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An internationally recognized quality assurance system for diagnostic parasitology in animal health and food safety, with example data on trichinellosis

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Abstract

A quality assurance (QA) system was developed for diagnostic parasitology and implemented for several diagnostic assays including fecal flotation and sedimentation assays, trichomonad culture assay, and the testing of pork and horse meat for *Trichinella* to facilitate consistently reliable results. The system consisted of a validated test method, procedures to confirm laboratory capability, and protocols for documentation, reporting, and monitoring. Specific system components included a quality assurance manual, training program, proficiency panels, inter-laboratory check-sample exchange program, assay critical control points, controls, and audits. The quality assurance system of the diagnostic laboratory was audited according to ISO/IEC Standard 17025 by an international third party accrediting body and accredited as a testing laboratory for the specific parasitology tests. Test results generated from the laboratory were reliable and scientifically defensible according to the defined parameters of the tests and were therefore valid for a variety of purposes, including food safety, international trade, and declaration of disease status in an animal, herd, farm, or region. The system was applicable to various test methods for the detection of parasites in feces or other samples, and a digestion test system developed for *Trichinella* was used as an example. A modified tissue digestion assay was developed, validated, and implemented by the Canadian Food Inspection Agency's Centre for Animal Parasitology for efficiency and quality assurance. The details of the method were properly documented for routine testing and consisted of a homogenization process, an incubation at $45 \pm 2\,^{\circ}C$, and two sequential sedimentations in separatory funnels to concentrate and clarify final aliquots for microscopic examination. To facilitate consistently reliable test results, 14 critical control points were identified and monitored, analysts were certified, and the test system

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verified through the use of validation data, proficiency samples, and training modules.

Keywords: Parasites; Quality assurance; Food safety; Meat inspection; Pork; Horse meat; Digestion assay; *Trichinella*

1. Introduction

Detection assays are important tools in the control of parasites and the prevention of animal and human parasitoses. Reliable test results are essential for clinical diagnosis, food safety, herd certification, and meeting export requirements. Recent increases in the number of outbreaks of human trichinellosis, despite increased testing requirements, have raised concerns about the validity of currently used assays and the reliability of test results, emphasizing the need for proper quality assurance (QA) in the test systems (Boireau et al., 2000). Internationally recognized guidelines for the implementation and use of the quality assurance measures are provided in ISO/IEC 17025-1999 "General requirements for the competence of the calibration and testing laboratories" (previously ISO/IEC Guide 25-1990) of the International Organization for Standardization/International Electrotechnical Commission (ISO/IEC). Laboratories with a QA system that meet these guidelines can attain international recognition through accreditation by an authorized third party such as the Australian National Association of Testing Authorities (NATA), the Standards Council of Canada (SCC), and the American Association for Laboratory Accreditation (A2LA).

An ISO/IEC compliant QA system could be established for most parasite detection assays, including fecal flotation, digestion test, ELISA, isoenzyme assays, and PCR. As part of an overall effort to ensure an effective and reliable food inspection system in Canada, the National Centre for Animal Parasitology developed a QA system for diagnostic parasitology and implemented a scope of specific tests for parasites, including gastrointestinal helminths and protozoa, *Tritrichomonas foetus*, and *Trichinella*. The QA system was accredited by the SCC. This report describes the development, implementation and accreditation of a testing laboratory, using the digestion method for the detection of *Trichinella* larvae in the pork and horse meat to demonstrate the components of a proper QA system.

2. Materials and methods

The requirements of ISO/IEC Standard 17025 (1999) were followed to establish a QA program as part of the Canadian Food Inspection System. The ISO/IEC Standard 17025 for testing laboratories covered 14 categories under management and 10 technical categories (Table 1). The site-specific plan for the laboratory to meet all the management and technical requirements was outlined in a Quality Manual, and the overall management of the plan was designated to a qualified employee (Quality Manager).

Within the overall QA system of the laboratory, test specific requirements for the *Trichinella* pepsin/HCl digestion assay were subdivided into the following five components: test method; method validation; confirmation of laboratory capability; documentation and reporting; monitoring of test system. An audit of the fully implemented QA system for the

Table 1
Elements of the standards for a testing laboratory as outlined by ISO/IEC 17025

Management	Technical
Organization	General requirements
Quality system	Personnel
Document control	Accommodation and environmental conditions
Review of requests, tenders and contracts	Test methods and method validation
Subcontracting	Equipment
Purchasing	Measurement traceability
Service to clients	Sampling
Complaints	Handling of test samples
Control of non-conforming tests	Assuring quality of results
Corrective actions	Reporting results
Preventive actions	
Control of records	
Internal audits	
Management reviews	

testing of *Trichinella* in the pork and horse meat was performed by the SCC, an independent body which conforms to the recommendations of the International Laboratory Accreditation Conference (ILAC) and accredits laboratories according to ISO/IEC Standard 17025.

3. Results

3.1. Test method

A modified pepsin/HCl digestion assay using a double separatory funnel procedure was written in the standard format of the Canadian Food Inspection Agency and employed in the QA system (Gajadhar et al., 1996, 1997). The primary modifications included the incorporation of two sequential separatory steps to clarify the sediment and concentrate larvae for detection, the use of 1:30 tissue homogenate to the digestion fluid, an incubation temperature of $45 \pm 2\,^{\circ}C$, and the monitoring of all critical control points in the assay. The test was capable of consistently detecting about 1 larva per gram in a 5-gram sample of the pork or horse meat when the written detailed protocol was followed (Forbes and Gajadhar, 1999). Fourteen critical control points in the *Trichinella* test method were identified and appropriately controlled (Forbes and Gajadhar, 1999). Briefly, the critical control points were:

1. preparation of solution (combining HCl and water before the addition of pepsin);
2. incubation parameters ($45 \pm 2\,^{\circ}C$ for 30 min);
3. completion of procedure (lack of undigested muscle on sieve);
4. stability of apparatus (undisturbed settling of digest for 30 min);
5. working order of equipment (unobstructed flow from stopcock);
6. proper use of equipment (rapid release of stopcock);
7. remedial measure (further collection of sediment if first collection is not sufficient);

8. further remedial measures as necessary (concentration of large volumes of collected sediment);
9. time sensitive requirement (collected sediment allowed to settle undisturbed for at least 1 min prior to examination);
10. quality of output (clarity of sample sediment for examination);
11. timely completion of test (same day examination of sediment);
12. adequate equipment (stereomicroscope with ≥10 X magnification and properly maintained);
13. re-processing of unsatisfactory output (clarification of an un-readable sediment);
14. complete transfer of sample (re-suspension of sediment and rinsing of container to ensure the complete transfer of larvae).

3.2. Method validation

The procedures used for validation of the *Trichinella* test method were performed on the tissues obtained from the experimentally infected pigs to determine the most suitable sampling sites and the detection limits (sensitivity) for 1, 3, or 5 g amounts of muscle containing different parasite loads (Forbes and Gajadhar, 1999). Some of the key validation results are summarized in Figs. 1 and 2. The validation data were used to determine optimal tissues and size for sampling, as well as sensitivity, specificity, range, accuracy, capacity, and other limitations of the test. These data helped to demonstrate the relevance of the test

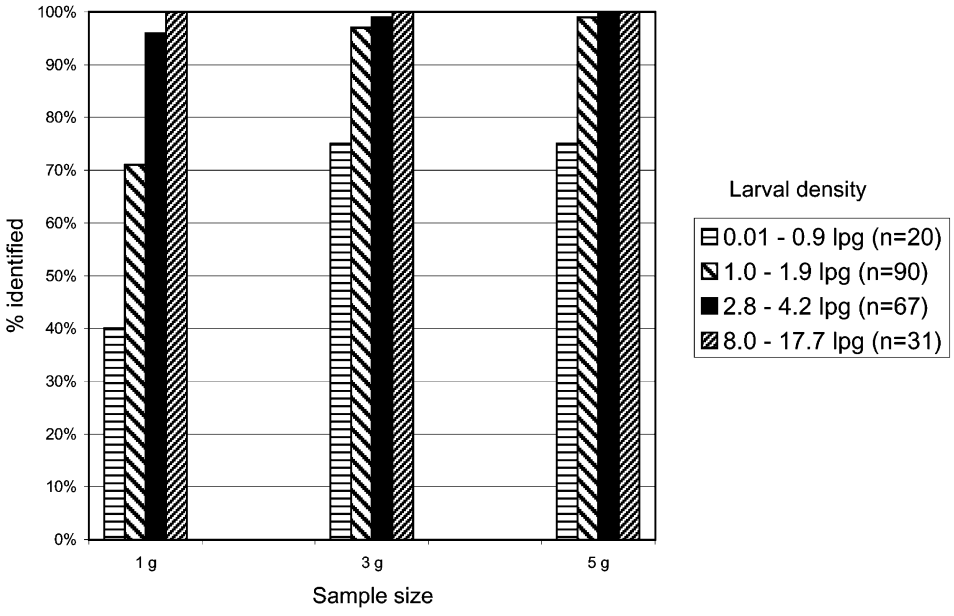

Fig. 1. Example of validation data for determining sample size for the pepsin/HCl digestion assay using samples from experimentally infected pigs. The data were used to determine the effectiveness of testing 1, 3, or 5 g of meat, according to the larval density.

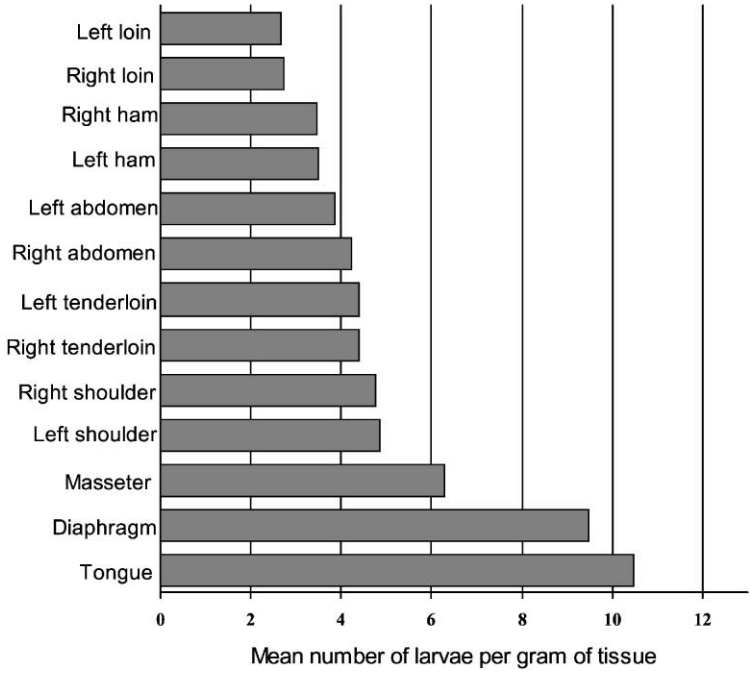

Fig. 2. Example of validation data for determining tissues for the sampling. Larval distribution (per gram of tissue) in various sites of 15 pigs experimentally infected with 40–1000 *Trichinella spiralis* muscle larvae. Raw data were transformed as previously described to account for the non-normal concentration of larvae within each tissue type (Forbes and Gajadhar, 1999).

method for its intended purpose in ensuring food safety and allowing for the economical testing of large numbers of animals under industry conditions. Analysis of the validation data yielded conclusions about the appropriateness of the test method under the various testing and sample conditions, as outlined in the 14 critical control points.

3.3. Confirmation of laboratory capability

Prior to processing diagnostic samples an audit of the laboratory confirmed its capability to perform the validated test as specified in the Quality Manual. The required facilities and equipments were present and functional, a training program was in place to teach and certify new analysts to perform the test, and the 14 critical control points identified in the assay were appropriately controlled by monitoring or through the use of controls (Forbes and Gajadhar, 1999). The effectiveness of the test system was confirmed by the use of intra-laboratory proficiency panels and an inter-laboratory check sample exchange program (Forbes et al., 1998). A standard operating procedure (SOP) for sample identification was present so that it was possible to track samples through the digestion process and link them to the appropriate submission information for subsequent trace back of any positive results to the carcass of the origin.

3.4. Documentation and reporting

Standard operating procedures were developed to provide the guidance for the control and review of the test related documentation. This included records to show that the test system was operating within the defined parameters and that the results were appropriately managed as per the requirements of ISO Standard 17025. Laboratory notebooks contained permanent records of all data, as well as notes generated by the analysts performing the test. Evidence of errors, complaints and problems, and how they were addressed were recorded and used to improve the system. Verification of transcribed data and results was a routine system requirement, and records were kept for a minimum of 5 years. The reporting of the test results required appropriate authorization and was in accordance with policies and details outlined in the Quality Manual of the laboratory.

3.5. Monitoring of test system

As part of the QA program, regular monitoring of the components of the test system was performed to ensure the continued effectiveness of the laboratory and the reliability of test results. The frequency of the monitoring was predetermined and included monitoring of the critical control points and critical equipment during performance of the assay, use of proficiency panels and controls, and internal and external audits as defined in the ISO Guide 1011 (ISO, 1991). A detailed description of the critical equipment and specific positive and negative controls, repeats and reference samples have been reported previously (Forbes and Gajadhar, 1999; Forbes et al., 1998).

4. Discussion

Traditional parasitology methods such as microscopic examination often rely on flexible or non-standardized criteria and subjective evaluation, which may contribute to erroneous or inconsistent results. Similarly, modern tests such as PCR assays may also produce unreliable results because of the practical problems including instrument variability, contamination, and the lack of or use of inappropriate controls (MacPherson et al., 1993). The QA system described in this report may be applied to both the modern and traditional assays to manage many practical problems and produce meaningful results. Examples of problems that can be overcome or managed by an effective QA system include: performance variability of instruments, fluctuations of environmental conditions such as temperature and humidity, false negatives and false positives, poor tests, inappropriate samples, and poor technical skills. Fecal flotation, the most widely used assay for parasites, is susceptible to many problems which could be readily managed by a proper QA system. A valid QA system is necessary to address the need for standardization and validation in fecal flotation tests.

The test systems in laboratories accredited to ISO Standard 17025 are expected to produce reliable results according to the specified parameters. The use of the test results as defined by specific needs dictates the accepted limitations of each test method, and usually does not require absolute sensitivity and specificity. For example, testing for helminths by fecal flotation does not usually require that every egg be recovered and identified. Similarly, in

order to prevent clinical trichinellosis, the digestion assay for the detection of *Trichinella* larvae in meat is satisfactory if a minimum sensitivity of 1 larva per gram of meat is achieved. Similarly, a test method used by an accredited laboratory has defined capabilities and limitations which are verified by the appropriate validation data and the use of pertinent controls. This information allows for the objective comparison of similar or different assays employed for the same purpose. Confirmed performance equivalency data for the modified tests or different assays, provides a scientifically valid rationale for reliable test used in different countries, for the same purpose such as meat or herd certification.

Ongoing demonstration of the testing competence includes thorough and complete documentation of all laboratory practices which are components of the verified QA system. These practices are listed in the QA Manual or SOPs to meet ISO Standard 17025 requirements. In 1998, Codex Alimentarius recommended that the laboratories involved in the import and testing of foods should be accredited to ISO/IEC Guide 25 or equivalent. ISO/IEC Standard 17025 has since been instituted to replace and extend the scope of ISO/IEC Guide 25 (ISO/IEC, 1990, 1999). Supplementary requirements for the accreditation of veterinary testing, including parasitologic assays, are available, and the specific accreditation criteria for laboratories testing for protozoa such as *Giardia* and *Cryptosporidium* have been established by NATA. Recognition of laboratories which meet the internationally accepted guidelines for QA can be achieved through accreditation by an authorized agency. The acceptance of test results between the countries is facilitated by mutual recognition agreements among the equivalent accrediting bodies. Test results from the accredited laboratories are globally acceptable for many purposes, including facilitation of international trade, declaration of disease status in an animal, herd, farm or region, and certifying food safety.

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