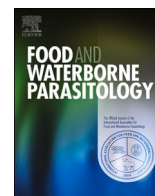




Contents lists available at ScienceDirect

# Food and Waterborne Parasitology

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## A review of testing and assurance methods for *Trichinella* surveillance programs

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### ARTICLE INFO

#### Keywords:

Magnetic stir Bar method  
Pork surveillance  
*Trichinella*

### ABSTRACT

While global cases of trichinellosis have fallen since pork regulation began, the disease remains a danger to pork and animal game consumers as well as a liability to producers. Managing food safety risk and supporting agricultural trade requires cost-effective and sensitive diagnostic methods. Several means exist to inspect pork for parasitic infections. Here, we review literature concerning the sensitivity, specificity, and cost of these methods. We found that artificial digestion coupled with optical microscopy to be the best method for verification of *Trichinella* larva free pork due to its cost efficiency, high specificity, and reliability. Serological techniques such as ELISA are useful for epidemiological surveillance of swine. While current PCR techniques are quick and useful for diagnosing species-specific infections, they are not cost efficient for large-scale testing. However, as PCR techniques, including Lateral Flow- Recombinase Polymerase Amplification (LF-RPA), improve and continue to reduce cost, such methods may ultimately succeed artificial digestion.

### 1. Introduction

Zoonotic parasites in the genus *Trichinella* cause the clinical illness trichinellosis. Humans acquire this disease by ingesting raw or undercooked meat infected with larvae. *Trichinella* is commonly associated with the consumption of domestic pork but in recent years more infections have been associated with high-risk meats: bear, cougar, wild boar, and horses (CDC, 2017; Gottstein et al., 2009). Of the eight species confirmed to infect humans, the most widespread is *T. spiralis* (Almeida et al., 2018; Gajadhar et al., 2019; Gayda et al., 2016).

*Trichinella* transmission can occur by domestic or sylvatic cycles. Domestic cycles occur through improper management in which livestock is fed infected meat scraps, consumes infected carcasses, or is reared in locations with infected animals such as rats and mustelids (Gottstein et al., 2009). This route of transmission is uncommon in the United States, Canada, and many countries in the European Union due to improved management practices and routine surveillance (Franssen et al., 2017; Gottstein et al., 2009). Transmission also occurs through the sylvatic cycle; infections alternate between hosts by the consumption of infected muscle tissues (Gottstein et al., 2009).

Once an organism ingests *Trichinella* spp. larvae, their gastric acid and pepsin liberate larvae from the muscle tissue. Larvae migrate to the small intestine where they molt four times and develop into adults within 48 h. After copulation, 5 to 7 days post-infection, females birth newborn larvae. These larvae then enter the bloodstream and invade striated muscle cells. Larvae from all *Trichinella*

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<https://doi.org/10.1016/j.fawpar.2021.e00129>

Received 29 March 2021; Received in revised form 28 July 2021; Accepted 3 August 2021

Available online 9 August 2021

2405-6766/© 2021 Published by Elsevier Inc. on behalf of International Association of Food and Waterborne Parasitology. This is an open access

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spp. induce muscle cell dedifferentiation and transformation into a nurse cell (nucleated mass surrounding the larvae) (Bogitsh et al., 2013; Pozio et al., 2001). However, only species that infect mammals induce the nurse cell to synthesize a collagen capsule that allows these larvae to withstand more extreme conditions (Pozio et al., 2001). The nurse cell larva complex remains infectious and survives in host muscle tissues until a new host ingests the infectious meat allowing the larva to continue its life cycle.

*Trichinella* can be distinguished based upon whether it encapsulates after muscle cell differentiation (Gajadhar et al., 2019; Gottstein et al., 2009). Those that form capsules have a narrow host range: they require temperatures between 37 and 40 °C to complete their life cycle and infect mammals) while non-encapsulated species have a wider host range (25 to 40 °C) and can infect mammals, birds, and reptiles (Gottstein et al., 2009; Pozio, 2005). *Trichinella*'s wide host range poses a public health risk.

*Trichinella* surveillance programs are essential to prevent outbreaks and reduce economic loss. As such, surveillance methods must be highly sensitive and specific to be suitable for detection. Here we compare available methods to aid swine surveillance programs and consider which approach(es) to use for various applications, taking into consideration sensitivity, detection speed, and cost. A

**Table 1**  
Summary of swine detection methods.

Test	a. Sensitivity (%)	b. Specificity (%)	c. Cost per Sample	d. Earliest Detection Post Infection	e. Lowest Larva Detection	References
ELISA (ES)	90.9–99.6%	98%	\$0.83* (includes personnel)  \$0.15	2–7 weeks	0.01 LPG	a. Gajadhar et al., 2009; Gómez-Morales et al., 2009; Nöckler et al., 2009 b. Gajadhar et al., 2009; Gómez-Morales et al., 2009; Nöckler et al., 2009 c. Pyburn, 2007; Supplementary Data 1 d. Bruschi et al., 2019; Gajadhar et al., 2009 e. Gamble et al., 1983
LF-RPA	100%	100%	\$5.36****, ****	17 days	100 fg	a. Li et al., 2019 b. Li et al., 2019 c. Supplementary Data 1 d. Gamble, 2014 e. Li et al., 2019
LAMP	High	High	\$5.90**	17 days	100 fg DNA	a. Li et al., 2012 b. Deng et al., 2019 c. Supplementary Data 1 d. Gamble, 2014 e. Lin et al., 2013.
Conventional PCR	High	High	\$3.98**	17 days	1 pg DNA	a. Lin et al., 2013 b. Kong et al., 2021; Liu et al., 2017 c. Supplementary Data 1 d. Gamble, 2014 e. Lin et al., 2013
qPCR	High	100%	\$3.78**	17 days	0.01 LPG 10 fg DNA	a. Alonso et al., 2011; Lin et al., 2013 b. Cuttell et al., 2012; Lin et al., 2013. c. Supplementary Data 1 d. Gamble, 2014 e. Alonso et al., 2011; Lin et al., 2013
Agglutination	80%	High	\$0.08	2–3 weeks	10 LPG	a. Gayda et al., 2016 b. Haralabidis et al., 1989 c. Supplementary Data 1 d. Gayda et al., 2016 e. Haralabidis et al., 1989
MSM	100%	Varied	\$1.72 * (includes personnel) \$0.06	17 days	1 LPG- 3 g/ 5 g samples 3 LPG- 1 g samples	a. Gajadhar et al., 2019; World, 2018; European Union Commission implementing regulation (EU) 2015/1375 of 10 August 2015 laying down specific rules on official controls for <i>Trichinella</i> in meat Of. J. EU, 2015 b. Marucci et al., 2013 c. Pyburn, 2007; Supplementary Data 1 d. Gamble, 2014. e. Forbes and Gajadhar, 1999; Gamble, 1996
Trichinelloscopy	43%	100%	Unavailable	17 days	6 LPG	a. Beck et al., 2005 b. Beck et al., 2005 c. Supplementary Data 1 d. Gamble, 2014 e. Beck et al., 2005
AAD Kit	100%	Varied	\$0.14	17 days	1 LPG- 3 g/ 5 g samples 3 LPG- 1 g samples	a. Gajadhar et al., 2018; Konecsni et al., 2017 b. Marucci et al., 2013 c. Supplementary Data 1 d. Gamble, 2014. e. Forbes and Gajadhar, 1999; Gamble, 1996

\* In house.

\*\* DNA extraction price included.

\*\*\* Prices for PCR and variants were not optimized for large scale screening.

\*\*\*\* PCR costs assume sample DNA is not pooled. MSM/AAD is pooled.

summary of our findings regarding specificity, sensitivity, and cost is found in Table 1.

## 2. Methods

We compiled literature using databases: PubMed, Google Scholar, Science Direct, ResearchGate, and EBSCO. Articles were assessed based on their application in surveillance testing and diagnosing *Trichinella*, whether they would be able to meet the international standards, and their relative cost compared to other methods. All years were taken into consideration up until March 2021. We evaluated detection methods used in quality assurance, comparing each to the magnetic stir-bar method (MSM). MSM is our reference, given its international acceptance as the gold standard for use in surveillance (Mayer-Scholl et al., 2017). We sought alternative methods matching MSM reliability in detecting infections likely to risk human health. At the conclusion of our literature review, we found 63 references suitable for use.

## 3. Results and discussion

### 3.1. Magnetic stir Bar method

The sensitivity of MSM is defined for samples of a specified size and larval burden. A one-gram sample suffices for reliable detection in muscle tissue infected with  $\geq 3$  larvae per gram (LPG). Five-gram samples reliably detect  $\geq 1$  LPG; while three-gram samples can detect as low as 1 LPG (Forbes et al., 1998; Gamble, 1998). The sensitivity of any detection method improves when the pathogen is abundant. Because larvae do not establish uniform distributions in muscle tissues, a larger mass of muscle must be digested to confidently detect sparser infections (Kapel et al., 2005). *Trichinella* spp. larvae can be detected in muscle samples 17 days after initial infection (Gamble, 2014). This time is consistent with all methods that use muscle tissue for detection.

Performing MSM incorporates quality assurance practices delineated by the International Commission on Trichinellosis (ICT) and developed by the ICT quality assurance committee (European Union Commission implementing regulation (EU) 2015/1375 of 10 August 2015 laying down specific rules on official controls for *Trichinella* in meat Of. J. EU, 2015; World, 2018); these follow International Organization for Standardization (ISO) ISO/IEC 17025 and 17,043 standards (Gajadhar et al., 2019; World, 2018). These guidelines facilitate accuracy in testing by ensuring laboratories have a quality management system which includes documentation of the management systems, validation in test methods with control points that are explicit and measurable, proficiency testing for analysts, documentation of results, procedures establishing improvements in methods, internal and third-party audits of performance, and lab certification (Gajadhar et al., 2019). The MSM is effective when laboratories meet quality assurance standards and follow the guidelines of the ICT. However, the precision of MSM is not guaranteed if those standards are not upheld. Genomic methods and serology each have positive and negative controls to inform the operator of mistakes. Artificial digestion lacks internal controls. As such proficiency testing must be done at regular intervals (Mayer-Scholl et al., 2017).

The official ICT protocol is summarized as follows. One gram samples cut from preferred muscles (tongue, diaphragm, masseter) free from fat and fascia are pooled from 100 pigs. These pooled samples are then digested in a pepsin-HCL (pre-heated 46–48 °C) solution, poured into a glass beaker, and incubated with a magnetic stir bar at 44–46 °C for 1 h. The digest is then poured through a wet sieve (180–200  $\mu\text{m}$ ) into a separatory funnel and rinsed with an additional 100 ml of tap water. The digest settles for 30 min. Then, 40 ml of the settled fluid is collected and sits for 10 min before being rinsed with tap water. 30 ml are discarded, and 10 ml of the sample is poured in a 1 cm gridded petri dish and analyzed using a stereomicroscope at 40 $\times$  magnification (Gajadhar et al., 2019).

Regulators have defined sources of diagnostic error for the MSM method. In an uncertainty study evaluating MSM, the maximal loss of larvae per individual sample was found to be 15%. Although, the study loss and tolerance derived is sample size-dependent and appears to be inflated: major larvae loss was found at sedimentation with separatory funnel (2%), washing step and sedimentation with a glass tube (6%), and microscopic examination (2%) - total 10% (Riehn et al., 2013). Other steps were estimated to have 2–3% error but aren't confirmed in the studies' recovery steps. Most larvae were lost in the first and second rinsing steps (Riehn et al., 2013). High-volume testing labs correctly identified nearly all positive samples but recovered only ~60% of the larvae for Proficiency Testing (PT) (Riehn et al., 2013). Laboratories that recovered >80% of larvae and reported no false negative or positive test results typically had low recovery (Riehn et al., 2013). High-grade facilities tended to miss larvae, illustrating the difficulty in implementing MSM to quantify larval burdens. Lab shortcomings only partially explained larvae loss, which systematically contributes to undercounts. In addition, as helminth larvae of different species can show a similar morphology under an optical microscope, misidentification of *Trichinella* spp. larvae can occur. Re-testing of a sample will often yield no result (Marucci et al., 2013).

Laboratories that do meet quality assurance guidelines document especially high accuracy. PT performance in National Reference Laboratories for Parasites had a 100% pass rate in 2014 (Marucci et al., 2016). Franssen et al., 2017 reported few false negatives for all samples- wild boar, domestic and free-range pigs - that were present in the pooled test sample. The estimated prevalence of false negatives in all pooled samples was  $3.73 \times 10^{-3}$  (95% CI  $2.17 \times 10^{-3}$ –  $4.9 \times 10^{-3}$ ), illustrating that the MSM method is sensitive to identifying true positives (Franssen et al., 2017). Recovery of larvae increased as larval density increased. However, samples containing fewer larvae would be at greater risk of being overlooked.

To understand the implications for MSM detection limits, we consider the typical infectious dose for human trichinellosis. Consuming 60–750 larvae poses risk; therefore, the ability to detect a larva in 1 g of pork defines a sensible public health standard (European Commission, 2005). However, Marucci et al., 2016 reported that 50% to 69.2% of the participating laboratories in the National Reference Laboratories for Parasites PT correctly identified the positive sample when samples were spiked at the lowest larval burden 1 LPG. Teunis et al., 2012 also found that consumption of 100 g pork infected with 200 larvae could fail to be detected by MSM.

Furthermore, Forbes and Gajadhar, 1999 reported a 73% sensitivity when samples were spiked with 1–1.4 LPG. When a larva infection was between 0.001 and 0.9 LPG, the sensitivity of the MSM method dropped to 40% (Forbes and Gajadhar, 1999). These findings illustrate that MSM, even when practiced by experienced inspectors, often fails to detect an infection when larval number is low. Outbreaks of trichinellosis in humans have been recorded with fewer than 3 LPG in frozen boar meat, and the average infective dose is suspected to be 150 worms (Van der Giessen et al., 2013; Teunis et al., 2012). Franssen et al., 2017 estimated that infections as low as 0.01 LPG can be missed during an inspection and lead to human trichinellosis. To account for samples between that range, more sensitive tests would be needed. We reviewed other methods and digestion techniques in relation to MSM performance. These include PrioCHECK™ Trichinella AAD Kit, trichinelloscopy, ELISA, PCR, LAMP, real time PCR (qPCR), and LF-RPA. .

### 3.2. PrioCHECK™ Trichinella AAD kit

The first method we considered is a variation in artificial digestion using the PrioCHECK™ Trichinella AAD Kit (AAD Kit). The AAD Kit utilizes a digestion buffer, a proprietary enzyme solution, and a digestion buffer additive. There are several benefits to the AAD Kit over the MSM method. The AAD Kit is safer. Although the additive used can cause minor skin/eye irritation if spilled, the digestion buffer (20×) is not hazardous (Digestion Buffer Additive, 2014; Digestion Buffer (20×), 2014). In contrast, the reagents used in artificial digestion are more harmful. Hydrochloric acid is caustic and volatile requiring a fume hood. While uncommon, pepsin can be immunogenic. A 22-year-old woman who worked as a *Trichinella* meat inspector developed allergic rhino conjunctivitis due to pepsin exposure (Marquès et al., 2006). Pepsin shortages and quality issues persist (Franssen et al., 2019). Reducing the digestion time from 30 min (MSM) to 20 min is a convenience the AAD Kit offers. The AAD Kit limits environmental contamination and occupational exposure since the higher digest temperatures (60 °C) kills larvae. A downside of the increased temperature is burn hazard. By killing the larvae, the AAD Kit may limit the ability to distinguish species since PCR assays succeed best when extracting DNA from live larvae. Nonetheless, success has been reported in applying the multiplex PCR assay to such larvae (Konecni et al., 2017).

Several studies report comparable diagnostic sensitivity for the AAD Kit and magnetic stir-bar method; each meets international standards for diagnosing *T. spiralis* in pork. Each test was able to detect 0.03 LPG and no false positives were recorded (Gajadhar et al., 2018). Samples spiked with larger than 25 *T. spiralis* larvae showed a greater than 90% recovery for both the AAD Kit and MSM (Gajadhar et al., 2018). However, the sensitivity of the AAD assay is variable and depends on species and tissue type. This requires validation for surveillance in other types of meat and for other species of parasites. Franssen et al., 2019 reported low recovery of *T. pseudospiralis* using the AAD method. Compared to MSM, which detected ~80% of *T. pseudospiralis* larvae (69.2–87.7%), the AAD Kit recovered only ~20% (12.3–30.8%;  $p < 0.0001$ ). The same study showed MSM outperformed the AAD Kit, to a lesser extent, when seeking to recover larvae of *T. spiralis* (89.9–96.7% vs. 69.2–87.7%, respectively). Recovery rates for the two methods were  $74\% \pm 10\%$  AAD Kit and  $90\% \pm 11\%$  MSM for pork recovery of the spiked diaphragm.

Each approach required additional clarification steps when seeking to identify *T. spiralis* from horse tongue tissue and pig diaphragm. More debris resulted from digesting these tissues when using AAD Kit which, as was found by Konecni et al., 2017 and Gajadhar et al., 2018, necessitated larger rinse volumes than the manufacturer's recommendation (300 ml). This could increase assay time and introduce further human error. Favorable for the AAD Kit, under the lowest spiked concentrations of *T. spiralis* (3LPG) in pork tongue, diaphragm, masseter, and loin yielded  $\geq 86\%$  recovery, but yielded 80% recovery for MSM (Gajadhar et al., 2018). Thus, the AAD Kit may allow for better detection of samples harboring fewer larvae. More evaluation on lower larvae burden is warranted.

### 3.3. Trichinelloscopy

Trichinelloscopy was the original method for *Trichinella* detection and surveillance. This method results in 0.5–1.0 g of muscles cut in small pieces about the size of a grain of rice (Boireau et al., 2007). Then samples are compressed between two thick glass slides and examined via an optical microscope at  $4\times$ – $40\times$  zoom (Beck et al., 2005; Boireau et al., 2007; Vignau et al., 1997). As with artificial digestion techniques, it relies on the operator's familiarity with the organism to be effective, but even experienced operators generally attain worse sensitivity than can be achieved through artificial digestion. Trichinelloscopy has fallen out of favor, in part owing to the recognition that it often fails to detect larvae of non-encapsulating species or larvae that have yet to encapsulate (Boireau et al., 2007). At  $<6$  LPG, trichinelloscopy has a sensitivity of only 43.4% and a specificity of 88% (Beck et al., 2005).

*Trichinella* surveillance programs need to reliably detect 1–3 LPG, meaning trichinelloscopy is not viable as the main method of certification (Gajadhar et al., 2009). Despite such drawbacks, trichinelloscopy is useful when quick analysis of samples must be done in the field, and when positive results are more valuable than negative.

### 3.4. Serology

Serological methods use excretory/secretory (ES) antigens to determine if an animal was infected by *Trichinella* spp. larvae. When a *Trichinella* spp. larva invades host tissues, the parasite protects itself from the host immune response and releases ES proteins into the bloodstream (Alonso et al., 2011; Gajadhar et al., 2009). These ES antigens stimulate an antibody response targeted by the *Trichinella* ELISA assay (Bruschi et al., 2019; Cuttell et al., 2014; Gajadhar et al., 2009). Serological tests are commonly used for population surveillance, but not for individual carcass detection (Gajadhar et al., 2019). ELISA (enzyme-linked immunosorbent assay) is a high-throughput method that is standardized and could be automated for large-scale testing (Gajadhar et al., 2009). Sensitivity of ELISA has been reported between 90.6% and 99.6% and its specificity is 98% making ELISA quite reliable (Gajadhar et al., 2009; Gómez-Morales et al., 2009; Nöckler et al., 2009).

However, ELISA assays have drawbacks, rendering them unsuitable as the main method of pork certification. ELISA requires anti-*T. spiralis* IgG antibodies to be present in the bloodstream. These responses commence 2–7 weeks after infection (depending on initial infective dose). Consistent levels of IgG thereafter persist which makes this method unsuitable to distinguish active infections from past infections (Bruschi et al., 2019; Gajadhar et al., 2009). This lag is important because larval infections can precede detection by ELISA (Bruschi et al., 2019; Gajadhar et al., 2009; Pozio et al., 2020). Another drawback (shared by artificial digestion and trichinelloscopy) is that the test cannot differentiate amongst parasite species (Almeida et al., 2018; Bruschi et al., 2019; Gajadhar et al., 2009). The ES antigen target is similar amongst all nine species. As such, ELISA can be a powerful tool to monitor swine for outbreaks of *Trichinella* but cannot determine the parasite species. Because of ELISA's limitations, western blot (WB) is coupled with ELISA to ensure positive sera is truly positive as WB has a sensitivity of 96% and a specificity of 99.6% (Bruschi et al., 2019; Frey et al., 2009). In addition, serum samples from animals infected by encapsulated and non encapsulated species can be distinguished by the WB patterns (Gómez-Morales et al., 2018). WB cannot distinguish between active (presence of infectious larvae in muscles) and non-active (absence of infectious larvae in muscles) infections. (Pozio et al., 2020). Due to its cost, time, and expertise required, WB would make a poor screening tool (Yang et al., 2016).

The agglutination assay binds the trichin-L protein to trichin-L IgG antibody- coated latex beads that form an immune complex in either digested meat samples or in blood samples (Haralabidis et al., 1989). This method has a sensitivity of 80%, compared to artificial digestion, has no cross reactivity with other helminth species, and can detect at least 10 LPG in less than three minutes (Gayda et al., 2016; Haralabidis et al., 1989). This works when positive results matter greatly, and negatives do not.

### 3.5. Detecting parasitic DNA

Genomic methods identify the presence of specific portions of the *Trichinella* genome. Such techniques have been used to help to understand parasite epidemiology and other factors relating to human health. The targets are usually conserved regions of the subunit ribosomal DNA -internal transcribed spacers; other targets (such as beta-carbonic anhydrase or mitochondrial ribosomal RNA genes) have been developed (Almeida et al., 2018; Atterby et al., 2009; Emameh et al., 2015; Li et al., 2012). Genomic methods include polymerase chain reaction (PCR), quantitative PCR (qPCR), loop mediated isothermal amplification (LAMP), and lateral flow-recombinase polymerase amplification (LF-RPA).

Amongst the many variants of PCR, the most promising may be quantitative real-time PCR (qPCR). qPCR has a detection limit 10 times lower than PCR, and 100 times lower than LAMP (Alonso et al., 2011; Lin et al., 2013). In addition, qPCR can be used on both fresh and frozen samples (Lin et al., 2013). *Trichinella* spp. larvae can also be detected in feces; although no field tests have been done and the duration of *Trichinella* spp. larvae excretion is narrow (1–3 weeks) so constant monitoring is needed to detect active infections (Kong et al., 2021). Both qPCR and LAMP were also able to detect *T. spiralis* in mouse feces at high larvae burdens (Kong et al., 2021; Liu et al., 2017). Unfortunately, qPCR and PCR require a high initial investment in technologies and training. LAMP still requires training but does not require a single temperature (no thermocycler needed) and can be done in a water bath if needed. Despite these differences, each method boasts high sensitivity and high specificity to *Trichinella* (Cuttell et al., 2012; Lin et al., 2013). Although, LAMP primers have high cross-reactivity within the *Trichinella* genus (Deng et al., 2019).

Recently, LF-RPA has been applied to pork samples. A newly designed assay for *Trichinella* targets the mitochondrial small subunit ribosomal RNA (rrnS) gene and can detect as little as 100 fg DNA of *Trichinella* (Li et al., 2019). The advantage of this technology is its quick results (<20 min), its 100% specificity and high sensitivity, and its ability to be used without a thermocycler; room temperature is sufficient (Li et al., 2019). Only Li, T et al., 2019 has shown its effectiveness in identifying a *Trichinella* spp. larvae infection; however, only at high larvae burdens. Field tests at low larval burdens have not been done. If this technology can combine the reliability of artificial digestion and PCR with the speed of serology, LF-RPA could be used to both certify and survey pigs as free of *Trichinella* spp. larvae.

Currently, multiplex PCR is the premier method of distinguishing *Trichinella* spp. larvae (Alonso et al., 2011; Cuttell et al., 2012; Pozio and Zarlenga, 2019). However, multiplex PCR is species specific which prevents this method from detecting other *Trichinella* spp. larvae. Since this method cannot detect multiple species at once it precludes it from surveillance. Other PCR variants can distinguish *Trichinella* spp. larvae but not to the scope of multiplex PCR. Almeida et al., 2018 showed qPCR can distinguish *T. spiralis*, *T. britovi* and *T. pseudospiralis*; although it was only tested on encapsulated species. Adding a high resolution melting assay (HRMA) to qPCR could allow for the identification of 8 *Trichinella* larvae: *T. spiralis*, *T. nativa*, *T. britovi*, *T. pseudospiralis*, *T. nelsoni*, *T. murrelli*, *T. papuae*, and *T. zimbabwensis* (Reslová et al., 2017). LAMP and LF-RPA have not been tested (Zarlenga et al., 2020). Sequencing has proven to be reliable in distinguishing *T. spiralis*, *T. pseudospiralis*, *T. papuae*, and *T. zimbabwensis* but due to cost, expertise, time, and lack of data on field samples, sequencing is a poor choice for surveillance (Mohandas et al., 2014).

### 3.6. Cost

Cost is an important consideration for any surveillance program. If too costly to perform at a large scale, effective methods become inefficient. Variance in the cost for each method is derived from the different expenses of materials and equipment required to labor time used to process each test. From a 2007 report from the US federal register, we found that ELISA tests are less costly, at \$0.83 per swine using commercial laboratories, and \$0.66 per swine, if processed by a slaughter facility (Pyburn, 2007). On the occasion that a result of an ELISA is positive, the findings would have to be confirmed by western blot, thus raising the cost. We also found that artificial digestion is estimated at \$1.72 per swine if processed by a commercial laboratory, and \$0.92 per digestion swine if processed by a slaughter facility (Pyburn, 2007).



We derived the cost for all methods by compiling reagents and non-reusable materials listed in research studies that tested *Trichinella* in swine and researched the cost of those materials from vendors to run the test (see supplementary data 1 for cost analysis). We estimated the cost for each method using one 1 g sample. It is important to note these cost estimates do not include labor costs, facility costs, or shipment/transport. These values are not uniform and are lab dependent. That said, these costs for simplicity purposes would be assumed to be standard for each method. Thus, our estimates provide information on what it would cost a facility to test for *Trichinella*. Genomic testing can be difficult to discern a price due to its variability in cost per test. Additionally, there is a lack of price clarity for materials, labor cost, and method cost.

Current methods of PCR require an amplification step to detect *Trichinella* DNA. Some methods, such as qPCR and LF-RPA, can be directly used on samples without the isolation step, but require an abundance of DNA for detection. This would reduce the overall cost, but due to the expected low number of *Trichinella* in pig muscles, this restriction impacts the effectiveness of qPCR and LF-RPA as a surveillance method. According to Li et al., 2019, mice that were infected with 300 larvae could achieve 100% sensitivity for the LF-RPA method. qPCR and LF-RPA have not been validated with smaller larva loads.

An additional difficulty in comparing cost between genomic methods and MSM is that MSM has been certified for use at 100 pooled samples, while data on PCR pooling is scarce. An *in vitro* experiment has shown that PCR could be used effectively on 100 pooled samples, but no *in vivo* experiments have been done with *Trichinella* to validate this claim (De Souza Rosés et al., 2019). Other pathogens have undergone more evaluation using *in vivo* experiments but are not as promising as what De Souza Rosés found. SARS-CoV-2 can be tested effectively with 10 samples per pool (Wacharapluesadee et al., 2020). Malaria can be tested with 4 per pool (Taylor et al., 2010). *C. fetus* in bulls can be tested at 5 samples per pool (García-Guerra et al., 2016). Regardless, future studies and *in vivo* experiments would benefit in finding the limit of PCR pooling detection of *Trichinella* spp. larvae.

We found that the MSM was the cheapest method, at about 6 cents/ sample. The most expensive method was LAMP at \$5.90. LF-RPA, conventional PCR, qPCR, ELISA, ADD Kit, and Agglutination were found to be \$5.36, \$3.98, \$3.78, \$0.15, \$0.14, and \$0.08 per sample respectively. Most of these costs come not from the DNA amplification itself, but rather from the DNA extraction. While LF-RPA is more expensive than PCR and qPCR, LF-RPA has the advantage of not relying on expensive machinery, which has a high initial investment and upkeep costs. However, LAMP also shares this advantage, needing only a water bath. These genomic methods are likely to be insufficient for surveillance testing due to their high cost. The ability to pool contributes to low cost methods such as MSM, AAD Kit, and Agglutination. Improvements may bring the costs of PCR methods closer to MSM and may obviate the need for isolation steps. If this happens, then LF-RPA could become a competitor due to its higher sensitivity. Out of the lower-cost methods, MSM is the most reliable method for the detection of *Trichinella* spp. larvae in striated muscle tissues.

#### 4. Conclusion

MSM remains the gold standard for *T. spiralis* testing of pork products and is internationally utilized as a reliable screening method. It carries a lower cost than most methods, and it is reliable. Serological tests could be used in conjunction with sophisticated biosecurity measures to monitor and control *Trichinella* exposure in our swine populations.

LF-RPA should be evaluated for its potential to provide a fast, convenient, and highly specific and sensitive test. Finding cost-effective methods for surveillance is an evolving effort. Understanding the true cost of various detection methods requires a more precise definition of all attendant costs, including labor costs we deemed insufficiently clear to include in our estimates. Protecting food safety requires cost-effective methods with sufficient sensitivity, specificity, and accuracy. Improving technology justifies reappraisal of surveillance methods. Further research should be conducted to evaluate the cost of emerging technologies, bringing transparency to knowledge gaps concerning efficacy and cost.

#### Declaration of Competing Interest

All authors declare no competing interests in the content of this review. The work was funded from public sources and performed in the public interest without regard for commercial interests of any kind, and with the sole desire to support science-based decision making and to advance public health.

#### Acknowledgements

We thank the following for their contributions. Dr. Ray Gamble for providing a proposed budget for the *Trichinella* Project. Valsin Fournet for helpful discussion and proofreading. We would also like to thank US Department of Agriculture, Agricultural Research Service, US Department of Agriculture Animal and Plant Health Inspection Service, The Hispanic Association of Colleges and Universities, and the Oak Ridge Institute for Science Education, US Department of Energy. for funding.

#### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.fawpar.2021.e00129>.

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