.9-99.0)
Alternative model 2²	96.0	(90.8-98.8)	99.5	(98.2-99.9)	97.3	(92.7-99.5)	98.3	(96.4-99.4)
Alternative model 3³	95.7	(90.6-98.6)	99.4	(98.0-99.9)	96.5	(93.0-98.7)	97.8	(95.9-99.0)
Alternative model 4⁴	96.1	(90.9-98.8)	99.5	(98.2-99.9)	97.4	(92.9-99.5)	98.3	(96.4-99.4)

1. Prior distribution for the sensitivity and specificity of the ELISA: mode=0.95, 10th percentile=0.90
2. Prior distribution for the prevalence in the negative (positive) population: mode=0.50, 95th (5th) percentile=0.95 (0.05)
3. Prior distribution for the sensitivity and specificity of the ELISA: mode=0.95, 10th percentile=0.90; prior distribution for the prevalence in the negative (positive) population: mode=0.50, 95th (5th) percentile=0.95 (0.05)
4. Probability that infection occurs in negative population (τ)=0.10

Evaluation of a new commercial enzyme-linked immunosorbent assay for the detection of porcine antibodies against *Trichinella* spp.

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Abstract

Trichinellosis is a zoonotic disease that is caused by the nematode *Trichinella* spp. Both European Union regulations as well as guidelines from the World Organization for Animal Health foresee the possibility to conduct serological surveillance for *Trichinella* spp. A newly developed commercial enzyme-linked immunosorbent assay (ELISA) was evaluated against 2 existing diagnostic techniques, namely an in-house ELISA, and an in-house Western blot. A total of 875 *Trichinella* larvae–negative samples of pigs and 93 *Trichinella* larvae–positive samples of both naturally and experimentally infected pigs were included in the study. Bayesian modeling techniques were used to correct for the absence of a perfect reference test. The sensitivity and specificity of the commercial ELISA was 97.1-97.8% and 99.5-99.8%, respectively. Sensitivity analysis demonstrated a high stability of the models. In a serological surveillance system, ELISA-positive samples should be tested by a confirmatory test. The Western blot is a suitable test for this purpose. Using the results of the models, the sensitivity and specificity of a test protocol using both ELISA and Western blot were 95.9% and 99.9%, respectively. The high sensitivity and specificity were achieved with a lower limit of detection than that of the routine artificial digestion test, suggesting that serological surveillance is a valuable alternative for surveillance for *Trichinella* spp. in pig production.

Introduction

Trichinellosis is a zoonotic disease that is caused by the nematode *Trichinella* spp. whose life cycle can take place in many different carnivorous and omnivorous animal species, including domestic pigs (23, 24). In order to prevent human clinical disease, domestic pigs are routinely tested for the presence of *Trichinella* spp. at slaughter plants in the European Union (EU) and Switzerland (1, 6).

The test prescribed for routine testing of domestic pigs is the artificial digestion test, which is typically applied by pooling up to 100 samples of at least 1 g of diaphragm tissue. This method is considered sufficiently sensitive to prevent human clinical disease (12, 27), but it was demonstrated earlier that reliable detection of *Trichinella*-positive samples using 1-g samples was only guaranteed when the sample contained more than 3 larvae/g (LPG). The sample size had to be increased to 3–5 g in order to detect samples containing a minimum of 1–2 LPG (9, 25).

The minimum infectious dose for humans was earlier estimated at 60–750 infective larvae (7). However, a dose-response curve was developed that showed that even a significantly lower number of larvae were able to cause disease in humans (26). These findings suggest that the pooled artificial digestion method may not be the optimal method for surveillance of *Trichinella* infections in domestic pigs and protection of public health under all circumstances.

Both the EU regulation (6) and the recommendations of the World Organization for Animal Health (28) foresee the possibility to apply serological techniques for surveillance purposes in domestic pigs. The enzyme-linked immunosorbent assay (ELISA) was already frequently used in pigs for research and surveillance purposes (2, 3, 11, 13, 17, 20). The assay is well adapted for automated systems (21), and ELISA techniques can be applied using both blood or meat juice samples (22).

An evaluation was conducted of a newly developed, commercially available ELISA (PrioCHECK[®] *Trichinella* Ab)^a for the detection of anti-*Trichinella* antibodies in pigs using serum and meat juice samples. Three different approaches were used. First, the artificial digestion method was used to assess the presence of *Trichinella* larvae in tissue. Second, an in-house ELISA was used to compare the efficacy of the commercial ELISA in correctly identifying seropositive and seronegative samples. Finally, the commercial ELISA was

evaluated against a Western blot test, which was considered as reference method for serological tests (10).

As previously demonstrated (10), none of the 3 reference tests could be considered to be a true gold standard. Allowing any of these tests to act as a gold standard would lead to a biased estimate of the sensitivity and specificity of the commercial ELISA (16, 18). Therefore, a Bayesian modeling approach was used to correct for imperfect reference tests. The test characteristics of the commercial ELISA were subsequently evaluated against the test characteristics of the 2 other serological tests.

Materials and methods

A total of 968 samples were included in the evaluation study (Table 1). Meat juice and diaphragm samples of 801 *Trichinella*-negative pigs were collected at Swiss slaughterhouses. These samples originated from finishing pigs, free-ranging pigs, and adult breeding pigs. The infection status of the pigs was determined at the slaughterhouse by pooled artificial digestion of 1 g (finishing pigs) or 2 g (adult pigs) diaphragm tissue according to the EU regulation (EG) no. 2075/2005. Additionally, 74 serum samples were used from *Trichinella*-negative pigs with known other nematode infections. These 74 samples were derived from and documented in a previous study on pig health in Switzerland (5, 14, 15).

A total of 93 samples from *Trichinella*-positive pigs were included. Seventy-seven meat juice samples from naturally infected pigs from Croatia (University of Zagreb) and 2 meat juice samples from naturally infected pigs from Italy were included.^b Larval densities in the naturally infected pigs ranged from 0.025 to 350 LPG and were unknown for 5 samples. Additionally, 14 meat juice samples from experimentally infected pigs were included.^{a,b,c} Pigs had been inoculated with *Trichinella spiralis* (12 pigs), *Trichinella pseudospiralis* (1 pig), and *Trichinella britovi* (1 pig) and slaughtered 50 to 61 days after inoculation. Additional information on the samples is available in Table 1.

The commercial ELISA^a was performed according to the manufacturer's instructions. Positive and negative control samples provided with the kit and serum samples were diluted 1:50, and meat juice samples were diluted 1:5 (final dilution) and incubated on plates coated with excretory/secretory antigen of *T. spiralis* produced according to the World Organization for Animal Health manual (27). A peroxidase labeled anti-pig IgG antibody was used as

secondary antibody. The test plate was read at 450 nm. The results were calculated in percentage positivity (PP) based on the positive control on the plate:

$$\frac{A_{450nm} \text{ sample}}{A_{450nm} \text{ positive control}} \times 100\% = X\% \text{ positivity}$$

Results obtained above or at the cut-off of 15% PP were considered positive. Results obtained below the cut-off of 15% PP were considered negative. The plate had to fulfill the validity criteria according to the manufacturer's instructions (positive control > OD_{450nm} 1, weak positive control >35 % positivity and negative control < OD_{450nm} 0.2). The cut-off of 15% PP was established based on ROC analysis in an in-house validation study performed by the manufacturer. The in-house ELISA, based on excretory/secretory antigen and the Western blot, based on somatic antigen derived from first larvae of *T. spiralis*, were conducted as described previously. The cut-off of the in-house ELISA was set at 23% PP, according to ROC analysis performed in a previous study (10).

The evaluation of the commercial ELISA was carried out as follows: all samples were first tested in the routine artificial digestion test according to the EU regulation (EG) no. 2075/2005. Then, all samples were tested in parallel using the commercial and in-house ELISAs. All digestion-positive samples and 295 digestion-negative samples were furthermore tested by Western blot. It was ensured that Western blot was carried out for all digestion-negative samples that gave discrepant results in the 2 ELISAs. The remaining digestion-negative samples for testing by Western blot were selected as a random sample. A confirmatory artificial digestion test with 20g diaphragm meat was conducted for those samples that were considered negative based on artificial digestion of 1–2 g, and where there was inconsistency between the ELISA results.

Data were analyzed using NCSS 2007^d and the freeware programs WinEpiscope 2.0^e and WinBUGS 1.4 (19). Beta distributions were established with the freeware BetaBuster.^f In an initial step, the sensitivity and specificity of the serological tests were calculated in NCSS 2007 using deterministic techniques assuming that the artificial digestion test was a gold standard test with a perfect sensitivity and specificity. In a second step, test performance of the serological tests was evaluated in WinBUGS 1.4 using Bayesian techniques as described previously (4).

The test performance of the commercial ELISA was evaluated against the performance of the in-house ELISA using a “2 dependent tests - 2 populations - no gold standard” model.

This model was selected, because both ELISAs were based on the detection of the same antigen and were therefore conditionally dependent from each other. The parameterization of this model was published previously (4). The model was run with 100,000 iterations including a burn-in phase of 2,000 iterations. The sensitivity and specificity of the in-house ELISA had a prior distribution of mode=0.90 and 10th percentile=0.60 (Beta(6.05; 1.56)). The prior distribution of the prevalence in the *Trichinella*-negative population had mode=0.02 and 95th percentile=0.10 (Beta(1.84; 42.11)). In the *Trichinella*-positive population the prevalence had mode=0.98 and 5th percentile 0.90 (Beta(42.11; 1.84)). Prior distributions for the correlation parameters (λ , γ) had mode=0.90 and 5th percentile=0.10 (Beta(1.32; 1.04)). The performance of the commercial ELISA was also compared with that of the Western Blot using the same “2 dependent tests – 2 populations – no gold standard” model. Number of iterations and burn-in phase were as described above. The prior distribution for the sensitivity and specificity of the Western Blot had a prior distribution with mode=0.90 and 10th percentile=0.60 (Beta(6.05; 1.56)). Prior distributions for the prevalence and correlation parameters were as described above.

Alternative models were developed to evaluate the sensitivity of the models to changes in the prior distributions. Prior distributions for the test characteristics were narrowed, and prior distributions for the prevalence were widened. In addition, models were created to allow for a zero prevalence in the *Trichinella*-negative population ($\tau < 1$).

Results

The results of the test series are summarized in Table 2. The commercial ELISA did not detect antibodies in 2 larvae-positive samples. These samples originated from pigs that were naturally infected with *T. spiralis*, having a larval burden of 0.4 and 32 LPG, respectively. The in-house ELISA gave positive results for 4 larvae-negative samples. These samples were obtained from adult breeding pigs. Tissue for confirmatory artificial digestion testing was only available for 3 of these samples. In none of these samples was *Trichinella* larvae detected. The in-house ELISA also did not detect antibodies in 2 larvae-positive samples. These samples originated from pigs that were naturally infected with *T. spiralis*, having a larval burden of 0.025 and 0.4 LPG respectively. The sample with a reported larval burden of 0.4 LPG was not detected by the commercial ELISA. The Western blot did not detect antibodies in 3 larvae-positive samples that contained 0.4, 1, and 32 LPG respectively. The sample containing 0.4 LPG was not detected by either of the 2 ELISAs. The sample containing 32

LPG was not detected by the commercial ELISA, but was detected by the in-house ELISA. The sample containing 1 LPG was detected by both ELISAs.

Under the assumption of an artificial digestion test with perfect sensitivity and specificity, the calculated sensitivity and specificity of the serological tests are presented in Tables 3 and 4 (deterministic model). The level of agreement (Kappa reliability test) between the 2 ELISA tests was 0.96 (95% CI: 0.90-1.00). Subsequently, the sensitivity and specificity of the commercial ELISA was evaluated using Bayesian models, which corrected for the false assumption of a reference test with perfect test characteristics. Various alternative models were run to assess the sensitivity of the models to changes in the model parameters. Results are presented in Tables 3 and 4. The sensitivity and specificity of the commercial ELISA were slightly higher in the deterministic model. The alternative models did not lead to major changes in the estimated sensitivity and specificity, demonstrating a good stability of the model.

Finally, the patterns of the absorbance values of the commercial and in-house ELISAs were compared. Results are presented in Figure 1, expressed by the PP. The PP of larvae-negative samples of the commercial ELISA was less variable, as demonstrated by smaller interquartile ranges, and was consistently lower than those of the in-house ELISA, as demonstrated by lower median PP values (values not shown).

Discussion

With a sensitivity of 97.1-97.8% and a specificity of 99.5-99.8%, respectively, the commercial ELISA had a better or at least similar performance to other ELISAs based on excretory/secretory antigen (3, 10, 20, 22, 27). All potentially cross-reactive samples were correctly classified as negative, giving further evidence for the good specificity of the commercial ELISA.

In earlier studies, ELISAs were able to detect antibodies in pigs with larval densities as low as 0.01 LPG (11, 27). In contrast, artificial digestion using 1-g samples can only reliably detect positive samples above the level of 3–5 LPG (9, 25). In the current evaluation study the commercial ELISA was correctly able to detect antibodies in samples from pigs with larval densities of 0.025 LPG which was the lowest larval load in this sample set. However, two samples with larval burdens of 0.4 and 32 LPG were not detected in the commercial ELISA but also tested negative in the in-house ELISA and/or the in-house WB, respectively.

ELISA technology, in general, results in a certain number of initial reactive samples in a surveillance program, depending on the prevalence of infection in the population, the sensitivity and specificity of the ELISA, and the number of tested samples. The test protocol therefore should include a second test to confirm any positive results obtained in the ELISA. The Western blot is a suitable test for this purpose (10). The sensitivity and specificity of the overall test protocol is thus based on the combined sensitivity and specificity of these 2 tests. Using Bayesian techniques, the sensitivity and specificity of such a test protocol would be 95.9% (95% probability interval [PI]: 90.4–98.9) and 99.9% (95% PI: 99.1–100.0), respectively. It is important to note that respective sensitivity and specificity are achieved with a much lower limit of detection than achieved by the routine artificial digestion test. Antibodies would also be detected in pigs with low larval densities, therefore giving more opportunities for the surveillance of *Trichinella* spp. in pig production.

In a *Trichinella* surveillance program that is conducted to ensure food safety, a high level of confidence in the negative test results of a serological surveillance system, i.e. a high negative predictive value (NPV), is of importance. The highest prevalence of *Trichinella* infections in domestic pigs at slaughter that was reported in an EU member state in 2006 was 0.000127% (8). With this prevalence, and the sensitivity and specificity of the test protocol reported above, the NPV was >99.9999%, meaning that less than 1 per million negative test results would have been a false-negative result if the abovementioned test protocol would have been applied. This demonstrates that high levels of sensitivity and specificity in combination with low prevalence levels for *Trichinella* infections in pigs create a high level of confidence in the negative results of a serological surveillance system. Serological surveillance is therefore an interesting alternative to routine artificial digestion at slaughter in pig populations where the prevalence of *Trichinella* spp. is very low.

It was thus concluded from the current study that the commercial ELISA is a suitable tool for the reliable detection of anti-*Trichinella* antibodies in pigs, and could be used within the framework of *Trichinella* surveillance programs. A serological surveillance system for *Trichinella* in pigs with a test protocol including an ELISA test and Western blot would provide a high sensitivity and specificity, combined with a low limit of detection.

Sources and manufacturers

- a. PrioCHECK® *Trichinella* Ab ELISA kit, Prionics AG, Schlieren-Zurich, Switzerland.

- b. Obtained from Professor E. Pozio, Department of Infectious, Parasitic and Immunomediated Diseases, Istituto Superiore di Sanita, Italy.
- c. University of Bern, Bern, Switzerland.
- d. NCSS Statistical Software, Kaysville, UT.
- e. Available at <http://www.clive.ed.ac.uk/cliveCatalogueItem.asp?id=B6BC9009-C10F-4393-A22D-48F436516AC4>.
- f. Available at <http://www.epi.ucdavis.edu/diagnostictests/betabuster.html>.

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Table 1. Description of the samples used in the current study*

Artificial digestion test/category	Nematode infection	No. of samples	Larval density (LPG)	Inoculation dose
Negative		875		
Finishing pig		265		
Free ranging pig		266		
Breeding sow		270		
Unknown age group, other nematode infection	Hyostrogylus	21		
	Ascaris	8		
	Trichuris	32		
	Strongyloides	1		
	Multiple infection	12		
Positive		93		
Naturally infected	<i>T. spiralis</i>	72	0.025–350	
	<i>T. britovi</i>	2	4–34	
	Unknown	5	Unknown	
Experimentally infected	<i>T. spiralis</i>	8	14.9–179.8	20,000
	<i>T. spiralis</i>	1	330	30,400
	<i>T. spiralis</i>	3	187–454	Unknown
	<i>T. pseudospiralis</i>	1	75	60,000
	<i>T. britovi</i>	1	1.65	60,000

* LPG = larvae/g.

Table 2. Testing results from the commercial enzyme-linked immunosorbent assay (ELISA), in-house ELISA and Western blot

	Artificial digestion	
	positive	negative
Commercial ELISA	+ 91	0
	- 2	875
In-house ELISA	+ 91	4
	- 2	871
Western blot	+ 90	0
	- 3	295

Table 3. Median (95% probability interval) for the sensitivity and specificity of the commercial enzyme-linked immunosorbent assay (ELISA) and in-house ELISA*

	Commercial ELISA				In-house ELISA			
	Sensitivity		Specificity		Sensitivity		Specificity	
Deterministic	97.8	92.5–99.4	100.0	99.6–100.0	97.8	92.5–99.4	99.5	98.8–99.8
Basic model	97.1	92.3–99.3	99.8	99.4–100.0	97.4	92.8–99.5	99.4	98.8–99.8
Alternative model 1	96.8	92.2–99.2	99.8	99.3–100.0	96.6	93.0–98.7	99.1	98.4–99.6
Alternative model 2	97.0	92.3–99.3	99.8	99.4–100.0	97.3	92.8–99.5	99.4	98.8–99.8
Alternative model 3	96.7	92.2–99.1	99.8	99.3–100.0	96.5	92.9–98.7	99.1	98.4–99.6
Alternative model 4	97.1	92.5–99.3	99.8	99.4–100.0	97.4	92.8–99.5	99.4	98.8–99.8

* Model 1: prior distribution for the sensitivity of the artificial digestion test mode = 0.95, 10th percentile = 0.90; model 2: prior distribution of the prevalence in the negative (positive) population mode = 0.50, 95th (5th) percentile = 0.95 (0.05); model 3: prior distribution for the sensitivity of the artificial digestion test mode = 0.95, 10th percentile = 0.90, and prior distribution of the prevalence in the negative (positive) population mode = 0.50, 95th (5th) percentile = 0.95 (0.05); model 4: probability that infection occurs in the negative population (τ) = 0.10.

Table 4. Median (95% probability interval) for the sensitivity and specificity of the commercial enzyme-linked immunosorbent assay (ELISA) and Western blot*

	Commercial ELISA				Western blot			
	Sensitivity		Specificity		Sensitivity		Specificity	
Deterministic	97.8	(92.5–99.4)	100.0	(99.6–100.0)	96.8	(90.9–98.9)	100.0	(98.7–100.0)
Basic model	97.8	(93.2–99.7)	99.5	(98.4–99.9)	97.0	(92.0–99.4)	99.6	(98.4–100.0)
Alternative model 1	97.4	(93.2–99.5)	99.2	(97.8–99.9)	96.3	(92.5–98.6)	98.9	(97.4–99.6)
Alternative model 2	97.7	(93.1–99.7)	99.5	(98.3–99.9)	97.0	(91.9–99.4)	99.6	(98.4–100.0)
Alternative model 3	97.4	(93.2–99.5)	99.2	(97.7–99.9)	96.2	(92.4–98.6)	98.9	(97.4–99.6)
Alternative model 4	97.8	(93.3–99.7)	99.5	(98.3–99.9)	97.0	(91.9–99.4)	99.6	(98.4–100.0)

* Model 1: prior distribution for the sensitivity of the artificial digestion test mode = 0.95, 10th percentile = 0.90; model 2: prior distribution of the prevalence in the negative (positive) population mode = 0.50, 95th (5th) percentile = 0.95 (0.05); model 3: prior distribution for the sensitivity of the artificial digestion test mode = 0.95, 10th percentile = 0.90, and prior distribution of the prevalence in the negative (positive) population mode = 0.50, 95th (5th) percentile = 0.95 (0.05); model 4: probability that infection occurs in the negative population (τ) = 0.10.

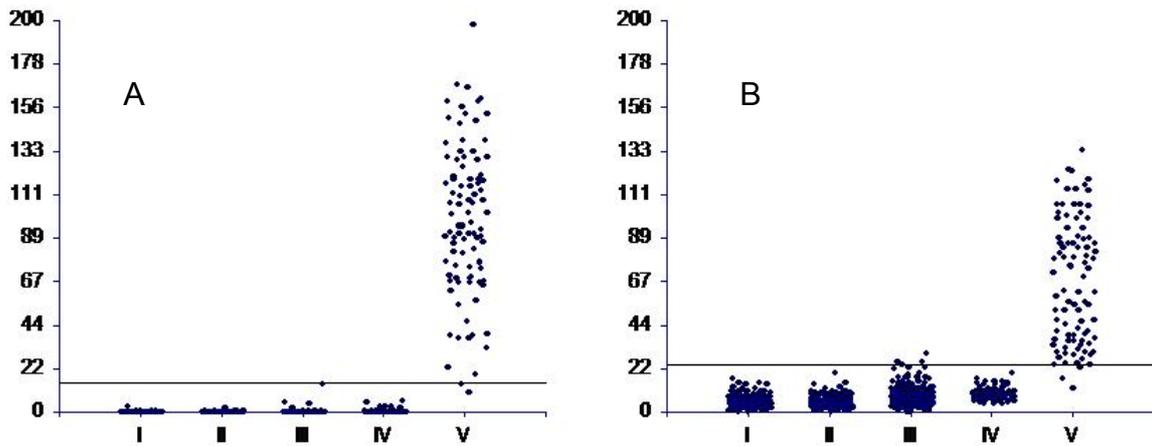


Figure 1. Distribution of percentage positivity (PP). A, PP-values of the commercial enzyme-linked immunosorbent assay (ELISA). B, PP-values of the in-house ELISA. Y-axis: PP-values. X-axis: categories (I—finishing pigs, II—free-ranging pigs, III—adult breeding pigs, IV—pigs infected with potentially cross-reacting parasites, V—*Trichinella*-positive pigs). The horizontal line indicates the cut-off value above which samples were considered positive.

Assessment of the prevalence of *Trichinella* spp. in red foxes and Eurasian lynxes from Switzerland

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Abstract

Trichinella spp. larvae have not been detected in Swiss pigs, horses or wild boar for many decades, whereas the parasite was repeatedly isolated from red foxes and Eurasian lynxes. Whenever the isolated larvae could be subjected to genotyping, *T. britovi* was found as infective agent. The present study was initiated to re-assess the epidemiological situation of *Trichinella* infection in Swiss carnivorous wildlife, namely in red foxes and lynxes. Tissue samples from 1'298 foxes were collected between 2006 and 2007, those of 55 lynxes between 1999 and 2007. The laboratory investigation for all samples included a standard artificial digestion method and a multiplex-PCR to investigate for respective species and/or genotypes upon positive samples. *Trichinella* spp. was found in 21 foxes (1.6%) and in 15 lynxes (27.3%). Multiplex-PCR performed on recovered larvae yielded *T. britovi* as infecting species in all cases. The geographic distribution of positive foxes showed two main clusters, one in Central Switzerland and one in the West of the country, where also many lynxes were found to be positive. While in foxes the prevalence for *Trichinella* infection was not statistically correlated with sex or age class, prevalence in lynx was significantly higher in males compared to females, and in adults compared to juveniles.

Introduction

In Switzerland, *Trichinella* spp. larvae have not been found in domestic pigs or horses for more than a century, and in wild boar (*Sus scrofa*) for several decades (1, 2, 3, 6, 13). However, serological investigations revealed positive wild boars (1), and larvae have been isolated from red foxes (*Vulpes vulpes*) and other carnivorous wildlife, such as lynxes (*Lynx lynx*). The most recent studies documented a prevalence of larvae of 0.9% in foxes and 30% in lynxes (1, 11). Interestingly, older studies carried out between 1968 and 1985 in foxes revealed prevalences ranging from 14% to 4% (3, 4, 5, 13), suggesting a decrease of the prevalence in foxes over time.

While some data exist on *Trichinella* prevalence in Swiss wildlife, so far little information is available on the infecting *Trichinella* species: a single fox was shown to be infected with *T. britovi* (1). In order to assess the risk of infection for domestic pigs and humans in Switzerland, additional knowledge is necessary about the epidemiology of *Trichinella* spp. in Swiss wildlife.

The aims of the present study were 1) to determine the current prevalence of *Trichinella* spp. infection in red fox and lynx in Switzerland in order to detect an eventual change in prevalence in these species in the past years; 2) to determine the genotype of the recovered *Trichinella* larvae; 3) to assess the geographical distribution of *Trichinella* spp., and 4) to investigate possible correlations between prevalence of *Trichinella* infection and sex and age in the two investigated carnivorous species.

Materials and methods

Fox samples

Between January 2006 and June 2007, 1'289 tongues from hunted foxes were collected either by state gamekeepers or local hunters. The tongues were sent natively to the Institute of Parasitology in Berne. The exact place where the samples originated from, the estimated age (juvenile <6 months, subadult 6-12 months, adult >12 months), and the sex of each fox were recorded by means of a standardized questionnaire. The age of the foxes was estimated by the hunters or gamekeepers, based on both body size and season of death.

The questionnaire was filled in for 90% of the samples. 56% of the samples originated from male and 44% from female foxes. The age distribution of the sampled foxes was 20% juvenile, 19% subadult, and 61% adult.

Lynx samples

The Eurasian lynx is a protected species in Switzerland. Currently, about 100 adult animals live in Switzerland, mainly in the Jura Mountains and the North-West Swiss Alps (14, 16). According to the Swiss Lynx Concept, all carcasses of free-ranging lynxes must be submitted to the Centre for Fish and Wildlife Health (FIWI) in Berne for a complete, standardized post-mortem examination. Between January 1999 and June 2007, muscle samples (diaphragm and/or masseter) were collected from 55 lynxes. Sex and age of each animal were recorded. Age was estimated according to body size and tooth wear, and age classes were chosen considering sexual maturity and therefore social behavior (12): juvenile (< 1year), subadult (1-<2 years for females, and 1-<3 years for males), and adult (≥ 2 resp. 3 years).

Information on sex was available for 54 animals (one carcass could not be specified) and age for all 55 animals: 23 lynxes were female and 31 were male, 24 were juvenile, 8 were subadult, and 23 animals were adult.

Artificial digestion technique

For fox specimens, each tongue was skinned and sagittally cut in half. One half of each tongue was subjected to an artificial digestion in a pooled assay of 5 samples (i.e. approximately 50 grams in total). If larvae were found in pooled samples, the other halves of the tongues of the pool were separately digested. For lynx samples, individual artificial digestion was performed with at least 10 grams of diaphragm and/or masseter muscle. The artificial digestion (magnetic stirrer) method was carried out according to the EU-regulation (EG) No. 2075/2005. Recovered larvae were picked from the digestion fluid, transferred to PBS and stored at -20°C for further analysis.

Purification of genomic DNA and PCR

Multiplex PCR was performed on all recovered larvae from foxes, and since 2002 also from lynxes. Extraction of genomic DNA of individual larvae (between 2 larvae and 10 larvae per positive sample) was performed according to Zarlenga et al. (15). Genotyping of the *Trichinella* isolates was performed by applying a multiplex, 2-step PCR exactly as developed and described by Zarlenga et al. (15).

Statistical analysis

Significance of differences in prevalence was tested using a 2-sided Fisher's Exact test (NCSS 2007, Kaysville, Utah, USA). Level of significance was set at $P < 0.05$.

Results

Fox

Twenty-one out of 1'289 fox samples were positive for *Trichinella* larvae, revealing an overall prevalence of 1.6% (95% confidence interval 1.0 - 2.5%) for *Trichinella* infection in Swiss red foxes (Table 1). *T. britovi* was identified by PCR as infecting species in all positive samples. The positive samples mainly originated from Central Switzerland and from the Western part of the country (Figure 1). Information on sex and age were available for 17 of the 21 positive animals: 10 were males and 7 were females. 7 foxes were juveniles, 2 subadults, and 8 adults. No statistically significant correlation was found between age and sex and the occurrence of *Trichinella* infection.

The prevalence of 1.6% is similar to the prevalence of 0.9% (1) and 1.3% (6) ($p > 0.05$), but significantly lower than the prevalences of 9% (3) and 14% (4) reported in the 1970ies ($P < 0.001$). The actual prevalence of 1.6% also tends to be lower than the prevalence of 4% determined in 1982 ($P = 0.06$; $N = 118$) (13).

Lynx

Fifteen out of the 55 lynxes were positive for *Trichinella* larvae, revealing an overall prevalence of 27.3% (95% confidence-interval 17.2-40.2%) in Swiss lynxes (Table 1). *T. britovi* was identified in all eight cases that could be submitted to PCR analysis. The positive lynxes originated both from the Jura Mountains and the North-Western Swiss Alps (Figure 2). Of the positive lynxes, 2 animals were females, 13 were males; 2 were juveniles, 2 were subadults, and 10 were adults. Statistically, there was a significantly higher prevalence of *Trichinella* infection in male lynxes compared to females ($P = 0.007$), and a statistically lower prevalence in juvenile animals compared to adults ($P = 0.008$).

Prevalence in lynxes was significantly higher than in foxes ($P < 0.0001$).

Discussion

The present study is the first to determine the genotype of *Trichinella* spp. in a large number of wild carnivores from Switzerland. *T. britovi* was the only species that could be identified in both foxes and lynxes. It was also the species identified in a fox in an earlier study (1). *T. britovi* is described to persist in a sylvatic cycle, its main reservoir in most regions of the EU being the red fox (10).

Our study revealed that the *Trichinella* prevalence in red foxes has remained stable at a low level (<2%) during the past decade (1, 6), and thus confirmed the suspected decrease of prevalence since the 1970ies (3, 4, 13). Regarding lynx, our prevalence of 27.3% corroborates previous data obtained from a smaller sample size (6 out of 20 lynxes collected in 1998-1999) (11). These results indicate that the prevalence of *Trichinella* sp. in lynxes has also been stable for the past 10 years. Two factors may explain the higher *Trichinella* prevalence in lynxes: 1) Foxes are a significant prey of lynx in Switzerland (7), and can therefore be considered as a source of infection for lynxes. 2) Lynxes can live up to 17 years in the wild (U. Breitenmoser, pers. comm.), while most foxes are shot before they reach 3 years of age (8). Thus, lynxes have a longer exposure time to potentially *Trichinella*-infected meat. This hypothesis is further sustained by our finding that adult lynxes have a higher prevalence of *Trichinella* infection than juveniles, as was also observed by Oksanen et al. (9) in Finnish lynxes.

Interestingly, we found that male lynxes present a higher prevalence compared to females. This result is again in accordance with the observations of Oksanen et al. (9), who reported the same finding in lynxes in Finland. However, the reason for this difference is unknown, since the food habits of female and male lynxes are comparable (7).

Geographical differences were obvious for the prevalence in foxes, with most cases originating from the canton of Fribourg and from Central Switzerland (Figure 1). Jakob et al. (6) found a similar geographical distribution of *Trichinella* infections in foxes. Interestingly, also many positive lynxes came from the canton of Fribourg and surrounding areas (Figure 2). However, positive lynxes furthermore originated from regions where all tested foxes were negative (Jura Mountains, Bernese Oberland). Data on lynxes from other Swiss areas are lacking, due to the sparse occurrence or even absence of the species in these parts of the country (16). Overall, these results indicate that the presence of *Trichinella* sp. in Swiss wildlife is not dependent on lynx occurrence. However, lynx might be a good indicator for the

existence of *Trichinella* infection in wildlife due to its top of the food-chain status and its high life expectancy.

The artificial digestion method was used in the present study. Previous studies done in foxes, in which artificial digestion was used in parallel with serology, revealed a higher prevalence by means of serology than digestion (1, 6). Serology also revealed a seroprevalence of 8.7% in wild boar (1), although *Trichinella* larvae have not been found in wild boar for many years (1, 2, 3, 6, 13). Low infection intensity in some individuals/species may be sufficient to induce a serum antibody response but not to allow direct demonstration of larvae by using the conventional digestion method. Thus, prevalences obtained by conventional digestion method might be underestimated.

To gain more exact information on the epidemiological situation of *Trichinella* in Switzerland, the seroprevalence of *Trichinella* in carnivores and in wild boar need to be (re)assessed. Furthermore, investigations elucidating the prevalence of *Trichinella* infections in rodents and other small omnivorous mammals potentially acting as hosts for the parasite, are necessary.

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Table 1. Number of positive per investigated animals per canton. Cantons with positive animals are indicated in bold.

Canton	Foxes	Lynxes
	Positive/investigated	Positive/investigated
Aargau	0/36	na*
Appenzell-A.	0/20	na
Appenzell-I.	1/20	na
Basel-Land	0/106	0/1
Basel-Stadt	0/17	na
Bern	0/104	6/27
Fribourg	5/148	4/7
Genève	na	na
Glarus	na	na
Graubünden	0/23	na
Jura	0/47	1/3
Luzern	3/125	na
Neuchâtel	0/35	0/2
Nidwalden	3/12	na
Obwalden	1/30	na
Schaffhausen	na	na
Schwyz	1/13	na
Solothurn	0/2	na
St. Gallen	0/38	0/2
Thurgau	0/132	na
Ticino	0/99	na
Uri	5/35	na
Valais	1/75	na
Vaud	1/49	4/13
Zug	0/35	na
Zürich	0/86	na
Total	21/1289 (1.6%)	15/55 (27.3%)

na: no samples available

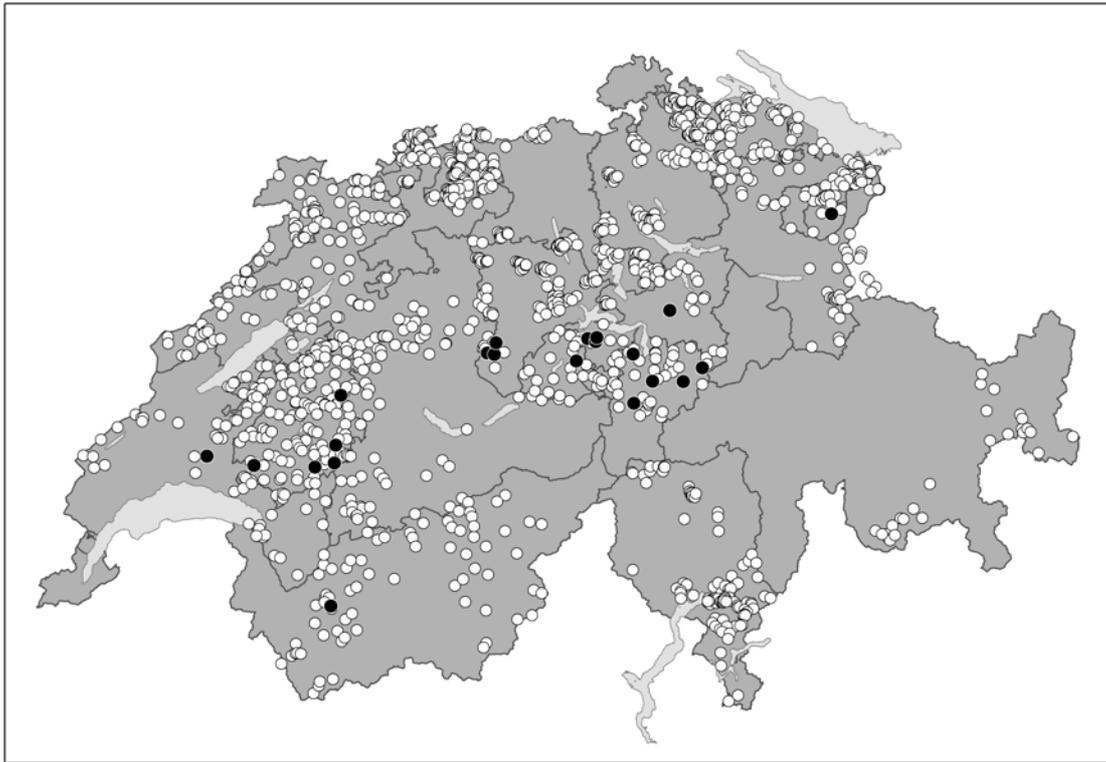


Figure 1. Map of Switzerland showing the geographical distribution of fox samples. Circles: *Trichinella*-negative samples, dots: *Trichinella*-positive samples.

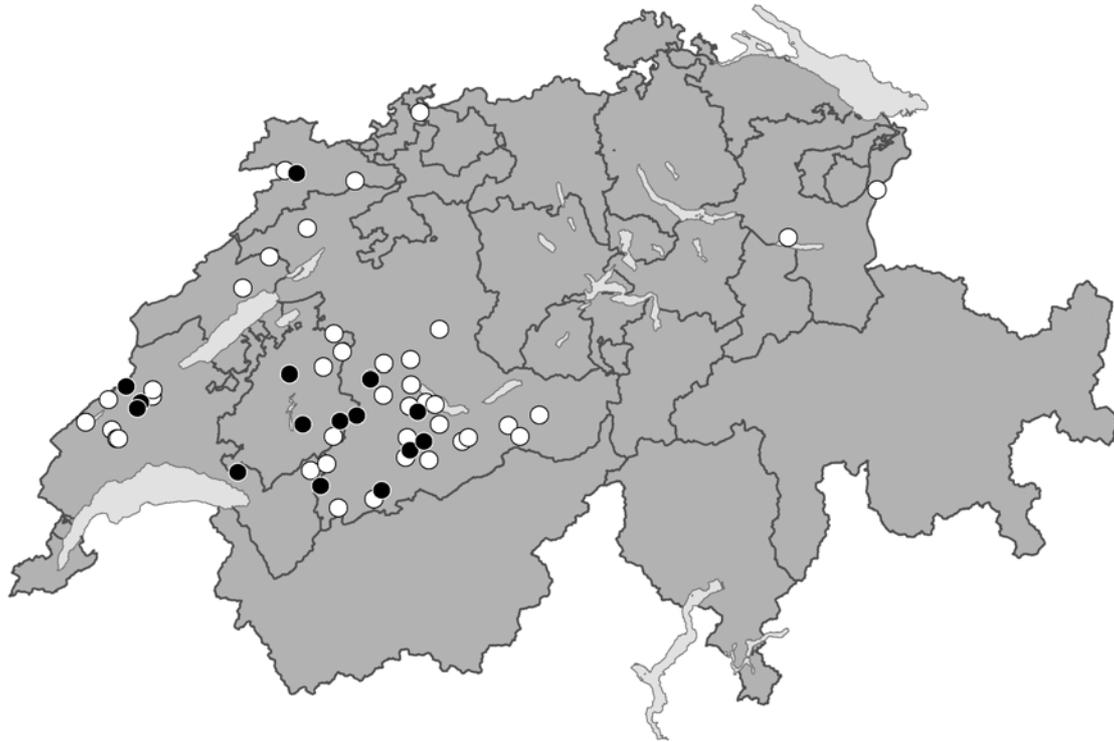


Figure 2. Map of Switzerland showing the geographical distribution of lynx samples. Circles: *Trichinella*-negative samples, dots: *Trichinella*-positive samples.

Occurrence of *Trichinella* spp. in wild boar in Switzerland

Based on:

Vorkommen von *Trichinella* spp. beim Wildschwein in der Schweiz

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Summary

Trichinellosis is a worldwide occurring zoonosis caused by the intracellular nematode *Trichinella* spp. One of the main infection sources for humans in Europe is raw or undercooked meat from wild boar. *Trichinella britovi* is prevalent in wild carnivores in Switzerland, thus a possible inclusion of wild boar in this wildlife cycle cannot be excluded. In order to assess the prevalence of *Trichinella* infection in wild boar, we tested 1'458 animals with both parasitological and serological methods. In none of the animals *Trichinella*-larvae could be recovered by the artificial digestion method (prevalence of larvae: 0% (95% CI 0.0-0.3)). Antibodies in meat juice were detected in 57 animals using a standardized E/S-Ag-ELISA. However, in the confirmatory Western Blot, only 3 animals remained seropositive (seroprevalence: 0.2% (95% CI 0.07%-0.60%)). The occurrence of wild boar positive for anti-*Trichinella*-antibodies indicates that meat inspection for *Trichinella*-larvae in this species is important to prevent human infections.

Introduction

The parasite *Trichinella* spp. can occur in many carnivorous and omnivorous animal species. Human infections, caused by oral intake of raw or undercooked muscle meat containing infectious *Trichinella* larvae, can lead to serious and potentially life-threatening disease. The main sources of infection for humans in Europe are domestic pigs, horses and wild boar (1, 13, 15, 18). *Trichinella*-larvae have not been detected in pigs and horses in Switzerland for more than a century, or in wild boar (*Sus scrofa*) for several decades (7, 9, 10, 11, 17). However, larvae have been detected repeatedly in foxes (*Vulpes vulpes*) and lynxes (*Lynx lynx*). The most recent study of *Trichinella* prevalence in wild carnivores demonstrated a prevalence of 1.6% in foxes and 27.3% in lynxes (3). *T. britovi* is therefore known to be present in Swiss wildlife, but *T. spiralis* can be considered to be absent in Switzerland.

In contrast to the inability to demonstrate *Trichinella*-larvae in wild boar in Switzerland, neighbouring countries Germany, France and Italy periodically report findings of *Trichinella*-positive wild boar (14, 16). Recently, also in Austria there was a report about the finding of a *Trichinella*-infected wild boar (16). However, there are indications that *Trichinella* infections occur in wild boar in Switzerland as well, because a previous serological investigation of 356 wild boar using ELISA detected antibodies in 8.7% of the tested animals (7).

The serological investigation using E/S ELISA has a high sensitivity, but false-positive results can occur (5). These would lead to an overestimation of the true seroprevalence and thus to an overestimation of the spread of infection. ELISA-positive results therefore need to be confirmed by a confirmatory test. The Institute of Parasitology in Bern (IPB) recently validated a Western Blot for use as a confirmatory test for *Trichinella* infections in domestic pigs (4). Now, the strategy to confirm ELISA-positive findings in pigs using Western Blot was also applied to wild boar.

Animals, materials and methods

Wild boar

A total of 1,458 samples of wild boar diaphragm tissue collected between 2005 and 2007 were tested. The IPB received 1,108 samples directly, because they were submitted for an official meat inspection for *Trichinella* spp. The remaining 350 samples were submitted to the Institute Galli-Valerio (Lausanne) for official meat inspection, and subsequently meat juice samples were sent to the IPB. All samples that were received at IPB directly were

accompanied by the official application form, from which information about the address of the sender, the age category (juvenile <12 months, sub-adult 1-2 years, adult >2 years) and the gender of the wild boar could be obtained. The age category was estimated by the hunters based on the body size of the animal and the season in which they were hunted. For the samples that were first submitted to Lausanne, only information about the address of the sender was available. Care was taken to consider hunters from all Swiss regions. The documentation did not provide any details about the exact locations where the animals were shot, but it confirmed that all of the wild boar originated from Switzerland.

Detection of larvae

From each diaphragm sample, at least 5 grams were tested in a pooled sample test with a maximum of 10 animals per pool (maximum of 50 gram diaphragm tissue per test). The samples were tested using the artificial digestion test with the magnetic stirrer method according to Annex I of EU Regulation 2075/2005.

Detection of antibodies

Meat juice was obtained by freeze-thawing of a part of each sample that was not used for the artificial digestion test (12). Antibody detection was done using an E/S-ELISA. Both the coated ELISA-plates and the negative and positive control sera, which were derived from experimentally infected pigs, were obtained from Dr. Nöckler, BfR, Berlin. The cut-off value above which samples were considered positive was set at 23% of the absorption value of the positive control, which was derived from validation of the ELISA for domestic pigs (4).

Each ELISA-positive meat juice sample was tested in a Western Blot that had previously been validated for its use in domestic pigs (4). The criteria for determining the specificity were left unchanged (a banding pattern of several, defined bands).

Statistics

For data analysis, data were exported from a Microsoft Excel database to a Microsoft Access database. Correlations between parameters were tested by a Chi-square test. Differences in prevalence were evaluated using a 2-sided Fisher's Exact test ($p < 0.05$) (NCSS 2007, Kaysville, Utah, USA).

Results

Data about gender and age were available for 46% and 44% of the animals, respectively. Fifty-two per cent of the tested wild boar were male, 48% were female. Ten per cent of the tested wild boar were juvenile, 59% were sub-adult and 31% were adult (table 1).

Detection of larvae

Larvae were not detected in any of the samples. Therefore the prevalence was 0% (95% CI 0.0%-0.3%).

Detection of antibodies

ELISA

Meat juice was obtained from 1,458 diaphragm samples. A total of 57 samples (3,9%; 95% CI 3.03%-5.03%) were positive in the ELISA.

Western Blot

All 57 ELISA-positive samples were tested by Western Blot. Only three samples showed the banding pattern indicative of a *Trichinella* infection (figure 1). Several other samples showed a single, non-specific band (figure 1) and they were classified as *Trichinella*-negative. Therefore, the seroprevalence of anti-*Trichinella* antibodies in wild boar in Switzerland was 0.2% (95% CI 0.07%-0.6%).

Risk factors

There were no statistically significant correlations between age or gender and seropositivity.

Discussion

In 1997, a study using the same E/S-ELISA found a seroprevalence of 8.7% (95% CI 6.2-12.1) in wild boar (7). At that time, a Western Blot was not yet available, and false-positive ELISA results could therefore not be excluded. In the current study, the Western Blot allowed a more accurate determination of the seroprevalence in wild boar. The ELISA test was used as a screening test and resulted in 3.9% (3.03-5.03) positive samples. Following confirmatory testing, a seroprevalence of 0.2% (0.07-0.6) was found. This seroprevalence was significantly lower than the 8.7% reported previously ($p < 0.0001$). Also when the results of the ELISA tests were compared directly (3.9% versus 8.7%), there was still a statistically significant reduction in the percentage of ELISA-positive samples ($p = 0.0003$). Therefore, we

can conclude that there has been a reduction of the seroprevalence for *Trichinella* spp. in wild boar over the last 10 years.

Until today, only *T. britovi* has been demonstrated in Swiss wildlife (3, 7), and therefore it is very probable that the antibody response in wild boar was caused by this species as well. *T. britovi* is mainly present in the wildlife cycle and is very well adapted to carnivores. Its main reservoir in Europe is the fox population (8). Via experimental infections, it was demonstrated that the intensity of a *T. britovi* infection in pigs, measured by the larval density in tissue, was very low (12). A low larval density in wild boar could result in a detectable antibody response when serological techniques are used, but it may not be possible to demonstrate larvae in muscle tissue using standard artificial digestion methods due to the limited sensitivity of these methods.

It was shown that samples with a larval density of 1 larva per gram could reliably be detected in pigs when samples of 5 grams tissue were used for the artificial digestion technique (2, 6). With lower larval densities, the probably reduces that infected pigs are detected.

In Germany, up to 500,000 wild boar are hunted annually and approximately 75% of those are tested for *Trichinella* spp. Based on these test results, the *Trichinella* prevalence in wild boar was estimated between 0.001-0.01% (14). Genotyping of recovered larvae almost exclusively resulted in *T. spiralis* (14, 16). The occurrence of *T. spiralis* in the German wild boar population is also relevant for Switzerland, because in contrast to *T. britovi*, *T. spiralis* is well adapted to pigs and *T. spiralis* infections in pigs lead to higher larval densities than infections with *T. britovi* (12). Since the probability of a human infection increases with an increase in the number of larvae ingested (8), *T. spiralis* is also very relevant from a public health perspective. A consistent genotyping of *Trichinella* larvae recovered from wildlife should therefore be maintained to recognize or exclude the presence of *T. spiralis* in Switzerland in a timely manner.

Even though in this study no *Trichinella* larvae were recovered from wild boar, the seroprevalence of 0.2% demonstrated that wild boar have a certain probability of being in contact with *Trichinella* spp. Official meat inspection of wild boar for *Trichinella* spp. therefore continues to be justified, in particular to prevent human infections.

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Table 1. Description of tested wild boar

Age	Male	Gender		Unknown	Total
		Female			
<1 year	34	23		8	65
1-2 years	177	166		41	384
>2 years	100	81		18	199
Unknown	37	56		717	810
Total	348	326		784	1458

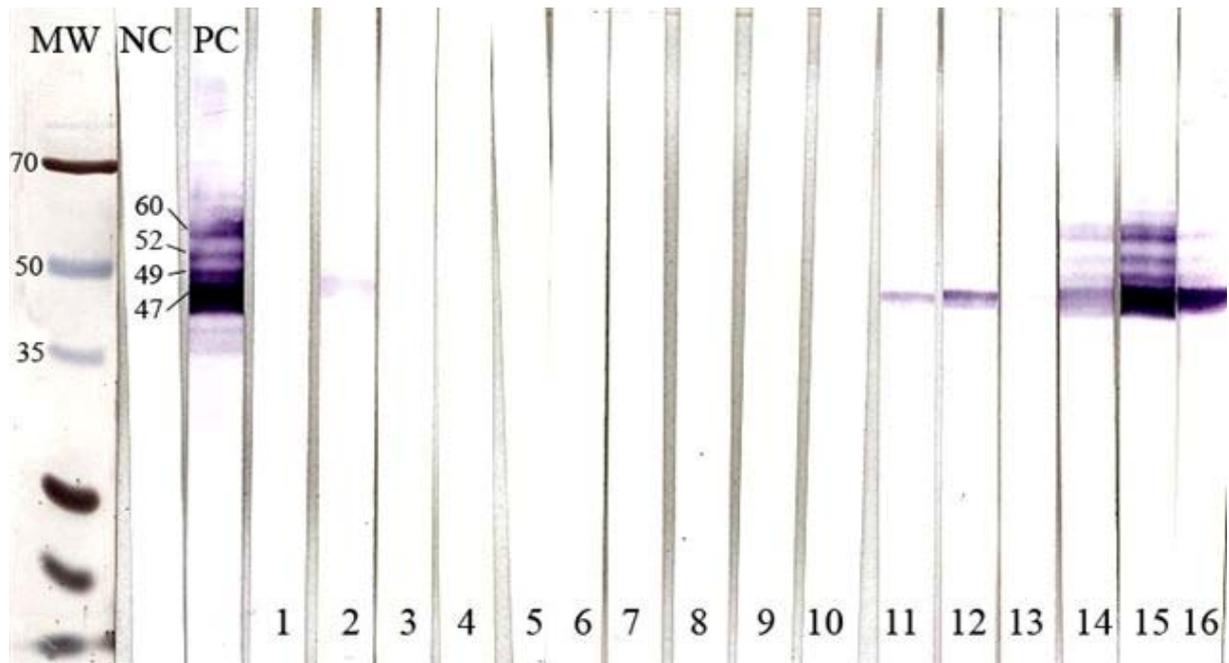


Figure 1. Western Blot for the detection of anti-*Trichinella* antibodies in meat juice from wild boar. MW: molecular weight marker; NC: negative control; PC: positive control; lane 1-13: negative meat juice samples, partially with non-specific reactions (lanes 2, 11, 12); lane 14-16: positive meat juice samples

A study to demonstrate freedom from *Trichinella* spp. in domestic pigs in Switzerland

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Impacts

- *Trichinella* spp. is a nematode that can occur in pigs and other omnivorous and carnivorous animal species and is the causative agent of trichinellosis in humans. *Trichinella britovi* has been previously found in wildlife in Switzerland.
- For the protection of public health, pork should be ensured free from infective larvae.
- Combining several diagnostic techniques, it was demonstrated that Swiss pigs are not infected with *Trichinella* spp., despite the presence of *Trichinella britovi* in Swiss wildlife.

Summary

Trichinellosis is a food-borne zoonotic disease caused by the nematode *Trichinella* spp. Many omnivorous and carnivorous animal species can act as host for this parasite, including domestic pigs. To protect public health, it should be ensured that pork does not contain infective *Trichinella* larvae. Surveillance for *Trichinella* spp. can be done using direct (larval detection) and indirect (antibody detection) diagnostic techniques. The aim of this study was to demonstrate the absence of infection in Swiss domestic pigs. An ELISA was used as the initial screening test, and sera reacting in ELISA were further investigated using both a Western Blot for serology and an artificial digestion test with 20 grams of diaphragm tissue for larval detection. A total of 7,412 adult pigs, 9,973 finishing pigs and 2,779 free-ranging pigs were tested. Samples from 17 (0.23%) adult pigs, 16 (0.16%) finishing pigs and 9 (0.32%) free-ranging pigs were ELISA-positive, but all of these sera were subsequently negative by Western Blot and by the artificial digestion method. Based on these findings, an absence of *Trichinella* infections in adult pigs (target prevalence 0.04%) and finishing pigs (target prevalence 0.03%) can be concluded. The results also demonstrated that the prevalence of *Trichinella* infections does not exceed 0.11% in free-ranging pigs, the group with the highest risk of exposure.

Introduction

Trichinellosis is a food-borne zoonotic disease caused by the nematode *Trichinella* spp., which can be hosted by many omnivorous and carnivorous animal species. The parasites are transmitted between hosts through oral intake of muscle tissue containing infective larvae (26, 28). Although trichinellosis is rare in the EU, with an incidence of 0,04 per 100.000 inhabitants (5), it is considered to be either an emerging or a re-emerging disease in certain parts of Europe. Since consumption of pork containing infective larvae is one potential source of infection for humans (2, 5, 23), it is important to ensure that the pork is free from *Trichinella* larvae in order to protect consumer health.

The probability of infection in domestic pigs is mainly influenced by access to wildlife (carrion) and access to feed containing meat scraps (26, 27). Three main risk groups have been determined within the domestic pig population (1). Finishing pigs, raised indoors and under controlled housing conditions, have the lowest probability of infection. In adult pigs the probability is higher due to the cumulative effects of longer lives. Pastured and backyard pigs, henceforth called free-ranging pigs, also have an increased probability of infection due to easier access to wildlife and absence of controlled housing conditions (1, 29).

There are two possible surveillance strategies for *Trichinella* spp. in domestic pigs. The first strategy is based on the detection of *Trichinella* larvae during meat inspection at slaughter. Typically, this inspection is done by the artificial digestion of 1-2 grams of diaphragm tissue from each pig (6). Although lower limits of detection can be achieved if more tissue is digested, the limit of detection by this test is 1-3 larvae per gram (LPG) (14). The second strategy is serological and based on antibody detection in either blood serum or meat juice samples (24). Although serological surveillance is uncommon, it has been established for use in a specific certification program to verify the effectiveness of control measures for *Trichinella* spp. in the USA (3) and is allowed for in EU regulation 2075/2005 governing *Trichinella* monitoring of the pig population in the EU.

The serological test most commonly applied in domestic pigs is the enzyme-linked immunosorbent assay or ELISA (32). Estimations for sensitivity of the ELISA ranged from 72.7-99.2% (22, 24, 25, 31) and for specificity from 90.6-99.6% (22, 24, 25, 31). False-positives can be essentially eliminated by performing an additional Western Blot (WB) resulting in an overall specificity of near 100% (13). These attributes, combined with effective

limits of detection as low as 0.01 LPG (15), make serological tests very attractive for surveillance programs aimed at demonstrating freedom from infection.

In Switzerland, artificial digestion at slaughter has been routinely performed for several years with intensity increasing from 15 to 87% of the pig population between 2001 and 2007 and no positive animals found (9). In 1992, a Swiss study using artificial digestion of 20 grams of diaphragm tissue to confirm ELISA-positive results also found no positive animals (20). These findings tend to indicate that the Swiss domestic pig population has been historically free from *Trichinella* spp..

The present study was conducted to confirm that the Swiss domestic pig population is currently free from *Trichinella* infection and was specifically designed to include all three previously mentioned risk groups and use serological techniques (ELISA and WB) to facilitate accurate detection at low larval densities.

Materials and methods

Origin of the samples

Three risk groups were defined: adult pigs (approximately 3-4 years old), finishing pigs (approximately 6 months old) and free-ranging pigs (approximately 6 months old). Pigs from all 26 Swiss cantons were sampled. Adult pigs and finishing pigs all originated from commercial farms and free-ranging pigs originated either from commercial pig farms or from backyard farms. Free-ranging pigs raised on the Alps were recorded separately from those raised in lower agricultural areas due to the different ecological environment including wildlife exposure that exists with higher altitudes and lower levels of human activity.

Sample size

Sample sizes were calculated for each of the three risk groups. The sample sizes for adult and finishing pigs allowed to demonstrate freedom from infection, which was defined as the absence of antibodies and larvae at target prevalences of 0.04% and 0.03% respectively with a confidence level of 95%. Meaning that *Trichinella* infection would be detected with 95% confidence if the prevalence exceeded these targets, which were derived from an earlier study by Jakob et al (20). Sample size calculations for the free-range risk group were complicated by the fact that no accurate population estimates were available. Therefore an estimated population of 50,000, i.e. 2% of the total pig population, was used. Furthermore, no target prevalence to demonstrate freedom from *Trichinella* spp. was available for this

group. The target prevalence for free-range pigs was therefore set at 0.1%, roughly three times higher than for finishing pigs, to reflect the increased risk in this group. The established sample sizes for adult pigs, finishing pigs and free-ranging pigs were 7,300, 10,000 and 3,000, respectively.

Sample collection

In the period between April 2006 and September 2007, diaphragm tissue samples of approximately 22 grams were collected from adult pigs, finishing pigs and free-ranging pigs at various Swiss slaughterhouses. These samples were refrigerated at 4°C and sent once a week to the Institute of Parasitology in Bern, which is the Swiss reference laboratory for trichinellosis. At slaughter, epidemiological data were collected on the origin of the pigs and the production label under which they had been produced.

Diagnostic testing

All of the diaphragm samples were tested by the routine artificial digestion method. Meat juice was also collected from all of the sample tubes after arrival of the diaphragm tissue at the laboratory by freezing-thawing of the samples and tested by ELISA. ELISA-positive samples were subsequently investigated by Western Blot and confirmatory artificial digestion.

Diaphragm samples of 1 gram (finishing pigs) or 2 grams of diaphragm tissue (adult pigs) were tested using routine pooled artificial digestion, according to EU-regulation (EG) No. 2075/2005, either at the slaughterhouse or at the ISO 17025 accredited Swiss *Trichinella* reference laboratory.

ELISA tests were conducted as previously described by Frey et al (13). In short, meat juice samples were diluted 1:10 and subsequently incubated on 96-well ELISA plates coated with the excretory/secretory antigen of larvae 1 of *T. spiralis*. The results were calculated as percentage positivity (PP) based on the A_{405nm} of the positive control on the plate. Cut-off values were determined for each risk group separately using ROC analysis. ROC analysis was carried out using meat juice and serum samples of 93 *Trichinella*-larvae positive pigs and 270 meat juice samples of *Trichinella*-larvae negative pigs for each risk group (13). These analyses resulted in cut-off values of 16% of the positive control value (PP) for finishing and free-ranging pigs, giving a sensitivity of 97.9% for both risk groups and a specificity of 99.2% for finishing and 99.6% for free-ranging pigs. For adult pigs, a cut-off value of 23% PP was selected, giving a sensitivity of 96.8% and a specificity of 99.0%.

Western Blots were also conducted as previously described (13). Briefly, meat juice samples were diluted 1:50 and incubated on Western blot strips prepared with the somatic antigen of larvae 1 of *T. spiralis*. A Western blot was considered positive when the following banding pattern became apparent: within a pattern of bands localizing between 35 and 65 kD, specific bands localized at 47 kD, 49 kD and 52 kD and two specific bands at 60 and 63 kD had to appear qualitatively, independent of the banding staining intensity. The sensitivity of the Western Blot was 95.8% and its specificity was 99.6% for all risk groups (13).

Confirmatory individual artificial digestion was performed with approximately 20 grams of diaphragm tissue. The artificial digestion (magnetic stirrer) method was carried out according to EU-regulation (EG) No. 2075/2005.

The theoretical overall sensitivity and specificity of the combined diagnostic tests were calculated using the values described above. Assuming independence of the tests, the sensitivity of the combination of ELISA, Western Blot and artificial digestion test was 97.9% for finishing and free ranging pigs and 96.8% for adult pigs. The specificity of the combined tests was 100.0% for all three risk groups.

Statistical analysis

Statistical analyses were performed using Microsoft Access 2007 (Microsoft Corporation, Redmond, WA, USA), NCSS 2007 (NCSS Statistical Software, Kaysville, Utah, USA), and the freeware applications WinEpiscope 2.0 and FreeCalc 2.0.

Results

Samples from 7,412 adult pigs, 9,973 finishing pigs and 2,779 free-ranging pigs were collected. Thirty-five per cent (35%) of the adult and finishing pigs were produced under a label that required regular access to outdoor areas and fifty-eight per cent (58%) were produced under a label that did not. Production label data were not available for 7% of the adult and finishing pigs. Of the free-ranging pigs, 9% were raised on the Alps and the remaining 91% in lower agricultural areas (Table 1). The median number of pigs sampled per farm was 8 (0.25-0.75 interquartile range 4-16).

All samples were negative in the routine artificial digestion method using 1-2 grams of diaphragm tissue. In the ELISA, 17 meat juice samples (0.23%) from adult pigs were antibody-positive, as well as 16 meat juice samples (0.16%) from finishing pigs and 9 meat

juice samples (0.32%) from free ranging pigs. None of the ELISA-reactive meat juice samples recognized any bands in the subsequent Western Blot, i.e. they were negative in the Western Blot and therefore considered false-positive in the ELISA.

From 16 ELISA-positive adult pigs, 8 ELISA-positive finishing pigs as well as 8 ELISA-positive free-ranging pigs an additional 20 grams of diaphragm tissue was individually tested by a confirmatory artificial digestion approach. No larvae were detected in any of these samples (Table 2).

Discussion

The assumption of independence of test errors may lead to an overestimation of the overall sensitivity and specificity of the combined tests, since two serological tests were included (19). However, the ELISA and the Western Blot were based on the use of different antigens, reducing the actual level of dependence between these two serological tests. Taking into account the imperfect overall sensitivity of the test methodology, this study demonstrated that the Swiss domestic pig population is both free from *Trichinella* larvae and from anti-*Trichinella* antibodies at a target prevalence level of 0.04% (adult pigs; $p=0.05$) and 0.03% (finishing pigs; $p=0.05$) respectively. It was not possible to demonstrate freedom from infection in the free-ranging pigs at the target prevalence level of 0.1%, because the number of samples was less than 3,000. However, it was possible to demonstrate that the prevalence in free-ranging pigs did not exceed 0.11% ($p=0.05$). These results were in line with findings from routine artificial digestion tests conducted at slaughterhouses in Switzerland, where in 2006 and 2007, 45% and 87% of all slaughtered pigs were tested without any positive result (8, 9).

Human *Trichinella* infections caused by pork consumption have been frequently attributed to free-ranging pigs (4). Free-ranging pigs have a higher probability of acquiring a *Trichinella* infection due to easier access to wildlife and a less controlled housing system and it has been demonstrated that *Trichinella* infections occur more frequently in this group (18, 30). In this study, the percentage of free-ranging pigs with a positive ELISA result (0.32%) was within expectations due to the imperfect specificity of the ELISA test. None of the ELISA-positive samples were positive in subsequent Western Blot tests and no larvae were detected in confirmatory artificial digestion tests. Therefore these ELISA results were considered false-positives.

Direct and indirect diagnostic techniques used for the purpose of surveillance are clearly distinct and each approach has advantages and disadvantages. The artificial digestion technique allows direct detection of the organism (32). However, it has been demonstrated that the sensitivity of the artificial digestion test using 1 gram samples is only 40% when the samples contained 0.01-0.9 LPG (10). In natural infections, 15-20% of the infected animals harbor ≤ 1 LPG (29), and consequently, infected pigs may not be detected reliably during routine testing at slaughter. Also the sensitivity of the confirmatory artificial digestion test is not known exactly, as it depends on the sample size and the larval density. Assuming a Poisson distribution of larvae in muscle tissue, the probability that a 20 grams sample with a larval density of 0.5 LPG contains >0 larvae is 100%. Therefore, for the purpose of this study, it was assumed that the sensitivity and specificity of the confirmatory artificial digestion test were 100%, with a limit of detection of 0.5 LPG.

In contrast, serological techniques were able to detect antibodies in pigs with larval densities at least as low as 0.01 LPG (15). This low limit of detection increases the range over which infected pigs can be detected. However, the imperfect specificity of the ELISA may be a disadvantage for serological surveillance for *Trichinella* spp., since positive ELISA-results can occur even when larvae cannot be detected (17, 24). Consequently, reported seroprevalence estimates tend to be higher than prevalence estimates based on artificial digestion (16, 20, 30). To increase the specificity of serological testing, ELISA-positive samples can be re-tested by Western Blot. This approach increases the specificity of the total serological test protocol to almost 100% (13).

The occurrence of *Trichinella* infections in the wildlife population affects the probability that domestic pigs acquire an infection through contact with wildlife. *T. britovi* is known to be present in Swiss wild carnivores, but there are no indications that *T. spiralis* or *T. pseudospiralis* are present as well. Recent studies showed a prevalence of *T. britovi* larvae of 1.6% in foxes and 27.3% in lynxes (12) and a seroprevalence of 0.2% in wild boar (11). In Switzerland, a large proportion of all pigs fall under a production label requiring regular access to outdoor areas (7) and some are raised on pasture, or on the Alps during the summer period. The high prevalence in the lynx population is of particular interest for pig production on the Alps, because lynxes occupy the same environment.

Results from routine artificial digestion tests over the last several years in Switzerland, as well as results from this study, demonstrate that it is possible to maintain *Trichinella*-free pig production even in areas where *Trichinella britovi* is circulating among the wild carnivores in the same environment. A prerequisite for this coexistence is a level of hygiene that prevents

pigs from having access to wildlife carcasses or feeding on meat scraps. Also, *Trichinella britovi* has a low infectivity for pigs (21), which significantly reduces the probability of infection for pigs that are unintentionally exposed. Nevertheless, in order to continue to guarantee food safety in Switzerland, any *Trichinella* surveillance system should focus on free-ranging pigs, which have a higher probability of being exposed to infected wildlife and becoming infected with *Trichinella* spp.

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Table 1. Number (%) of pigs raised per housing system

Housing system	Adult pigs		Finishing pigs		Free ranging pigs		Total	
Raised under label requiring regular access to outdoor areas	2,820	(38%)	3,299	(33%)	-	-	6,119	(30%)
Raised under label not requiring access to outdoor areas	3,544	(48%)	6,482	(65%)	-	-	10,026	(50%)
Raised on pasture	-	-	-	-	2,530	(91%)	2,530	(13%)
Raised on alps	-	-	-	-	249	(9%)	249	(1%)
No information	1,048	(14%)	192	(2%)	-	-	1,240	(6%)
Total	7,412	(100%)	9,973	(100%)	2,779	(100%)	20,164	(100%)

Table 2. Overview of the diagnostic test results

Category	Total samples	Routine artificial digestion	ELISA	Western Blot	Confirmatory artificial digestion
Adult breeding pigs	7,412	7,412 negative	7,395 negative		16 negative
			17 positive	17 negative	0 positive
					1 N.A. ¹
					8 negative
Finishing pigs	9,973	9,973 negative	9,957 negative		8 negative
			16 positive	16 negative	0 positive
					8 N.A.
					8 negative
Free ranging pigs	2,779	2,779 negative	2,770 negative		8 negative
			9 positive	9 negative	0 positive
					1 N.A.
					8 negative

1) No material available for confirmatory artificial digestion

Qualitative assessment of the probability of human exposure to *Trichinella* spp.

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Impact

- *Trichinella* spp. is a nematode that can occur in many animal species, is transmitted orally and can cause clinical disease in humans.
- For an effective and efficient protection of public health, risk management measures should be focused on those meat sources that pose the highest risk for human health.
- It was demonstrated that in Switzerland pork from free-range pigs and game meat have the highest probability of containing *Trichinella* larvae and risk management should be targeted towards these two categories.

Summary

Trichinellosis, a zoonotic disease caused by the nematode *Trichinella* spp., is transmitted orally and occurs in a wide range of animal species and in humans, but clinical disease is restricted to humans. A qualitative risk assessment was conducted to assess the probability that *Trichinella* spp. occurs in Swiss wildlife and domestic animals, and the probability of human exposure to *Trichinella* spp. in Switzerland. *Trichinella* inactivation steps for raw meat were not considered. *Trichinella britovi* is known to occur in several wildlife species in Switzerland. The probability of human exposure to *Trichinella* spp. through wild boar meat used for private consumption was very low, because not all carcasses are submitted for official meat inspection. Approximately 1-3% of all pigs in Switzerland are raised under free-range conditions with access to pasture and lower biosecurity standards. Due to the presence of *T. britovi* in wildlife, the probability of infection in free-range pigs was very low, for other pigs this probability was negligible. Testing for *Trichinella* spp. at slaughter is compulsory for all susceptible animal species, but small slaughterhouses may be exempt from this obligation. If free-range pigs are slaughtered in such slaughterhouses, the probability of human exposure through pork from free-range pigs is not lowered due to absence of a risk reduction step. Therefore, assuming a worst-case scenario it was concluded that the probability of human exposure through pork from free-range pigs was very low. No data were available to assess the importance of privately imported game meat. To reduce the overall probability of human exposure to *Trichinella* spp., monitoring and meat inspection activities should be targeted at free-range pigs. Also, awareness of hunters and travellers should be increased regarding the risks related to the consumption of game meat that was not tested for *Trichinella* spp. and possibilities for risk reduction.

Introduction

Trichinellosis is a zoonotic disease caused by infection with the nematode *Trichinella* spp. Clinical disease is largely restricted to humans, even though infections can occur in a wide range of omnivorous and carnivorous animal species (36, 45, 56).

In Europe, pork, game meat and horse meat are the main sources of infection for humans, however especially in Western Europe many human cases are acquired abroad. *Trichinella* infections in domestic pigs and horses were rarely detected in most EU countries during the last years (8, 11-13).

In Switzerland, six outbreaks of human trichinellosis were reported in the 20th century in which the source of infection could be determined. Four outbreaks were related to consumption of dog meat and two outbreaks to nutria meat and lynx meat, respectively (38, 39, 43). The source of infection could not be identified in five other cases diagnosed in the 20th century.

Since January 2007, all pigs, horses, wild boar and other animals susceptible to *Trichinella* spp. and destined for human consumption must be tested at slaughter or at game handling establishments in Switzerland (5, 9). Prior to this date, testing in Switzerland was only compulsory for commercially marketed wild boar, although limited testing of pig carcasses was conducted in export-approved slaughterhouses. There is no evidence that *Trichinella* larvae were isolated from domestic pigs since the beginning of the 20th century. On the contrary, *T. britovi* was isolated from Swiss wildlife on various occasions (32, 35, 43, 59).

Based on the results of testing of pigs and data on human trichinellosis, the Swiss Federal Veterinary Office considers the domestic pig population to be free of *Trichinella* spp. Therefore, the implementation of compulsory testing of all pigs at slaughter was perceived as an increased use of surveillance resources without a significant benefit for public health. However, the presence of *T. britovi* in certain wildlife species indicates that there is potential for transmission of infection to the domestic animal population and for human exposure.

The objective of this qualitative risk assessment was to assess the probability that *Trichinella* spp. occurs in the wildlife and domestic cycle in Switzerland and the probability of human exposure to *Trichinella* spp. in Switzerland through the consumption of domestically produced or imported meat.

Materials and methods

A qualitative risk assessment was conducted based on the recommendations for import risk assessment published by the World Organisation for Animal Health (OIE) (69). A risk pathway was developed that included all animal species and routes of transmission that were considered potentially relevant for human exposure to *Trichinella* spp. in Switzerland (figure 1). The following definitions were used to describe probabilities (10):

- Negligible: the event is so rare that it does not merit to be considered;
- Very low: the event is very rare but cannot be excluded;
- Low: the event is rare but does occur;
- Medium: the event occurs regularly;
- High: the event occurs very often;
- Very high: the event occurs almost certainly.

To incorporate the level of uncertainty in our estimates, the following descriptions were used (10):

- Low: there are solid and complete data available; strong evidence is provided in multiple references; authors report similar conclusions;
- Medium: there are some but no complete data available; evidence is provided in small numbers of references; authors report conclusions that vary from each other;
- High: there are scarce or no data available; evidence is not provided in references but rather in unpublished reports or based on observations or personal communications; authors report conclusions that vary considerably between them.

The role of each animal species and meat type in the occurrence and transmission of *Trichinella* spp. in Switzerland was assessed based on the amount of supporting evidence for this. The more evidence was available to support occurrence or transmission in a specific animal species or meat type, the higher their probabilities were assessed to be. When evidence showed inconclusive results, the uncertainty of the estimates was increased.

This assessment did not include any inactivation steps of raw meat, such as cooking, smoking or salting. The final conclusions that were drawn related to the probability of occurrence of live *Trichinella* larvae in raw meat. This assessment also did not include illegal actions.

Results

The risk questions were “what is the probability that *Trichinella* spp. occurs in the Swiss wildlife and domestic cycle” and “what is the probability that consumers in Switzerland are exposed to *Trichinella* spp. through domestically produced or imported meat”.

Evidence on the role of wildlife

The size of the Swiss lynx (*Lynx lynx*) population is estimated at around 100 animals, whereas the wolf (*Canis lupus*) population consists of a few individuals (68, 70). The prevalence of infection in the lynx population was estimated at 27.3% (32). Larvae were also found in one examined wolf (59). Isolated larvae were all identified as *T. britovi*.

The Swiss fox (*Vulpes vulpes*) population is significantly larger: hunting statistics showed that in 2000-2008 between 27,097 and 39,936 foxes were hunted annually (18). In a recent survey, larvae were detected in 21 of 1289 foxes (1.6%) investigated. All recovered larvae were identified as *T. britovi* (32). These results were similar to those found in earlier studies (35, 43). There is no routine monitoring of *Trichinella* spp. in foxes. In neighboring countries, both *T. spiralis* and *T. britovi* were reported to occur in foxes (42, 46, 51, 61). Marketing of fox meat is not allowed in Switzerland (4). Although consumption of fox meat occurs among hunters (7), the annual volume of fox meat consumed nationally was considered to be negligible.

The size of the Swiss wild boar (*Sus scrofa*) population was estimated between 25,000 and 66,000 animals (48), with between 3,611 and 8,748 animals being hunted annually in 2000-2008 (18). Testing of wild boar is compulsory unless they are used for private consumption. Larvae have not been detected in wild boar for many years (28, 40, 41). In the period 2000-2008, between 1,237 and 4,145 wildlife animals were negatively tested for *Trichinella* spp. (20, 22, 24, 26, 27, 30), the large majority of these tests related to meat inspection of wild boar. However, in a recent survey a seroprevalence of 0.2% was found in wild boar (31), indicating that some wild boar may have been exposed to *Trichinella* spp. In neighboring countries, infections with *T. spiralis*, *T. britovi* and *T. pseudospiralis* were found in wild boar (42, 51, 52).

Data about the occurrence of *Trichinella* spp. in other wildlife species in Switzerland are absent or scarce, and all date from more than 20 years ago. Infections were previously found in badgers (*Meles meles*), European polecats (*Mustela putorius*), pine martens (*Martes martes*) and stone martens (*Martes foina*) (39-41). Data are also limited in neighboring

countries. In one study in the north-western part of Italy bordering Switzerland, the prevalence of *T. britovi* in stone martens and badgers was estimated at 7.9% and 1.9% respectively (61). In Germany, one study reported a prevalence of 5% of *T. spiralis* in raccoon dogs (*Nyctereutes procyonoides*) (51).

Rodents are frequently mentioned in combination with *Trichinella* infections as likely carriers; however, out of all rodent species natural infections were only identified in the black rat (*Rattus rattus*), the brown rat (*Rattus norvegicus*) and the bandicoot rat (*Bandicota bengalensis*) (56). The only relevant data from Switzerland date from 1975-1985, when 3 muskrats (*Ondatra zibethica*), 5 brown rats (*Rattus norvegicus*) and 733 rodents of the family muridae were tested, all with negative findings (39, 41). The home range of brown rats was found to be limited to a few hundred meters only (66), and the role of rats in the transmission of infections from one farm to another therefore is unlikely. This statement was supported by studies from Pozio (55) and Stojcevic et al. (65).

Carnivorous birds are only considered susceptible for *T. pseudospiralis* (56). Birds in Switzerland were never examined for *Trichinella* spp., nor was *T. pseudospiralis* ever isolated in any other animal species. Reports of *T. pseudospiralis* in neighboring countries are rare. In 1998, two cases of *T. pseudospiralis* in owls (*Strix aluco* and *Athene noctua*) were reported in central Italy. A few cases in wild boar were reported at the Mediterranean coast in France and in Germany (42, 52).

Evidence on the role of feed

Commercial kitchen waste (including waste from restaurants, industry, other large kitchens) contains approximately 1% meat (23), and an official license is required for commercial processing, marketing or feeding of kitchen waste. To prevent transmission of highly contagious diseases, it must be treated at boiling temperature for 20 minutes (3, 19). *Trichinella* larvae are already inactivated instantly at 62.2°C, although for home cooking it is recommended to achieve a core temperature of 71°C (33, 34). Therefore, the required heat treatment for commercial kitchen waste well exceeds the inactivation threshold for *Trichinella* spp.

A license is not required for feeding of kitchen waste from the own household to own pigs (3, 19). As a result, there were no data available about the frequency and volume of kitchen waste from own households that is fed to pigs, nor about the contents. Also, there were no data available to assess compliance with the requirements for heat treatment. However, it was assumed that feeding of own kitchen waste is negligible in commercial pig production.

It is generally assumed that horses do not eat meat, but horses ingested meat patties when offered (49). Therefore, horse feed must be considered as a potential route of transmission for *Trichinella* spp. Horse feed cannot contain slaughter waste or kitchen waste materials (2), but roughage may contain traces of tissue from wildlife, when such an animal is killed during harvest (18).

When rodents have access to feed, pigs and horses could become infected when [1] a *Trichinella*-infected rodent died in the feed, [2] this rodent was not detected by the farmer, [3] this feed was fed to pigs or horses and [4] the rodent was ingested by a pig or horse. Manual feed distribution would allow the farmer to detect any rodents in the feed.

Cannibalism, for example by tail biting, in a pig herd could contribute to maintaining an infection cycle within a pig herd (37), because *Trichinella* larvae were detected in pig tail musculature (64). Ingestion of infected synanthropic or sylvatic animals by pigs could lead to a new infection (55). Under free-range conditions, access of synanthropic and sylvatic animals cannot be prevented entirely.

Evidence on the role of domestic animals

Switzerland has a domestic pig population of approximately 1.5 million animals and currently approximately 2.6-2.7 million pigs are slaughtered annually (30). Animal-friendly housing systems including outdoor access are very common in Switzerland. Outside pens mostly have concrete or partially slatted floors (6, 29), feeding is mostly done indoor, and pens are well-fenced for reasons of biosecurity. Therefore, these outdoor housing systems were considered to be equivalent to indoor housing systems. Between 1996 and 2008, the percentage of pigs provided with outdoor access increased from 5% to 63% (17). Additionally, a very small percentage of all pigs are raised under free-range conditions, including access to pasture. There were no exact data available about the percentage of pigs that are raised under these conditions, but estimates did not exceed 1-3% (W. Zimmerman and E. Fuschini, pers. comm.). Fencing of pasture does not prevent the entry of wildlife, including wild boar and foxes, nor does it prevent piglets from exiting the pasture. During the summer period, small but unknown numbers of pigs are also raised on the higher pastures of the mountains. These two free-range housing systems were considered to have a significantly different biosecurity level than the outdoor and indoor housing systems.

A recent survey detected neither larvae nor antibodies in 9,973 finishing pigs from outdoor and indoor housing systems, 7,412 breeding sows and 2,779 free-range pigs; 249 of the latter group had been raised on the higher pastures of the mountains (63). These results

confirmed earlier studies (35, 43, 67). Between 2001 and 2008, 8.7 million pigs from all types of housing systems were tested during meat inspection, without any positive finding (21, 24, 26, 27, 30). Although all pig carcasses must be tested for *Trichinella* spp. at slaughter since 2007, an exception is still made for slaughterhouses with a very small slaughter capacity (5). These very small slaughterhouses account for over 90% of all licensed slaughterhouses, but they slaughter less than 8% of all pigs. Products of these slaughterhouses cannot market their products outside of Switzerland. There is currently no system in place that allows to estimate the proportion of free-range pigs and pigs from other housing systems going through these small slaughterhouses. In a worst-case scenario it should be assumed that all free-range pigs are slaughtered in small slaughterhouses and no free-range pigs are tested for *Trichinella* spp. during meat inspection.

The mean annual domestic pork production in 2007-2008 was 181,000 tons (60), of which 1,810 to 5,430 tons (1-3% of the total volume) were estimated to be from free-range pigs. Following through with the worst-case scenario it was assumed that no risk reduction step via meat inspection occurred for pork from free-range pigs.

The horse population in Switzerland consists of approximately 55,000 animals (27, 30), and the number of slaughtered horses gradually decreased from 5,400 in 2000 to 3,000 in 2008 (60). Raising of horses for meat production is uncommon in Switzerland, most horses are kept for leisure or sports purposes. Data about *Trichinella* testing in horses are very scarce. In 1992/1993, 3 of 106 tested horses were serologically positive, but larvae were not recovered from any (43). In 2005 and 2006, 14 and 13 foals respectively were negatively tested at slaughter (24, 26). In 2007 and 2008, 1,730 and 1,743 horses and foals were tested at slaughter, also without any positive finding (27, 30).

Finally, cats and dogs were considered, because dog meat was involved in various human outbreaks of trichinellosis in Switzerland in the 20th century. Between 1968-1985, 3 of 616 tested dogs and 1 of 256 tested cats were positive for *Trichinella* spp. (39-41). Cats and dogs can be considered at risk of acquiring an infection, because they eat rodents, scavenge on carcasses of wildlife or are fed with meat scraps. The large majority of the cat and dog population is domesticated, and do not depend on scavenging behavior for feed. Nevertheless, cats and dogs in rural areas have more access to potentially infected wildlife than those in urban areas. In Finland, it was shown that the prevalence of *Trichinella* infections in rural dogs was higher than in urban dogs in areas with a sylvatic cycle of *Trichinella* spp. (53). There is no official evidence that dog and cat meat is still eaten in Switzerland, but there are internet sources claiming this is the case. However, the number of

people exposed to this meat must be very limited on a national level and the volume of meat consumed annually was considered to be negligible.

Evidence on the role of imported meat

In 2006-2008, the annual volume of imported pork was low with around 11,000-20,000 t, or 5-10% of the total pork consumption. Approximately 90% of all imports originate from Germany and Austria (60). Horse meat was mostly imported; in 2006-2008, 4,700-5,000 t of horse meat were imported annually covering more than 90% of the domestic consumption (60). Traditionally, horse meat was mainly imported from USA and Canada (14, 15), but in 2007 Mexico became the second largest supplier after Canada (16). Imported game meat covers around 80% of the domestic consumption and in 2006-2008, 3,700-3,900 t of game meat were imported annually (60). It was not possible to differentiate between different animal species.

Commercially imported meat from *Trichinella*-susceptible animals must be tested for *Trichinella* in the exporting country. *Trichinella* testing of pork can be forgone when pigs were raised in *Trichinella*-free holdings recognized by the European Commission, or when it has undergone a freezing treatment to inactivate any *Trichinella* larvae if present (25).

Alternatively, meat can be imported privately (without import permit) for own consumption from EU-member states, where pigs and horses must also be tested. However, testing of privately imported wild boar and other game meat for private consumption lies within the responsibility of the importer. Swiss statistics and laboratory results do not allow to determine the amount and origin of privately imported game meat, nor if it was tested for *Trichinella* spp. The probability that game meat is contaminated depends on the country of origin as well as on the animal species.

Risk estimation

The results of the risk estimation are summarized in table 1. In the wildlife cycle, the probability of infection increased when animals were placed higher in the food chain. Other carnivorous animal species were considered to have the same probability of infection as foxes, but due to an absence of sufficient data, the uncertainty was higher. Only meat from privately consumed wild boar had a very low probability of harbouring *Trichinella* larvae, because risk reduction via meat inspection was only partial as data indicated that not all carcasses were submitted for meat inspection.

Feed played a negligible role in transmission of infection to livestock. However, the probability that rodents were eaten by pigs could not be assessed and the probability that wildlife was eaten by free-range pigs was very low.

Only pork from free-range pigs had a very low probability of harbouring *Trichinella* larvae. This was caused by the fact that it had to be assumed that free-range pigs were not subjected to *Trichinella* testing at meat inspection, and thus no risk reduction step occurred. This assumption reflected a worst-case scenario, and therefore the uncertainty of the probability estimate was increased to medium.

Finally, it was determined that commercially imported meat and privately imported pork and horse meat from the EU had a negligible probability of harbouring *Trichinella* larvae. The probability for privately imported game meat could not be assessed due to a lack of data.

Discussion

In case of *Trichinella* spp., pork, horse meat and game were frequently mentioned as potential sources for human infection in Europe (8, 13, 50). However, to our knowledge, a risk assessment was never conducted to evaluate the relative importance of these pathways as a potential source of infection for humans. Such an assessment can be a tool to determine through which source humans are most likely to become infected, and thus to determine where risk reduction efforts should be concentrated.

This risk assessment identified two potential sources for human exposure to *Trichinella* spp.: non-commercial wild boar meat and pork from free-range pigs. Other domestic sources played a negligible role either because the animal species had a negligible probability of being infected or because the volume of meat consumed annually was negligible. Commercially imported meat and privately imported pork and horse meat did not play a role either due to required risk reduction measures.

In Switzerland, a sylvatic cycle of *T. britovi* is evident, although larvae were not isolated from wild boar (31, 32). In neighboring countries with a sylvatic cycle of *Trichinella* spp., *T. spiralis* and *T. britovi* larvae were also isolated from wild boar (42, 51, 52). The inability to demonstrate larvae in wild boar tissue in Switzerland despite serological evidence (31), may be related to the fact that only *T. britovi* was detected in Switzerland. *T. britovi* is less adapted to pigs than to foxes and other carnivores, and larvae may therefore not establish in

high densities and may not persist long in porcine tissue (44). However, an antibody response to the infection remains detectable for an extended period of time. Consequently, meat inspection of wild boar remains an important risk reduction measure to lower the probability of human exposure to *Trichinella* spp. When comparing the current hunting and meat inspection statistics it must be concluded that many wild boar for private consumption may not be submitted for official meat inspection. Information campaigns targeted at hunters should be reinforced to stress the importance of *Trichinella* testing to prevent human infections. Even though the probability of human exposure through fox meat was estimated to be negligible, the related uncertainty was high due to absence of data regarding the volume consumed. Information campaigns should therefore also increase awareness about *Trichinella* spp. in foxes and possible risk reduction measures such as testing and thorough cooking.

Pork from free-range pigs was considered to have a very low probability to lead to human exposure to *Trichinella* spp., however with a medium uncertainty. Currently, it is not possible to trace free-range pigs through slaughter, and therefore it was not possible to verify which proportion of all free-range pigs were tested for *Trichinella* spp. in the large slaughterhouses. Taking a worst-case approach, it was assumed that no free-range pigs had been tested for *Trichinella* spp., and thus no risk reduction occurred before pork reached the consumer.

Inherent to the free-range system, feeding of free-range pigs is not as controlled as of pigs in outdoor and indoor housing systems, and sylvatic and synanthropic animals have more freely access to pig enclosures. Also, piglets can temporarily leave their pasture and roam around in surrounding areas. Contact with infected sylvatic or synanthropic animals constitutes a potential for transmission of infection, although still oral intake of muscle tissue containing larvae is needed to transmit infection (36, 45, 56). Therefore, the probability of infection in free-range pigs in Switzerland remains higher than in pigs in outdoor and indoor housing systems.

This assessment indicated a difference in probability of infection between pigs from free-range systems in comparison to other systems. Therefore, the efficiency of the current monitoring and meat inspection efforts could be increased by targeting free-range pigs, while the efforts for testing of pigs from other housing systems could be reduced. Schuppers et al (62) demonstrated that a risk-based surveillance system focusing on free-range pigs can provide an equivalent level of consumer protection as the current traditional meat inspection, while reducing the number of tested pigs. A prerequisite for such a risk-based inspection system is that free-range pigs could reliably be identified and selected for testing. A recent

publication also proposed that alternative surveillance approaches can be used for pigs depending on the occurrence of *Trichinella* spp. in domestic pigs and wildlife (57).

The probability of human exposure to *Trichinella* spp. through commercially imported meat was considered to be negligible. However, imported horse meat was implicated in several human outbreaks in neighboring countries (1, 47, 54, 58). According to both Swiss and EU regulations, horses as well as other susceptible animals must be tested for *Trichinella* spp. at slaughter, and imported meat should be tested in the country of origin (5, 9, 25). The conclusion related to commercially imported meat is valid under the assumption that the testing requirement is complied with appropriately. If otherwise, it would be necessary to assess the production processes and feeding systems for each animal species in each exporting country separately in order to account for the lack of an adequate risk reduction step.

Privately imported game meat from EU member states poses a certain probability for human exposure in Switzerland. Unfortunately, no data were available regarding the countries of origin, the types of animals, the volumes of meat nor the application of tests to qualify this probability reliably. Nevertheless, information campaigns should increase awareness among travelers about the risks of consuming game meat that was not tested for *Trichinella* spp.

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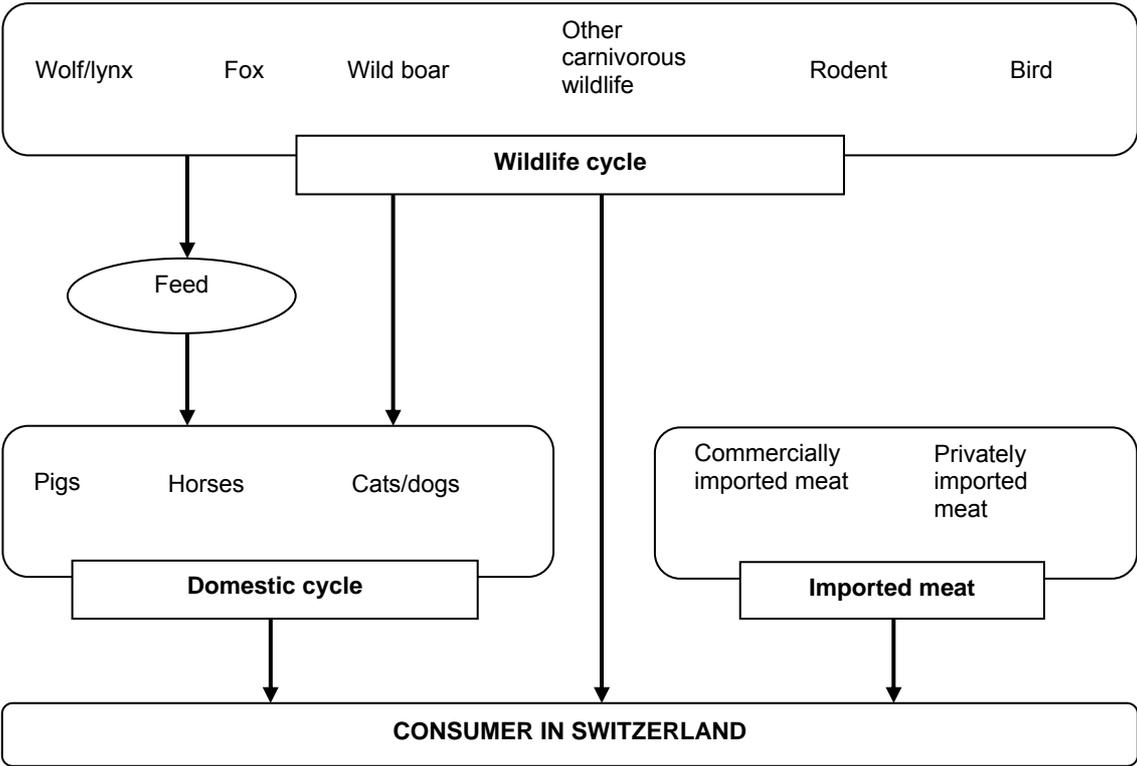
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Table 1. Risk estimation of the occurrence of *Trichinella* spp. in live animals and meat

Cycle	Animal species or meat type	Probability	Uncertainty
Wildlife	Lynx/wolf	High	Low
	Fox	Medium	Low
	Fox meat	Negligible	High
	Wild boar	Low	Medium
	Commercial wild boar meat	Negligible	Medium
	Private wild boar meat	Very low	Medium
	Other carnivorous animal species	Medium	Medium
	Rodents	Very low	High
	Carnivorous birds	Negligible	High
	Feed	Commercial kitchen waste	Negligible
Private kitchen waste		Negligible	High
Roughage for horses		Negligible	Medium
Rodents eaten by pigs		Cannot be assessed	
Wildlife eaten by pigs in controlled housing		Negligible	Medium
Wildlife eaten by pigs in free-range housing		Very low	Medium
Domestic	Pigs in controlled housing	Negligible	Low
	Pigs in free-range housing	Very low	Medium
	Pork from free-range pigs	Very low	Medium
	Horses	Negligible	Medium
	Cats/dogs in urban area	Negligible	High
	Cats/dogs in rural area	Very low	High
	Meat from cats/dogs in rural area	Negligible	High
Import	Commercial import of pork or horse/game meat	Negligible	Medium
	Private import of pork/horse meat	Negligible	Medium
	Private import of game meat	Cannot be assessed	

Figure 1. Risk pathway for the exposure of consumers in Switzerland to *Trichinella* spp.



Comparing the demonstration of freedom from *Trichinella* infection of domestic pigs by traditional and risk-based surveillance

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Summary

Traditionally, the routine artificial digestion test is applied to assess the presence of *Trichinella* larvae in pigs. However, this diagnostic method has a low sensitivity compared to serological tests. The results from artificial digestion tests in Switzerland were evaluated over a time period of 15 years to determine by when freedom from infection based on these data could be confirmed. Freedom was defined as a 95% probability that the prevalence of infection was below 0.0001%. Freedom was demonstrated after 12 years at the latest. A new risk-based surveillance approach was then developed based on serology. Risk-based surveillance was also assessed over 15 years, starting in 2010. It was shown that by using this design, the sample size could be reduced by at least a factor of four when compared with the traditional testing regime, without lowering the level of confidence in the *Trichinella*-free status of the pig population.

Introduction

Nematodes of the genus *Trichinella* are the causative agents of trichinellosis, a zoonotic disease with clinical symptoms in humans ranging from mild to fatal. *Trichinella* spp. also occur in many carnivorous and omnivorous animal species, but animal infections do not lead to clinical signs (17, 28). Transmission of infection occurs via the intake of meat containing infective larvae (22, 27). Appropriate heat or freezing treatment are effective to inactivate larvae (12), and therefore human infections are caused by the consumption of raw or undercooked meat. Wild boar meat, horse meat and pork are the main sources for human infection in Europe (4).

Testing of all slaughtered pigs for the presence of larvae is mandatory in the European Union (EU) and Switzerland to prevent human disease (5). Despite routine testing at pig slaughter in Switzerland since 2001, no larvae have ever been detected (7). A recent study also did not detect anti-*Trichinella* antibodies in domestic pigs (32). Presence of antibodies without direct detection of the parasite would be an indicator for the presence of low-grade *Trichinella* infections that are not detectable by routine artificial digestion.

EU Regulation 2075/2005 requires that one gram (finishing pigs) or two grams (adult pigs) of diaphragm tissue per pig are tested using the routine artificial digestion method during meat inspection. The sensitivity of this method depends on the larval density of the positive samples. Above a larval density of 3-5 larvae per gram (LPG), a sensitivity of 100% was achieved, but below one LPG the sensitivity dropped to 40% (11). Because approximately 15-20% of naturally infected pigs harbored larval densities of less than one LPG (29), infected pigs may not be detected reliably by this method. Despite the large financial efforts involved in testing of all slaughtered pigs during meat inspection, this surveillance is not adequate to prevent human consumption of pork containing low larval densities. However, if surveillance continues over several years without detecting any infected pigs, these surveillance data can be used to demonstrate that the domestic pig population of a country is free from *Trichinella* infection (18, 19).

Instead of applying the routine artificial digestion method to all pigs during meat inspection, a risk-based surveillance programme could be developed that targets high-risk pigs and uses a diagnostic test protocol with a high sensitivity. Targeted sampling of high-risk pigs increases the confidence that infection is truly absent when all samples are tested negative, whereas a diagnostic test system with a high sensitivity increases the probability of detecting infection if

present. Such a risk-based surveillance programme should provide at least an equivalent level of consumer protection as the current meat inspection programme.

The probability of infection of a pig depends on age and housing conditions. In older pigs this probability is higher due to the cumulative effect of longer lives (29). Housing conditions determine access to potentially infected wildlife (carrion) and feeding of slaughter and kitchen waste, both of which are important routes of infection (26, 27). Swiss pig production meets high hygiene standards, thus reducing the importance of feeding of waste materials, but *T. britovi* is known to occur in Swiss wildlife (10, 16). Domestic pigs with outdoor access therefore have a higher probability of being exposed to *Trichinella* spp. than pigs entirely raised indoors.

The first goal of this study was to evaluate the probability that the Swiss slaughter pig population is truly free from *Trichinella* larvae based on the data from the current meat inspection programme, and to model the future probability of freedom if this surveillance is continued in its current format. The second goal was to develop a risk-based surveillance programme for *Trichinella* spp. in domestic pigs that provides an equivalent probability of freedom from infection in the Swiss pig population.

Materials and Methods

Target population

The target population for this study consisted of all slaughtered pigs in Switzerland, the unit of surveillance being one slaughtered pig. The time period for analysis was 1 year.

Model

Disease freedom is usually defined as a certain level of confidence that the true prevalence is below a specified design prevalence (20). Freedom from *Trichinella* infection of the target population can be demonstrated when all pigs tested within the surveillance programme have negative test results. The achieved probability of freedom depends on the number of tested pigs and the test characteristics of the diagnostic test. The probability of freedom increases when all test results are negative for multiple surveillance time periods. A Bayesian approach (18, 19) was used to calculate the probability of freedom using data from multiple surveillance time periods. The model depends on several parameters:

- The design prevalence P^* ;

- The sensitivity of the surveillance system SSe ; and
- The probability of introduction $PIntro$.

At the beginning of each time period tp , a certain prior probability exists that the target population is infected. This probability is reflected by $PriorPinf_{tp}$. At the end of tp it is possible to calculate the posterior probability of freedom $PostPfree_{tp}$ using Bayes' theorem assuming perfect specificity of the surveillance system (18, 19):

$$PostPfree_{tp} = \frac{1 - PriorPinf_{tp}}{1 - PriorPinf_{tp} * SSe_{tp}} . \quad (1)$$

Two alternative designs were calculated and compared. In the first design, the surveillance programme was based on the use of the routine artificial digestion test at slaughter. Slaughtered pigs were tested without consideration of their relative risk of infection, so no risk groups were included in the first design. Data from the routine artificial digestion test were used that were available for the period 2001-2007. Data from 2007 were extrapolated until 2015 to obtain a 15-year surveillance period, assuming the surveillance system would not change from 2008-2015, and no positive results would be recorded. This assumption was considered reasonable, because the data from 2007 reflected a full-scale testing programme in Switzerland and the size of the slaughter pig population has remained stable over the last seven years.

In the second design, a risk-based, serological surveillance programme was considered. An ELISA was used as screening test, and a Western Blot assay (WB) was used as a confirmatory test for any ELISA-positive samples (9, 32). The target population was divided into different risk groups depending on age and housing conditions, and groups with a higher risk were sampled more intensively than groups with a lower risk. The risk-based surveillance programme was also modelled for a 15-year period starting in 2010, directly following nine years of surveillance in design one.

The model was built as a scenario tree with multiple branches (Table 1). First, the total pig population was stratified according to the risk factors age and housing condition. Then, the probability of infection for a randomly selected pig in each of the different strata was determined. Clustering at herd level was not included in the model, because trichinellosis is not a contagious disease and the mere presence of an infected pig therefore does not increase the probability of infection for nearby pigs.

For infected pigs, the diagnostic test system could either correctly confirm this status (outcome=positive), or fail to detect the infected pig (outcome=negative). The specificity of the surveillance system was considered to be 100%. The assumption of perfect specificity is common for programmes demonstrating freedom (1, 20), because a positive finding after confirmatory investigations would imply the loss of the “free status” and the surveillance to demonstrate freedom would be replaced by surveillance to regain the “free status”. Also, the specificity of the WB was 100% or very near (8, 9, 24).

The models were created in Microsoft Excel with the add-in @Risk (Palisade Inc. Newfield, New York, USA). The models were stochastic models with appropriate probability distributions as inputs, and were run with 10000 iterations. A regression analysis was conducted in @Risk to identify the input parameters with the greatest influence on the model outcome (probability of freedom from infection).

Slaughter pig population

In the period 2001-2007, 2.6-2.8 million pigs were slaughtered annually in Switzerland (Table 2). Routine artificial digestion tests had been implemented voluntarily since 2001 and were made compulsory in 2007 (2), though an exception is made for small-scale slaughterhouses that only market their products locally. The results of the routine artificial digestion tests are presented in Table 2. For the risk-based surveillance programme a slaughter pig population of 2.7 million pigs per year was assumed. The slaughter statistics did not allow differentiation between age categories or housing conditions. Therefore, these data had to be derived from other sources.

In 2006, the adult pig population was estimated at 155000 animals (30). Assuming an annual replacement rate of approximately 40%, around 62000 adult pigs were slaughtered in 2006, representing 2.3% of the total slaughter pig population. This percentage was similar to the numbers presented for Denmark (1). The proportion of slaughtered finishing pigs ($PrP_{finishing\ pig}$) was thus modelled as $Pert(0.97, 0.98, 0.99)$ to allow for small variations in the actual proportions and the proportion of slaughtered adult pigs ($PrP_{adult\ pig}$) as $1 - PrP_{finishing\ pig}$.

A large proportion of the Swiss pig population is kept in production systems with access to outdoor areas. According to the annual report of the Swiss Federal Office of Agriculture (6), 61% of all finishing pigs and 58% of all adult pigs have access to outdoor areas. In the majority of cases, these outdoor areas consist of small, confined areas with concrete floors (housing condition: outdoor). Rarely, pigs are kept on pasture under extensive conditions (free-range), but no estimates for the number of pigs in this category were available. Using

expert opinion, it was estimated that 2% of all finishing pigs and 1% of all adult pigs fell in this category. The remaining pigs (37% of all finishing pigs and 41% of all adult pigs) were assumed to be produced under intensive conditions without outdoor access (indoor). To account for uncertainty around these point estimates, the proportions of indoor finishing pigs ($PrP_{finishing\ pig,\ indoor}$) and indoor adult pigs ($PrP_{adult\ pig,\ indoor}$) were modelled as $Pert(0.32, 0.37, 0.42)$ and $Pert(0.36, 0.41, 0.46)$ respectively. The proportion of outdoor finishing pigs ($PrP_{finishing\ pig,\ outdoor}$) was modelled as $Pert(0.56, 0.61, 0.66)$ and of outdoor adult pigs ($PrP_{adult\ pig,\ outdoor}$) as $Pert(0.53, 0.58, 0.63)$. The proportion of free-range finishing pigs was then calculated as $1-(PrP_{finishing\ pig,\ indoor} + PrP_{finishing\ pig,\ outdoor})$ and of free-range adult pigs as $1-(PrP_{adult\ pig,\ indoor} + PrP_{adult\ pig,\ outdoor})$.

Design prevalence and effective probability of infection

P^* was set at 0.0001%, as defined by EU Regulation 2075/2005. Though P^* applied to the whole target population, the effective probability of infection (EPI) differed between the different risk groups. However, the average EPI of all pigs still equalled P^* .

The EPI for a pig is derived from the relative risks (RR) associated with the applicable levels of each of the risk factors specified, *i.e.* age and housing condition. For each risk factor, RR is the risk of infection in its risk category relative to the risk in the lowest risk category for that risk factor. No cases of *Trichinella*-positive pigs have been reported in Switzerland, and also in other Western European countries there is a lack of data to reliably determine the RR of individual pigs in the different risk groups.

The RR of adult pigs in comparison to finishing pigs is derived from the longer life span and thus the increased probability of infection at some time during life. Finishing pigs are slaughtered at around 6 months of age, and the average breeding sow is slaughtered at around 3.5 years of age (assuming five litters per sow). If the probability of infection during life increased linearly, at slaughter a breeding sow would have a seven times higher probability of having acquired an infection than a finishing pig. To account for uncertainty around this assumption, two different RR for adult pigs in comparison to finishing pigs were used: $RR_{adult} = 5$ and $RR_{adult} = 10$.

The RR of pigs raised under outdoor or free-range housing conditions in comparison to pigs under indoor housing conditions is determined by the differences in biosecurity of these housing conditions and thus the probability that pigs in these different housing conditions have contact with infected wildlife or contaminated kitchen or slaughter waste. No estimates

for RR were available, therefore two different increments were selected. First, it was assumed that the RR increased by a factor five between housing conditions ($RR_{outdoor} = 5$ and $RR_{freerange} = 25$). Second, it was assumed that the RR increased by a factor ten between housing conditions ($RR_{outdoor} = 10$ and $RR_{freerange} = 100$).

Combining these two risk factors (age and housing condition) into a matrix, four schemes were developed (Table 3). Relative risks were then adjusted to give adjusted risks (AR), such that the average AR over the target population was one (18, 19). For age:

$$\sum_{l=1}^L (AR_l * PrP_l) = 1 \quad (2)$$

in which the target population was divided into L different age categories, and PrP_l was the proportion of animals in the target population belonging to age group l . This process was repeated for the risk factor housing condition using the appropriate conditional proportions. Then (18, 19):

$$EPI_{l,m} = AR_l * AR_{l,m} * P^* \quad (3)$$

where m denoted categories of housing condition.

Diagnostic tests and the sensitivity of the surveillance system

For the routine artificial digestion test, samples of up to 100 pigs can be pooled. It was demonstrated that the sensitivity of a pooled assay with 100 samples did not exceed 40% in case of larval densities below one LPG (11), a situation that occurs in 15-20% of the pigs infected under field conditions (29). As a conservative approach for design one, it was therefore assumed that the sensitivity of the routine artificial digestion test (Se_{AD}) was 40% and it was modelled as $Pert(0.35, 0.40, 0.45)$ (1).

For design two, an ELISA and WB were used as screening and confirmatory test respectively. Various studies evaluated the sensitivity of the ELISA (Se_{ELISA}) and reported values from 72.7% to 99.2% (8, 21, 23, 25, 33). Se_{ELISA} was therefore modelled as $Pert(0.60, 0.95, 1)$. The Western Blot was recently validated with reported sensitivities of 95.8% to 98.1% (8, 9, 24). The sensitivity of the WB (Se_{WB}) was therefore modelled as $Pert(0.90, 0.98, 1)$.

The SSe is an estimate of the probability that the surveillance system detects infection in the target population if the prevalence exceeds P^* . SSe is calculated as (18, 19):

$$SSe = 1 - (1 - Se_u)^N \quad (4)$$

in which Se_u is the probability that a randomly sampled animal (unit) is both infected and detected and N is the total number of animals in the surveillance system. Equation (4) assumes independence of animals with regard to the probabilities of being infected and detected. In design one, no risk groups were included and Se_u was therefore calculated as:

$$Se_u = P^* * Se_{AD} \quad (5)$$

In design two, an animal in any of the risk groups can give a positive outcome, so Se_u was calculated as:

$$Se_u = \sum_{l=1}^L \sum_{m=1}^M PrSSC_{l,m} * EPI_{l,m} * Se_{ELISA} * Se_{WB} \quad (6)$$

in which $PrSSC_{l,m}$ was the proportion of pigs processed that belonged to the l th age stratum and the m th housing condition stratum.

Probability of introduction

T. britovi is present in Swiss wildlife (10), and constitutes a risk for introduction of infection into the domestic pig population. However, no records of infected domestic pigs exist in Switzerland, and $Plntro$ therefore cannot be derived directly. Alban et al (1) conservatively determined $Plntro$ for the Danish domestic pig population as one divided by the time since the last outbreak, resulting in 1/76. Since this was a conservative estimate, we considered it valid to use a similar $Plntro$ for the Swiss pig population. We modelled $Plntro$ as a Beta distribution with 0 introductions in 75 years (Beta(1, 76)), resulting in a median annual probability of introduction of 0.91% (95% probability interval 0.03-4.7). Taking into account the higher proportion of pigs having access to outdoor areas in Switzerland and the presence of *T. britovi* in wildlife, we also modelled $Plntro$ as a Beta distribution with 0 introductions in 50 years (Beta(1, 51)), resulting in a median annual probability of introduction of 1.3% (0.05-7.0).

Results

Design 1: traditional *Trichinella* surveillance

The SSe increased gradually from 14.95% in 2001 to 62.02% in 2007, because the sample size increased annually during this period. From 2008-2015 the SSe remained equal to the SSe in 2007, because the number of pigs tested was kept constant. The $PriorPinf_{2001}$ was set at 50%, because no other information was available. Depending on the selected $PIntro$, Switzerland could demonstrate freedom from *Trichinella* infection in domestic pigs with 95% confidence by the end of 2010 or 2012 (Figure 1).

The input parameters Se_{AD} and $PIntro$ had the largest influence on the model, although their relative importance changed over time. For example, when $PIntro = \text{Beta}(1, 76)$, the regression coefficients of Se_{AD} and $PIntro$ changed from 0.64 and -0.77 respectively after year two to 0.12 and -0.99 respectively after year 15. Regression coefficients were very similar when $PIntro = \text{Beta}(1, 51)$.

Design 2: risk-based *Trichinella* surveillance

Also in the risk-based surveillance, freedom from infection must be demonstrated with at least 95% probability. The $PriorPinf_{2010}$ (the year in which the risk-based surveillance programme started) was calculated using the $PostPinf_{2009}$ of design one. This was considered appropriate, because the risk-based surveillance programme started immediately after the completion of the traditional surveillance in 2009. The sampling was targeted towards the higher risk groups, and included almost all adult pigs, almost all free-ranging finishing pigs, a large number of outdoor finishing pigs and a small number of indoor finishing pigs. The minimum sample size was determined by increasing the sample size by steps of 10000 samples until freedom from infection was demonstrated (Table 4). For $PIntro = \text{Beta}(1, 76)$, the required sample sizes ranged from 120000 (scheme four) to 360000 (scheme one). For $PIntro = \text{Beta}(1, 51)$, the required sample sizes ranged from 260000 (scheme four) to 620000 (scheme one). Figure 2 shows the probability of freedom from infection achieved by the risk-based surveillance programme from 2010-2024 under scheme one.

The SSe differed for each of the four schemes due to different sample sizes, and was also influenced indirectly by $PIntro$, because a higher $PIntro$ resulted in higher sample sizes. After the required sample sizes had been established, the SSe was determined. For

$PIntro = Beta(1, 76)$, the median SSE of schemes 1-4 varied between 51.3-52.4%. For $PIntro = Beta(1, 51)$, the median SSE of schemes 1-4 varied between 61.1-61.3%.

After one year of surveillance, the model was mainly influenced by four input parameters. For $PIntro=(1, 76)$, in scheme one the regression coefficients were $PIntro_{(design\ 2)}=-0.72$, $PIntro_{(design\ 1)}=-0.60$, $Se_{AD}=0.29$ and $Se_{ELISA}=0.10$. After 15 years, two main input parameters remained: $PIntro_{(design\ 2)}=-0.98$ and $Se_{ELISA}=0.11$. Regression coefficients were very similar for the other schemes.

Discussion

This study demonstrated that surveillance by routine artificial digestion test is not capable of demonstrating freedom from *Trichinella* infection in the domestic pig population at the desired level of confidence based on data from a single year in Switzerland. To achieve this, a much larger slaughter pig population would be required than is available in Switzerland. Freedom from *Trichinella* infection by traditional surveillance can only be demonstrated when historical data are incorporated. The method developed by Martin et al (18, 19) allowed this, by assuming that the posterior probability of freedom achieved in year $t-1$ could be used to derive the prior probability of freedom in year t . However, even when historical data were incorporated, freedom from infection could no longer be demonstrated when the sample size was reduced to one million pigs per year (data not shown). Therefore, Switzerland would need to continue testing almost all slaughtered pigs at slaughter if routine meat inspection alone was used to demonstrate freedom from infection.

The sample size could be reduced significantly when serological tests were used and the different risk groups within the pig population were taken into account. Depending on the scheme selected, the annual sample size was reduced by at least a factor of four without a loss in the probability of freedom from infection. Also, freedom from infection was already demonstrated after one year of risk-based serological surveillance.

Alban et al (1) developed a risk-based surveillance model for *Trichinella* spp. in domestic pigs in Denmark. In this model all adult pigs and all finishing pigs with outdoor access were sampled, whereas finishing pigs from indoor housing systems were not sampled. However, this model used the routine artificial digestion test instead of serology. Serology has two advantages over the routine artificial digestion test. First, especially with low larval densities the diagnostic sensitivity of ELISA and WB is higher than of routine artificial digestion (8, 11,

21, 23, 25, 33). Second, the number of larvae triggering a detectable antibody response is much lower than the number of larvae that can be detected reliably by routine artificial digestion test (13), leading to a higher analytical sensitivity of serology. Thus, the probability of detecting low-grade infections in pigs increases when serology is used, which additionally supports claims of freedom from infection when all samples are negative.

In the present calculations, a positive outcome was defined as detection of antibodies by both ELISA and Western Blot. Detection of larvae was not included, which is usually considered a reference for determining the infection status of a pig (12, 14). However, presence of antibodies indicates that the tested pig has previously been in contact with *Trichinella* spp. False-positive results of the ELISA were excluded by the use of a WB. The combination of both tests was previously shown to have a specificity of at least 99.8% to 99.9% (8, 9). In case antibodies were demonstrated by WB, investigations should be initiated on the farm of origin to assess the opportunities for exposure of pigs to *Trichinella* spp..

The sensitivity analysis showed that *Plntro* was the most important input variable for the model. Very limited data were available to estimate *Plntro*. The first approach was to use a similar value as used by Alban et al (1), who already discussed that this value was a conservative estimate. However, the situation in Denmark is different from Switzerland. *T. britovi* is known to occur regularly in Swiss wildlife (10, 16), whereas *Trichinella* spp. is rare in Danish wildlife (3). Also, outdoor housing of pigs is much more common in Switzerland than in Denmark (1, 6). Therefore, in a second approach an even more conservative *Plntro* was used to take these two differences into account. Also, the sampling in the risk-based surveillance model was heavily targeted towards pigs in the higher risk groups. Despite the increased *Plntro*, freedom from infection could still be demonstrated in the Swiss domestic pig population.

There are very few data about the relative risks of pigs acquiring a *Trichinella* infection. It is generally accepted that pigs with outdoor access as well as adult pigs have a higher probability of infection, but this probability was never quantified. Ribicich et al (31) determined that *Trichinella* infections occurred in pigs raised outdoor but not in pigs raised in confinement or partial confinement, however a relative risk could not be determined. Also in other studies, infections were detected more frequently in pigs in outdoor access than in pigs in indoor housing systems (15, 34), however relative risks were not calculated. Alban et al (1) arbitrarily defined four scenarios with different relative risks for the high risk group, ranging from 5.5 to 69. In this study also four different schemes for the relative risk were used to compensate for the uncertainty around the estimates. Scheme one was considered to be the

most conservative scheme, because the relative risks were minimal. This scheme therefore also lead to the highest required sample sizes.

Ability to identify and trace pigs of the different risk groups clearly is a crucial element for the successful implementation of a risk-based surveillance system. Currently, such identification and traceability is only possible in Switzerland with an unjustifiably high input of resources. Production labels (for example organic production) are poor indicators for the actual pig housing conditions, because farmers may voluntarily exceed the minimum label requirements. Improvement of the pig identification system should be considered before a change to a risk-based surveillance for *Trichinella* spp. is feasible in Switzerland.

In conclusion, this study demonstrated that risk-based serological *Trichinella* surveillance is able to achieve a probability of freedom from infection equivalent to routine artificial digestion, while the required sample size can be reduced by at least a factor of four.

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Declaration of interest

None.

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Table 1. Scenario tree structure for risk-based serological *Trichinella* surveillance in domestic pigs in Switzerland, assuming perfect specificity of the surveillance system

Age	Housing condition	Animal status	ELISA result	Western Blot result	Outcome
Finishing pigs	Indoor	Infected	Positive	Positive	Positive
			Negative	Negative	Negative
		Uninfected			Negative
	Outdoor	Infected	Positive	Positive	Positive
			Negative	Negative	Negative
		Uninfected			Negative
	Free-range	Infected	Positive	Positive	Positive
			Negative	Negative	Negative
		Uninfected			Negative
Adult pigs	Indoor	Infected	Positive	Positive	Positive
			Negative	Negative	Negative
		Uninfected			Negative
	Outdoor	Infected	Positive	Positive	Positive
			Negative	Negative	Negative
		Uninfected			Negative
	Free-range	Infected	Positive	Positive	Positive
			Negative	Negative	Negative
		Uninfected			Negative

Table 2. Number of pigs slaughtered and tested for *Trichinella* spp. in Switzerland in 2001-2007¹

Year	Pigs slaughtered	Pigs tested	Per cent tested	Positive results
2001	2,745,186	404,881	14.7%	0
2002	2,729,495	404,674	14.8%	0
2003	2,646,905	484,623	18.3%	0
2004	2,608,978	488,768	18.7%	0
2005	2,712,779	916,791	33.8%	0
2006	2,801,133	1,249,091	44.6%	0
2007	2,782,708	2,420,008	87.0%	0

1) Source: Federal Veterinary Office, Swiss Zoonoses Reports 2005-2008.

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2009

Table 3. Relative risks of *Trichinella* infection associated with age and housing condition in four combinations (schemes), and adjusted prevalence (effective probability of infection) for each risk group separately. Design prevalence for whole population $P^* = 0.0001\%$.

Scheme	Risk group	Population proportion	Relative Risk	Effective probability of infection
1	Finishing pigs	98.0%	1	
	Indoor	37.0%	1	0.000024%
	Outdoor	61.2%	5	0.000119%
	Free range	1.8%	25	0.000596%
	Adult pigs	2.0%	5	
	Indoor	41.0%	1	0.000131%
	Outdoor	58.1%	5	0.000653%
	Free range	0.9%	25	0.003265%
	2	Finishing pigs	98.0%	1
Indoor		37.0%	1	0.000011%
Outdoor		61.2%	10	0.000111%
Free range		1.8%	100	0.001113%
Adult pigs		2.0%	5	
Indoor		41.0%	1	0.000065%
Outdoor		58.1%	10	0.000648%
Free range		0.9%	100	0.006482%
3		Finishing pigs	98.0%	1
	Indoor	37.0%	1	0.000022%
	Outdoor	61.2%	5	0.000109%
	Free range	1.8%	25	0.000545%
	Adult pigs	2.0%	10	
	Indoor	41.0%	1	0.000239%
	Outdoor	58.1%	5	0.001195%
	Free range	0.9%	25	0.005976%
	4	Finishing pigs	98.0%	1
Indoor		37.0%	1	0.000010%
Outdoor		61.2%	10	0.000102%
Free range		1.8%	100	0.001019%
Adult pigs		2.0%	10	
Indoor		41.0%	1	0.000119%
Outdoor		58.1%	10	0.001187%
Free range		0.9%	100	0.011865%

Table 4. Minimum required sample size to demonstrate freedom from *Trichinella* infection of the Swiss domestic pig population with at least 95% confidence after 15 years of negative risk-based serological surveillance

Risk group Scheme ²	<i>P_{intro}</i> ¹ =Beta(1, 76)				<i>P_{intro}</i> =Beta(1, 51)			
	1	2	3	4	1	2	3	4
Finishing pigs	306,000	119,000	157,500	66,000	573,500	358,750	441,000	208,000
Indoor	15,300	5,950	7,875	3,300	28,674	17,937	22,050	10,400
Outdoor	244,800	65,450	102,375	29,700	501,813	295,969	374,850	150,800
Free-range	45,900	47,600	47,250	33,000	43,013	44,844	44,100	46,800
Adult pigs	54,000	51,000	52,500	54,000	46,500	51,250	49,000	52,000
Indoor	22,140	20,910	21,525	22,140	19,065	21,012	20,090	21,320
Outdoor	31,320	29,580	30,450	31,320	26,970	29,725	28,420	30,160
Free-range	540	510	525	540	465	513	490	520
Total	360,000	170,000	210,000	120,000	620,000	410,000	490,000	260,000

1) Probability of introduction

2) Scheme 1-4 each have a different combination of relative risks for the risk factor age (finishing pigs vs. adult pigs) and housing conditions (indoor vs. outdoor vs. free-range)

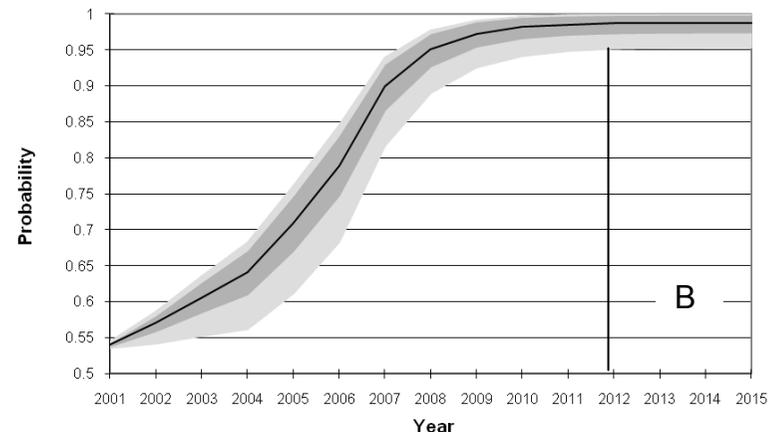
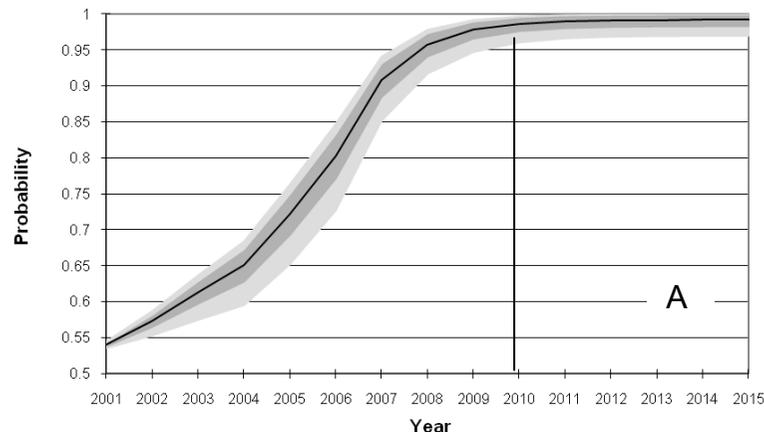


Figure 1. Probability of freedom from *Trichinella* spp. infection of the Swiss slaughter pig population at a design prevalence of 0.0001% achieved at the end of each surveillance year using routine artificial digestion without considering risk groups in the pig population. Vertical line: year at which end the probability of freedom exceeds 95%, as expressed conservatively by the lower limit of the 95% confidence interval. Black line: mean. Dark grey area: \pm one standard deviation. Light grey area: 95% confidence interval. A: Probability of introduction $P_{Intro} = \text{Beta}(1, 76)$, B: $P_{Intro} = \text{Beta}(1, 51)$.

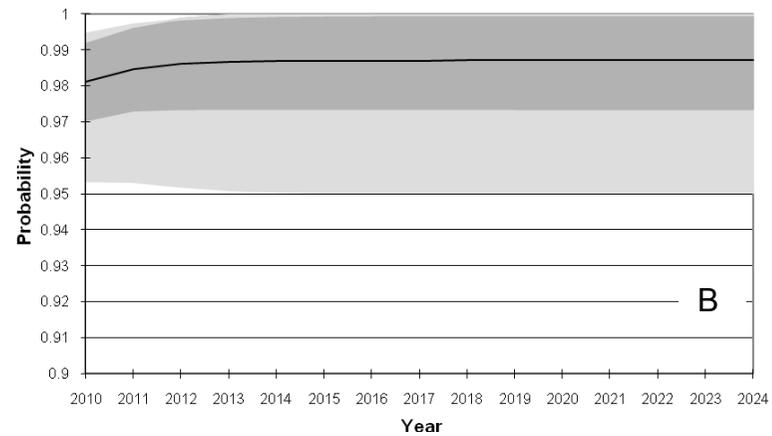
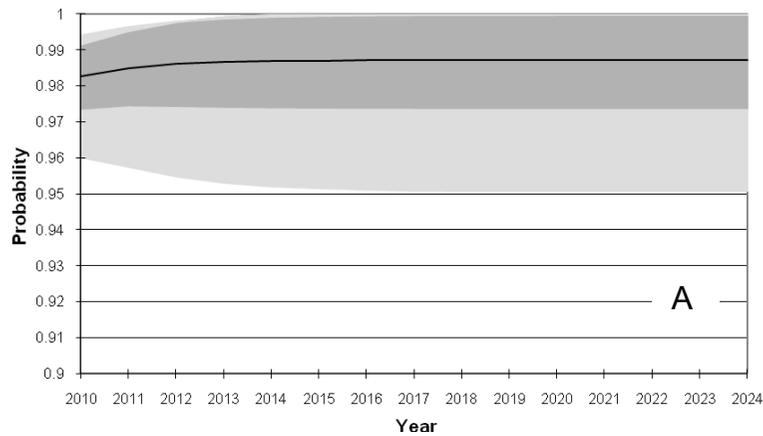


Figure 2. Probability of freedom from *Trichinella* spp. infection of the Swiss slaughter pig population at a design prevalence of 0.0001% achieved at the end of each surveillance year using ELISA and Western Blot assay and considering risk groups in the pig population. Black line: mean. Dark grey area: \pm one standard deviation. Light grey area: 95% confidence interval. A: Probability of introduction (P_{Intro})= $\text{Beta}(1, 76)$, B: P_{Intro} = $\text{Beta}(1, 51)$.

Discussion

“The important thing in science is not so much to obtain new facts as to discover new ways of thinking about them” (Sir William Bragg, 1862-1942).

Discussions about *Trichinella* surveillance in Europe have continued since the initiation of this project. It even appears as if EU Regulation 2075/2005 renewed governmental interest in alternative strategies for *Trichinella* surveillance. This regulation considered that the routine artificial digestion test was an appropriate test to protect public health (4). Historically, little attention had been paid to quality assurance of *Trichinella* testing on an international level, but in 2007 the Community Reference Laboratory for *Trichinella* conducted a ring trial to detect *T. spiralis* larvae in pork among the National Reference Laboratories. The results of this ring trial demonstrated that there were significant differences in laboratory performance, in particular between laboratories using a validated or accredited diagnostic method and those not using such method, and in some laboratories even samples with high numbers of larvae had not been detected (2). These findings undermined the trust that *Trichinella*-positive animals could always reliably be detected by laboratories using the artificial digestion technique, not due to weaknesses of the diagnostic test itself but due to weaknesses of the laboratories. Also, in most countries, routine testing for *Trichinella* is conducted decentralized by laboratories attached to slaughterhouses, which could potentially lead to further complications in assuring the quality of the diagnostic tests.

Serology was evaluated very critically in the EU. In 2005, the EFSA considered that no serological test with an acceptable sensitivity and specificity was available for use in pigs and approved in the EU, and *Trichinella* freedom should be demonstrated by using the artificial digestion test (6). However, in another opinion the EFSA stated that serology could be a useful tool to monitor *Trichinella*-free farms provided that a validated test would be available (7). The EU Regulation also mentioned the option to use serology for monitoring purposes, once a validated test would be available (4). To overcome this problem, an inter-laboratory comparison of an in-house ELISA for use in pigs was organized for the first time by the Community Reference Laboratory. The results showed that this ELISA was robust enough to be used for monitoring purposes (1). In 2010, serology is still not considered a valid alternative for meat inspection of individual carcasses, but it is acknowledged that serology is suitable for monitoring of domestic pigs and that an ELISA was validated and accredited by the Community Reference Laboratory, and further efforts for its standardization are undertaken (11). In the USA, serology is better accepted. An official *Trichinella* certification

program for pig farms has been established, in which serology can be used to verify the “free”-status of certified pig farms (3, 12).

Four years after regulation 2075/2005 was implemented EU-wide it is realized that it has not necessarily led to a significantly increased protection of public health, because within the boundaries of the regulation high-risk pigs are still often not tested at slaughter. It has however, led to testing of millions of low-risk pigs (11). Therefore, new strategies of *Trichinella* surveillance are being discussed. An expert group proposed that the type and intensity of *Trichinella* surveillance could be dependent on the *Trichinella*-status of the region. Four categories were proposed (11):

- Endemic regions where *Trichinella* is present in both wildlife and livestock;
- Low risk regions where there is a low risk that *Trichinella* is present in finishing pigs from controlled housing systems;
- Regions with a negligible risk in finishing pigs from controlled housing systems;
- Regions with a negligible risk in all finishing pigs.

In these categories there is no need for *Trichinella* surveillance in wildlife except for in the last category. This new approach is different from approaches discussed in 2005, when it was considered that *Trichinella*-free regions could not be defined and that only on officially *Trichinella*-free farms a reduced testing would be possible (6, 7). Regulation 2075/2005 foresees the recognition of negligible risk regions, although without further defining “negligible risk” (4). However, practice has demonstrated that a status of “negligible risk” is extremely hard to obtain, as only Denmark has been able to achieve this status (5). Other EU member states informally expressed interest as well, but they all failed to obtain this status until today. Certification of *Trichinella*-free farms has also not been used by EU member states due to the high administrative efforts related to it. This reality has urged risk managers to search for alternative surveillance strategies, a first attempt of which was mentioned above.

The move from meat inspection for *Trichinella* using routine artificial digestion tests for all carcasses to a normal *Trichinella* surveillance activity using either routine artificial digestion tests or serology requires a change of thinking about efforts that are undertaken to protect public health. The meat inspection approach has its strengths, but also its weaknesses, such as the possibility to miss pigs harbouring only a low larval density in their muscles, problems with quality assurance in the laboratories, and the enormous amounts of pigs that are being tested without any positive finding. A serological surveillance system also has its strengths, but also its weaknesses, such as the possibility to miss infected pigs during the early stages

of infection. Also, serology is often used in combination with a reduced testing program, meaning that not all pigs are tested anymore. This implies that the pig production system, including housing system, should ensure that pigs do not get infected, and serology is only used to confirm the absence of infection. The papers presented in this thesis have demonstrated that both systems are able to provide an equivalent – not equal – level of protection for consumers.

A similar approach with reduced testing is already being applied for other zoonotic, foodborne hazards. Switzerland is considered to be free from brucellosis in cattle, sheep and goats, a zoonosis that can be transmitted via milk, among other routes. This “free” status is based on the results of investigations of abortion material and is supported by an annual risk-based surveillance program in small ruminants. The sample size of the surveillance program is designed to provide a 99% confidence that infection would be detected if more than 0.2% of the herds are affected (10). *Listeria monocytogenes* is another important zoonotic agent that can be transmitted to humans via dairy products. Despite the facts that human cases of listeriosis occur in Switzerland and that *L. monocytogenes* has been detected in several dairy products (8, 9), not every single liter of milk is tested. Instead, an official monitoring program is in place and the Swiss research institution Agroscope offers a *Listeria* Monitoring Program for industry partners including sampling and consultancy services to strengthen hygienic production practices (8, 9). Finally, campylobacteriosis is the most frequent foodborne illness in Switzerland, mostly caused by *Campylobacter jejuni* or *C. coli* (9). A study conducted in 2008 documented that, depending on the month, 32% to 90% of the broiler farms were positive for either *C. jejuni* or *C. coli*, and at least 52% of the broiler carcasses were contaminated after slaughter (9). This obviously leaves a large potential for exposure of consumers to contaminated poultry meat. However, instead of testing all broiler carcasses at slaughter, the Swiss poultry industry uses an approach of monitoring at slaughter in combination with a system of positive incentives for *Campylobacter*-negative farms.

The examples above document that a reduced and/or risk-based surveillance approach for zoonotic, foodborne hazards is possible and already exists. It is often combined with strategies to strengthen health and hygiene management on farm and in food processing companies. Such an approach can also be applied for *Trichinella* spp. However, it is important to keep in mind that “zero risk” does not exist. The current meat inspection system for *Trichinella* does not achieve “zero risk”, and a risk-based surveillance system will not achieve it either.

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