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Validation of a new commercial serine protease artificial digestion assay for the detection of *Trichinella* larvae in pork

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ABSTRACT

Trichinella is a zoonotic nematode parasite transmitted by the ingestion of raw or under-cooked meat. Control of the parasite is essential to facilitate public health and trade in products from susceptible food animals, including pork and horse meat. The standard method for detecting *Trichinella* muscle larvae uses pepsin enzyme and hydrochloric acid (HCl) in an artificial digestion procedure. A new artificial digestion assay using serine protease was recently developed and commercialized (PrioCHECK™ Trichinella AAD) for the detection of *Trichinella* larvae in the muscle of infected animals. The assay uses no hazardous substances such as HCl or pepsin. Activation of the enzyme requires an elevated digestion temperature of 60 °C which kills the parasite and reduces the risk of contaminating the environment with *Trichinella*. Compared to the pepsin/HCl method, digestion time for the PrioCHECK Trichinella AAD assay is reduced by a third. A recent study demonstrated these features of the new assay and its suitability for digesting various muscles from domestic and wild animals. To further validate the assay's performance relative to the conventional pepsin/HCl digestion method several comparative studies were conducted using samples from different muscle sites spiked with low levels of encapsulated first stage *Trichinella* larvae (L1). Multiple muscle samples were collected from diaphragm, tongue, masseter, and loin of 3–4 month old pigs. Samples were spiked with 3, 4, 5, or 25 *Trichinella spiralis* L1. A total of 320 meat samples of 100 g each were used to compare the diagnostic proficiency of the PrioCHECK Trichinella AAD assay with the pepsin/HCl digestion method. Comparative and validation data produced from these studies showed that both methods are capable of consistently detecting *Trichinella* in 100 g samples which contained as few as 3 L1 or 0.03 larvae per gram of meat. Overall, the PrioCHECK Trichinella AAD assay performed satisfactorily according to international guidelines of the World Organization for Animal Health (OIE), European Union (EU) and International Commission on Trichinellosis (ICT) for the detection of *Trichinella* infection in pork.

1. Introduction

Trichinella is a meat-borne nematode parasite that infects many species of animals. It is prevalent in wild carnivores and omnivores, with potential for spillover between susceptible species of wildlife and livestock (Pozio, 2015). Historically there has been a close association between *Trichinella spiralis* and pigs, but other species of the parasite can also infect porcine hosts (Pozio and

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Murrell, 2006). Raw or under-cooked pork has been a common source of transmission to humans. Although *Trichinella* infection is not recognized as a cause of disease in animals, any species of the parasite can cause mild to serious illness in humans, and in some cases even death (Gottstein et al., 2009). In a recent global ranking of foodborne parasites, *T. spiralis* was listed as the most important parasite for trade, and 9th in terms of public health (FAO/WHO, 2014). Adequate cooking of pork is usually recommended for protecting consumers from *Trichinella* infection. Other control measures are not as straight forward and may include freezing or curing to kill the parasite or biosecurity and management production practices to prevent exposure of pigs (Gamble et al., 2000; Alban and Petersen, 2016; ICT, 2016). A common tool for establishing or monitoring the effectiveness of control measures is the artificial digestion method which is used to detect first stage *Trichinella* larvae in muscle tissues of host species. In the EU alone, meat samples from millions of pigs are tested annually for the presence of *Trichinella* (Pozio, 2014).

The traditional artificial digestion assay uses pepsin enzyme activated by hydrochloric acid (HCl) and incubation, followed by sedimentation and microscopy for the detection of *Trichinella* larvae in meat, and has been described as a standard method (ISO 18743:2015). Although the assay is widely employed as a diagnostic tool for detecting *Trichinella* infection, it is more commonly used for regulatory certification related to trade and food safety to ensure negligible risk of *Trichinella* infection. The artificial digestion method processes individual samples or pools of samples weighing up to 100 g (ICT, 2014; OIE, 2017). A serine protease artificial digestion assay (PrioCHECK Trichinella AAD) was recently developed and evaluated using limited numbers of muscle samples from domestic and wild animals for the detection of *Trichinella* (Konecsni et al., 2017). The results for pork indicated test performance that was comparable with the standard pepsin/HCl digestion method, and confirmed lab advantages of convenience, safety and speed.

Reliable performance that is fit for purpose requires method validation, quality assurance measures, and suitable samples. The present large scale study was designed to generate experimental data to validate the performance of the PrioCHECK Trichinella AAD assay for detecting *Trichinella* in fresh (unfrozen) pork tongue, diaphragm, masseter and loin to meet various international standards for trade and food safety.

2. Materials and methods

2.1. Experimental design

To demonstrate the performance of the PrioCHECK Trichinella AAD assay a total of 160 pork samples from four muscle sites were spiked with proficiency samples containing encapsulated *T. spiralis* first stage larvae (L1) and tested for parasite recovery. An additional 160 matching samples were tested using the traditional pepsin/HCl digestion method for detecting *Trichinella* in pork.

Specifically, ten fresh muscle samples collected from each of diaphragm, tongue, masseter, and loin spiked with 3, 4, 5, or 25 *T. spiralis* L1 were tested using the PrioCHECK Trichinella AAD assay (Table 1). A matching set of spiked samples was also tested by the traditional pepsin/HCl digestion method. Prior to spiking, a 100 g sample from each muscle site used was tested by the pepsin/HCl method to ensure *Trichinella*-negative status of the source tissue.

2.2. Sample collection and preparation

All pork muscle samples used in this study were collected from a local commercial abattoir. Sufficient samples of each of the four muscle sites were collected from as few carcasses as possible to minimize variability. *Trichinella*-free muscles were trimmed to remove visible fat and fascia, vacuum packed and kept at 4 °C for use within 6 weeks. After confirming the *Trichinella*-negative status of each site, 100 g muscle samples containing proficiency samples with precise numbers of embedded encapsulated L1 were prepared as previously described (Forbes et al., 1998; Konecsni et al., 2017). The experimental infection and use of rats to produce *T. spiralis* L1 for the proficiency samples were approved by the University of Saskatchewan Animal Care Committee in accordance with the guidelines of the Canadian Council on Animal Care.

2.3. Digestion assays

All aspects of the digestion assays used in this study, including sample preparation and stereo-microscopic examination, were performed by trained and certified analysts in a laboratory accredited or certified for *Trichinella* digestion testing according to

Table 1
Numbers of 100 g pork samples collected from four muscle sites spiked with encapsulated larvae (L1) of *Trichinella spiralis* and tested by the serine digestion assay (PrioCHECK Trichinella AAD) or standard pepsin/HCl digestion method.

Muscle	PrioCHECK Trichinella AAD				Pepsin/HCl			
	3 L1	4 L1	5 L1	25 L1	3 L1	4 L1	5 L1	25 L1
Diaphragm	10	10	10	10	10	10	10	10
Tongue	10	10	10	10	10	10	10	10
Masseter	10	10	10	10	10	10	10	10
Loin	10	10	10	10	10	10	10	10
Total	40	40	40	40	40	40	40	40

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