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A comparison of detection methods of *Alaria alata* mesocercariae in wild boar (*Sus scrofa*) meat

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ABSTRACT

Distomum musculorum suis (DMS), the mesocercariae of *Alaria alata*, is typically found accidently during examination of wild boar meat for *Trichinella* spp. The aim of the study was to compare DMS detection methods. Briefly, 232 wild boar meat samples were tested by mesocercariae migration technique (AMT) as a reference method; of these, 104 were found to be positive. Selected positive samples were tested again with the three other methods: compressorium method (Compressor), digestion with magnetic stirrer (Digestion) and by modified digestion with Pancreatin® bile and pancreatic enzymes (D + P). The results were analyzed by logistic regression, the non-parametric Kruskal-Wallis test and the Mann-Whitney *U* test. Of the 43 samples found positive by the AMT, 20 were found positive by Digestion and 25 by D + P. The Compressor identified DMS in seven of the 19 tested samples. The Digestion and D + P methods gave similar intensities (P = 0.506), i.e. 1.4 and 1.3 DMS respectively, but the AMT detected seven times higher number of parasites. The probability of detection of DMS in the meat sample by the Digestion or by D + P was higher than 0.5 when at least seven (Digestion) or five (D + P) DMS were present in the sample (AMT). The Compressor was the least sensitive method: at least 14 DMS must be present in the meat sample for detection. AMT should be considered the most accurate method of DMS detection.

1. Introduction

Alaria alata, a member of the *Trematoda* genus, has a particularly interesting life cycle with carnivores as definitive hosts (Bruzinskaite-Schmidhalter et al., 2012; Tăbăran et al., 2013; Takeuchi-Storm et al., 2015; Bindke et al., 2017; Duscher et al., 2017; Lempp et al., 2017; Kołodziej-Sobocińska et al., 2018), snails as the first intermediate host, and amphibians as the second (Möhl et al., 2009; Portier et al., 2012; Patrelle et al., 2015). However, the mesocercarial stage of *A. alata*, *Distomum musculorum suis* (DMS) (Duncker, 1896), may also exist in paratenic hosts, mostly wild boar: a species particularly susceptible to infestation due to its omnivorous feeding habits and preference for mud biotypes.

Recent studies on the status of alariosishave recognized the parasite as a zoonotic agent (Woolhouse and Gowtage-Sequeria, 2005). However, the only reported cases have been caused by *Alaria americana* (McDonald et al., 1994; Kramer et al., 1996). As *A. alata* is closely related to *A. americana* it is also classified as zoonotic agent, inter alia by Swiss Agency for the Environment, Forests and Landscape (SAFEL). Due to the potential risk of infection in humans and the wide spread of *A. alata* throughout Europe, more sensitive detection methods for its presence in wild boar meat are needed.

A number of well-standardized methods (macroscopy and coprology) are commonly used for detecting adult *A. alata* (Popiołek et al., 2007; Szafrańska et al., 2010; Karamon et al., 2018); however, the detection of DMS is arguably a more important issue for public health.

A variety of methods are used to detect DMS in wild boar worldwide, such as digestion with magnetic stirrer (Digestion), modified digestion with Pancreatin® bile and pancreatic enzymes (D + P) and compressor analysis (Compressor). Many infestations were diagnosed accidently

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during inspection of meat for *Trichinella* spp. (Jaksic et al., 2002; Portier et al., 2011, 2014; Michalski and Wiszniewska-Laszczych, 2016). The latest and most highly-recommended methods are based on the *A. alata* mesocercariae migration technique (AMT) (Sailer et al., 2012; Riehn et al., 2012; Paulsen et al., 2013; Ozoliņa&Deksne, 2017; Rentería-Solís et al., 2018; Strokowska et al., 2020).

The aim of our study was to compare the prevalence and the sensitivity of DMS infection in wild boars obtained by four methods – AMT, Digestion, D + P, Compressor. Three methods (AMT, Digestion, D + P) were compered in terms of intensity of infection.

2. Materials and methods

2.1. Sample collection and partition

Initial samples (n = 232) were collected in north-eastern Poland from wild boar as part of planned hunts by the Polish Hunting Association. The following tissue samples were collected: diaphragm pillars, intercostal muscles, tongues, limb muscles and chewing muscles. The samples included adipose and peritoneal tissue. If more than one type of tissue was collected from one animal samples from different organs were pulled together. The samples ranged in size from 100 g to 250 g. After collection, the samples were delivered to the laboratory to test for *Trichinella* spp. and DMS. Until analysis, all samples were stored at a temperature of 0–4 °C for a maximum of seven days.

Next, the samples were tested as presented in Fig. 1 (Fig. 1). From each of the 232 initial samples, 30 g were separated to be tested, cut into approximately 5 mm fractions and tested with AMT as described by Riehn et al. (2010). In total, 104 samples were found to be positive by AMT.

Of the 104 positive samples, 19 were subjected to further testing with the Compressor. Only 19 samples were used as these demonstrated the minimum intensity for detection, i.e. 33 mesocercariae per 100 g of meat (Enemark et al., 2015). Briefly, 3 g samples were taken, and Compressor examination was then conducted as described in Polish legislation: Commission implementing regulation (EU) 2015/1375 of August 10, 2015 laying down specific rules on official testing for *Trichinella* in meat.

In addition, 43 of the 104 positive samples, demonstrating at least six mesocercariae per 100 g of meat, were analyzed by Digestion (30 g sample) as described previously (Annex I, Chapter I of the regulation (EC) No. 2075/2005; Mayer-Scholl et al., 2017) and by D + P (30 g sample) as described by Riehn et al. (2010).

2.2. Statistical analysis

The prevalence of infection (the number of DMS-positive meat samples) was determined using four methods: AMT, Digestion, D + P and Compressor. However, infection intensity (the numbers of DMS present in infected meat samples) was compared using only three methods, *viz.* AMT, Digestion and D + P: Compressor was not used for this comparison, because it requires a different sample size. As AMT is regarded as the most reliable method (Riehn et al., 2010), the AMT results were used as reference values. As intensity did not present a normal distribution, the methods were compared using the nonparametric Kruskal-Wallis test. Following this, a pairwise comparison was conducted with the Mann-Whitney *U* test.

Logistic regression was performed to indicate the sensitivity of the tested methods, i.e. the threshold value at which each one can detect the presence of DMS in a meat sample. Three models were built, in which the dependent binary variable was the presence or absence of DMS according to the Digestion, D + P or Compressor methods. The numbers of DMS determined in the AMT acted as a covariate in all models. All statistical analyses were performed using SPSS software (version 24.0, IBM Corporation, Armonk, NY).

3. Results

3.1. Prevalence and infection intensity

According to the reference AMT, the prevalence of DMS was almost 45% and the mean intensity 10.6. Of the 43 samples found to be positive by the AMT, the parasite was detected in 20 by Digestion and 25 by D + P samples. The Compressor identified DMS in only seven of 19 samples found to be positive by AMT.

The mean intensity was similar for both Digestion and D + P (P = 0.506), i.e. 1.4 and 1.3 DMS respectively. In contrast, the AMT detected seven times higher number of parasites (Fig. 2).

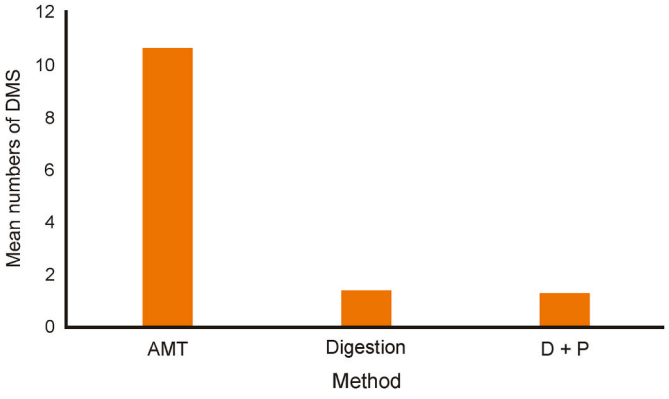

Fig. 2. Mean numbers of DMS in meat sample (30 g) and comparison with the Kruskall Wallis test ($\chi 2 = 64.34.03$; df = 2; $P < 0.001$, N = 43 in all cases) and pairwise comparison with the Mann-Whitney *U* test (statistical difference was stated in comparison of pairs: AMT-Digestion and AMT-D + P, $p < 0.001$ in both cases).

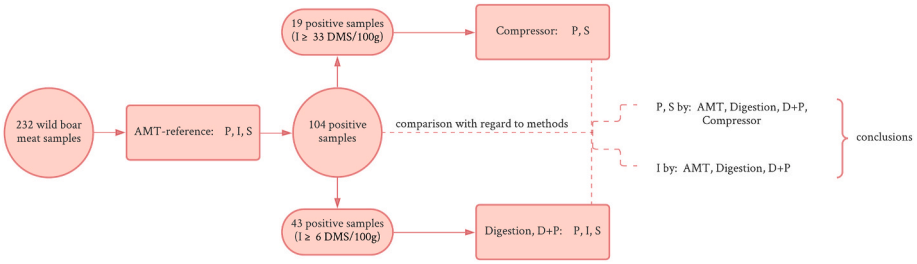

Fig. 1. The scheme of the experiment. Legend: P – prevalence of infection has been determined, I – intensity of infection has been determined, S – sensitivity of method has been determined, DMS – Distomum musculorum suis (mesocercariae of *Alaria alata*), AMT-reference – *Alaria alata* mesocercariae migration technique – reference method, Compressor – compressor analysis, Digestion – digestion with digestion stirrer, D + P – modified digestion with Pancreatin® bile pancreatic enzymes.

3.2. Sensitivity

The Digestion and D + P allowed for the construction of a well-fitted logistic regression model, which explained 88.4% and 81.4% of the full classification of the observed values (Nagelkerke's R square = 0.669 for Digestion and 0.55 for D + P). Both models were statistically significant ($\chi 2$ = 29.908, P < 0.001, B_0 = −3.904, B_{AMT} = 0.629, n = 43 for Digestion; $\chi 2$ = 22.589, P < 0.001, B_0 = −2.76, B_{AMT} = 0.584, n = 43 for D + P method). None of the tested methods were as sensitive as AMT. The probability of detection of DMS in the meat sample using the Digestion or D + P was higher than 0.5 when at least seven or five DMS respectively were present in the sample, as detected by AMT (Fig. 3). Therefore Digestion was less sensitive than the D + P method. The logistic regression model based on the Compressor also demonstrated statistically significant results, but the number of observations was much lower than in other models ($\chi 2$ = 19.679, P < 0.001, B_0 = −17.141, B_{AMT} = 1.227, n = 19). The model was characterized with a high proportion of correctly-classified observations (89.5%) and the highest Nagelkerke's R-square value (0.881). However, the Compressor was also the least sensitive of the three methods: at least 14 DMS must be present in the meat sample (detected by AMT) to be detected with a probability higher than 0.5.

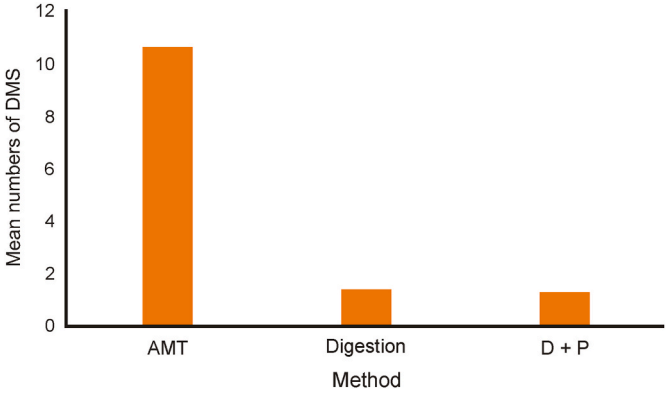

Fig. 3. Probability of detection of DMS using various methods (Digestion, D + P, Compressor) in relation to numbers of DMS in meat sample (basing on AMT method) (n = 43 for D + P, Digest, n = 19 for Compressor, for details, see Methods).

4. Discussion

Our findings confirms that the AMT method was far more sensitive than the other tested methods: Digestion, D + P and Compressor. The four methods compared in the present study are characterized by significantly different sensitivities, and therefore it is impossible to compare the DMS infection data obtained by them. Despite this, the Digestion and D + P appear demonstrate similar sensitivity with regard to determining the intensity of mesocercariae infection.

Digestion only detected DMS in 20 of 43 samples tested positively with AMT, with similar results being presented by Ozoliņa and Deksne (2017). In addition, Riehn et al. (2010) report that the Digestion, used as a reference for Trichinella detection, gives a high probability of false negative results regarding the presence of DMS; however, Riehn et al. (2013) note that AMT correctly identified 38 more DMS-positive samples than the Digestion. This might be due to the differences in size of DMS and *Trichinella* larvae and the different predilection sites of the two parasites (Möhl et al., 2009; Riehn et al., 2010; Gajadhar et al., 2019). Furthermore the Digestion solution was at a low pH, which may have negatively impacted the survival and motility among the DMS. As such, detection of DMS by the Digestion is rather accidental and is only suited to severe infections (Riehn et al., 2010; Portier et al., 2011). In contrast, the bile and pancreatic enzymes (Pankreatin) used in the modified digestion (D + P) method provides higher vitality of DMS (Riehn et al., 2010); even so, the treatment could still result in damage. Although the D + P was found to be more sensitive than Digestion in the present study, the sieves it uses are too small for mesocercariae and it should be considered as less efficient than AMT (Riehn et al., 2013).

Of the tested methods, the Compressor was found to be the least sensitive. It is also not currently recommended for routine *Trichinella* diagnosis in domestic animals due to lack of sensitivity: Commission Implementing Regulation (EU) 2015/1375 of August 10, 2015 laying down specific rules on official controls for *Trichinella* in meat (Text with EEA relevance). The accuracy of the method is dependent on the skill and experience of the examiner, as well as the size of the examined sample; as such, it is able to detect DMS only in heavily-infected meat samples and should not be used for diagnostic purposes (Gavrilović et al., 2019; Gazzonis et al., 2018).

As alariosis is considered a re-emerging disease in Europe, and one that is dangerous for human health (Möhl et al., 2009; Strokowska et al., 2020), it is especially important to re-evaluate the regulations regarding DMS inspection, with a recommendation that AMT should be regarded as the most valuable diagnostic tool. Moreover, AMT is a cheap and easy-to-use method, it can be used in laboratories with basic equipment, without the need to use advanced equipment or chemical reagents which in addition to high specificity and sensitivity makes it a perfect diagnostic tool. On this basis there is no reason to use other and less sensitive methods. A uniform verification procedure also offers the opportunity to better compare the results of different working groups and to derive measures for consumer health protection.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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