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ORIGINAL RESEARCH ARTICLE

Detection of *Trichinella* spp. larvae in domestic pigs (*Sus scrofa domesticus*) and golden jackals (*Canis aureus*) using artificial digestion and a microfluidic device: a comparative study

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Abstract. Nematodes of genus *Trichinella* are zoonotic parasites and are among the most widespread parasites of swine, badgers, bears and predatory animals like wolfs, foxes or jackals. This study aimed to compare the detection capacity (for *Trichinella* spp. larvae) of artificial digestion and an experimental microfluidic device. A total of 20 samples positive for *Trichinella* spp. in trichinoscopy (10 domestic pigs and 10 golden jackals) were tested (5 g/animal) with both artificial digestion method and a microfluidic device. Artificial digestion is the gold standard method for direct detection of *Trichinella* larvae in meat samples. A microfluidic device is a new approach regarding *Trichinella* spp. detection, and in this study, a newly designed experimental device was used (Bionics Biomicrofuidics lab, Budapest). All of the tested pig and golden jackal samples came out positive in the artificial digestion method, but also in the microfluidic device. The total number of counted larvae was significantly higher in artificial digestion (695 larvae in jackals; 486 larvae in pigs) than in the microfluidic device method (291 larvae in jackals; 263 larvae in pigs). When the experimental device was used, the larval structure was more visible than in the golden standard method. The results described here indicate that the microfluidic device can be used in the direct detection of *Trichinella* larvae in domestic and wild animals, allowing the visualization of their structure, but it's not as efficient as artificial digestion in counting the number of larvae.

Keywords: *Trichinella* spp.*;* artificial digestion; microfluidic device.

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Detectarea larvelor *Trichinella* spp. la porcii domestici (*Sus scrofa domesticus*) şi şacalii aurii (*Canis aureus*) folosind digestia artificială şi un dispozitiv microfluidic: un studiu comparativ

Rezumat. Nematodele din genul *Trichinella* sunt paraziţi zoonotici şi sunt printre cei mai răspândiţi nematozi ai suinelor, bursucilor şi a animalelor de prada precum lupii, vulpile, sau şacalii. Acest studiu a avut drept scop compararea capacităţii de detectare (pentru larvele de *Trichinella* spp.) a digestiei artificiale cu un dispozitiv microfluidic experimental (Laboratorul de biomicrofluidică Bionics, Budapesta). In total 20 de probe pozitive (pentru *Trichinella* spp.) de porc domestic şi şacal auriu au fost testate (5g/animal) în ambele protocoale. Digestia artificială este metodă standard pentru detectarea directă a larvelor de *Trichinella* în probele de carne. Dispozitivul microfluidic este o nouă abordare în ceea ce priveşte detectarea *Trichinella* spp. Acest protocol se bazează pe un dispozitiv experimental nou proiectat. Toate probele de porc şi de şacal auriu testate au ieşit pozitive în metoda digestiei artificiale, dar şi în dispozitivul microfluidic. Numărul larvelor a variat, un număr mai mare fiind identificat în digestia artificială (695 larve la sacali; 486 larve la porci) fata de dispozitivul microfluidic (291 larve la sacali; 263 larve la porc). În dispozitivul experimental structura larvelor a fost mai vizibilă fata de metoda standard. Rezultatele descrise indică faptul că dispozitivul microfluidic poate fi utilizat în detectarea directă a larvelor de *Trichinella* la animalele domestice şi sălbatice, permiţând vizualizarea structurii lor, dar nu este la fel de eficient ca digestia artificială în numărarea larvelor.

Cuvinte cheie: *Trichinella* spp. digestie artificială; dispozitiv microfluidic.

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Introduction

Trichinosis is a cosmopolitan disease of warm-blooded animals that can also infect human beings, causing different digestive symptoms and in some cases even death (Ribicich et al., 2020). Both adults and larvae have parasitic behavior and live in the same host. The larvae migrate in the host, causing negative effects especially in humans (Şuteu and Cozma, 2012; Franssen et al., 2017). There are more and more evidence that suggest that this parasite can also be affected by climate change (Pozio, 2020).

The magnetic stirrer digestion (artificial digestion) is the gold standard method for the direct detection of *Trichinella* spp. larvae in muscle samples (Gamble et al., 2000). This method is mandatory for meat inspection according to EU regulation (EU 2015/1375). Trichinoscopy and, more recently, artificial digestion were previously used for the identification of *Trichinella* spp. in wild animals in Romania (Blaga et al., 2009a; Blaga et al., 2009b; Ciobotă et al., 2015; Boros et al., 2020).

However, there is a need for low-cost, rapid, and reliable diagnostic results in veterinary medicine. Point-of-care tests have huge advantages over existing laboratory-based tests, because of their intrinsic low cost and rapidity (Busin et al., 2016; Sivakumar and Lee, 2020). Microfluidics focuses on the behavior, precise control, and manipulation of different kinds of fluids that are geometrically constrained to a small scale at which surface forces dominate volumetric forces (Li et al., 2017). Microfluidic devices are made of silicon, glass, and polymeric material, because of their chemical compatibilities. Polymers that are used in the fabrication of microfluidic devices may include polydimethylsiloxane (PDMS) and polymethyl methacrylate (PMMA). Plastics devices are more attractive because they are disposable and are manufactured at a low cost (Sequeira et al., 2002). The dimensions of the channels in the devices have a wide range and cover a large span of depths or widths of 10–100s of micrometers (Greenwood & Greenway, 2002; Olmos et al., 2020). These devices have

been used in a large variety of areas, including bioanalysis (Khandurina & Guttman, 2002), pharmaceuticals forensic and clinical applications (Huikko et al., 2003). Recent advances in the field of microfluidics made new and exciting opportunities for human health diagnostics, and there is now a potential for these new technologies to be applied in the field of veterinary medicine diagnostics (Busin et al., 2016; Picco, 2020).

Microfluidics is a new approach on detecting and counting different species of parasites, including *Trichinella*, the current study aiming to compare the capacity of artificial digestion and a newly designed experimental microfluidic device to detect *Trichinella* spp. larvae in wild and domestic animals.

Materials and methods

Artificial digestion

For artificial digestion, 5 g of muscle tissue were used from each animal. Each pig and jackal sample was digested individually, and the necessary calculations for water (100 ml), pepsin (0.5 g), and chlorhydric acid (0.8 ml) were made. This method respected all the conditions described by Gamble and others (2000), to detect and number the larvae of *Trichinella* spp.

Microfluidic device

The microfluidic device was designed in Hungary (Bionics Biomicrofluidics Laboratory, Budapest). A total of 10 ml from the liquid obtained in artificial digestion were also used in this device. The fluid was introduced in a 10 ml syringe that was connected to the experimental device with a small cylindrical tube. Another cylindrical tube was used for the evacuation of the filtered fluid. The filtration area of the device was located in between the entry and exit points of the digestion fluid. The liquid was pushed thru the filtration area with constant pressure and a speed of 50 ml/h. The device (CMF 11-07) was examined under the microscope (Olympus BX61) during the filtration process at × 4 and × 10 magnification in order to detect the number of *Trichinella* larvae. The assessments were performed with a DP72 camera and Cell^F software (Olympus Corporation, Japan).

Statistical analysis

The distribution of data was evaluated using Shapiro-Wilk normality test. Chi-square goodness of fit and non-parametric Wilcoxon signed rank test were used to compare the number of *Trichinella* spp. larvae detected by artificial digestion and microfluidic device in both animal species. A p value less than 0.05 was considered statistically significant. Data were analyzed in R software v. 4.0.5.

Results and discussion

The results of this study show that all the 20 swine and golden jackal meat samples that were found positive in trichinoscopy, were also positive in artificial digestion and when using the microfluidic device. Artificial digestion was able to detect a significantly higher number of *Trichinella* larvae (table 1, table 2) than the microfluidic method in both swine, and jackals as seen in table 3. The morphological structure of the larvae was more visible in the experimental device than after artificial digestion.

Table 1. Comparative results in jackals (*C. aureus*). The table shows the number of animals and the results obtained in the two protocols from each animal.

Animals	Results artificial digestion	Results microfluidic device (CMF 11-07)
Jackal/ Nr.ctr 1	19 larvae	9 larvae
Jackal/Nr. ctr 2	261 larvae	106 larvae
Jackal/Nr. ctr 3	21 larvae	21 larvae
Jackal/Nr ctr 4	26 larvae	12 larvae
Jackal/Nr. ctr 5	14 larvae	3 larvae
Jackal/Nr. ctr 6	22 larvae	5 larvae
Jackal/Nr. ctr 7	34 larvae	3 larvae

Jackal/Nr. ctr 8	234 larvae	71 larvae
Jackal/Nr. ctr 9	61 larvae	41 larvae
Jackal/Nr. ctr 10	26 larvae	20 larvae
Total	**695 larvae**	**291 larvae**

Table 2. Comparative results in pigs (*S. scrofa domesticus*). The table shows the number of animals and the results obtained in the two protocols from each animal.

Animals	Results artificial digestion	Results microfluidic device (CMF 11-07)
Swine/ Nr.ctr 1	5 larvae	2 larvae
Swine/Nr. ctr 2	35 larvae	31 larvae
Swine/Nr. ctr 3	21larvae	19 larvae
Swine/Nr ctr 4	41 larvae	36 larvae
Swine/Nr. ctr 5	15 larvae	6 larvae
Swine/Nr. ctr 6	19 larvae	18 larvae
Swine/Nr. ctr 7	51 larvae	36 larvae
Swine/Nr. ctr 8	39 larvae	33 larvae
Swine/Nr. ctr 9	45 larvae	25 larvae
Swine/Nr. ctr 10	215 larvae	57 larvae
Total	**486 larvae**	**263 larvae**

Table 3. Comparative results for swine (*S. scrofa domesticus*) and jackals (*C.aureus*) regarding statistical findings.

	Swine	Jackal
Chi-square goodness of fit	$p < .001$	$p < .001$
Wilcoxon signed rank test	$p = .002$	$p = .009$

However, the experimental device allows the microscopical examination of larvae with × 4 or × 10 magnification, while in case of the golden standard method the examination is performed with a stereo microscope (Olympus BX61) (figure 1). Retained residues were observed in both protocols (60% of the exanimated samples contained residues), but this didn't influence the numbering of the larvae in neither of cases. When comparing the results from the microfluidic device method obtained in swine to the ones from golden jackals we can mention that the number of larvae identified in jackals (291 larvae) was higher (table 1) than in swine (236 larvae) (table 2). This might be caused by the differences in the degree of infestations in the used meat samples. In both animal species the larvae were retained in the filtration area from the beginning of the filtration protocol (100% efficacy) and none of the larvae escaped (figure 2) (larvae lost 0%).

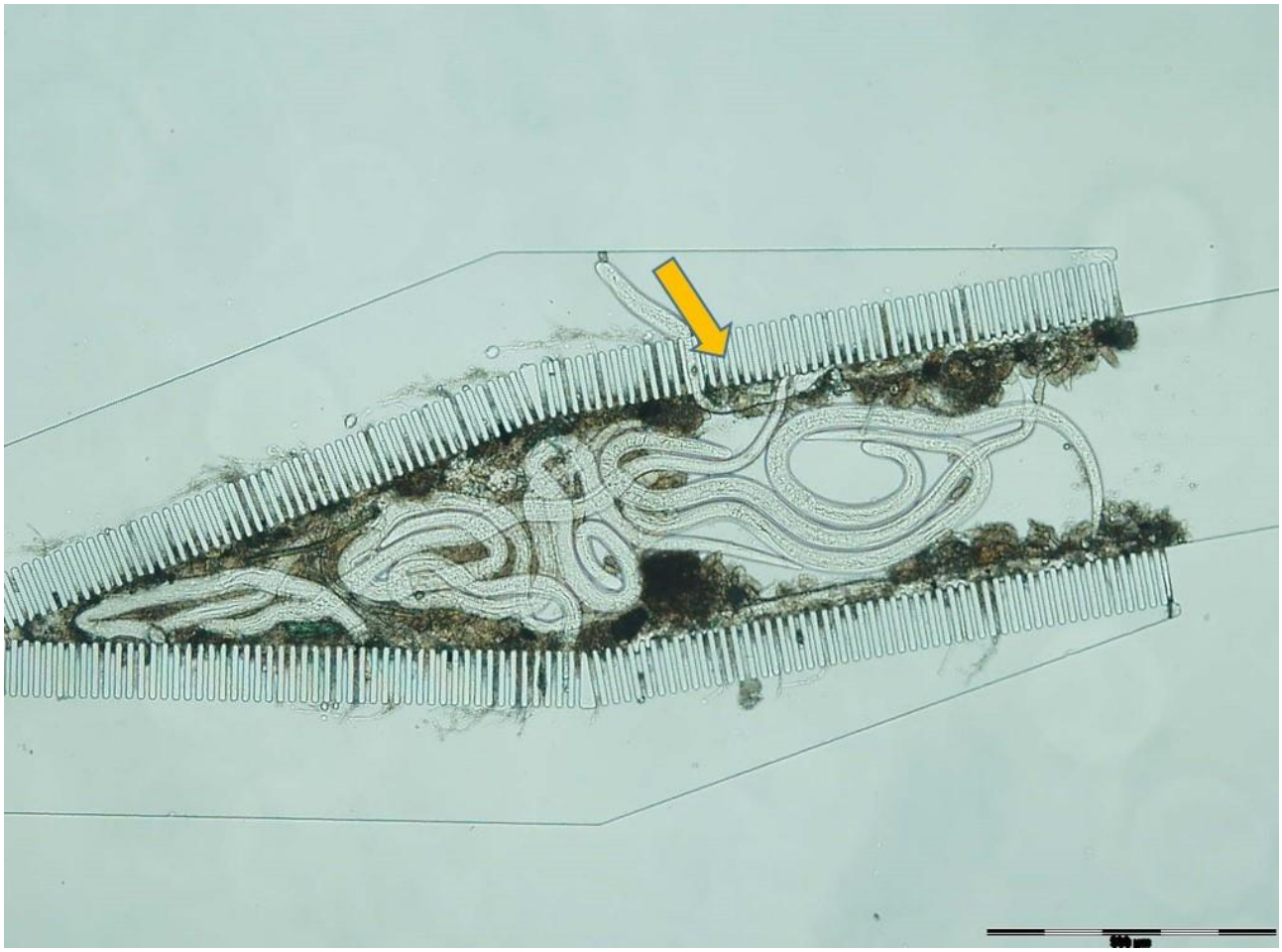

Figure 1. The first filtration area of the microfluidic device. The picture shows the trapped *Trichinella* spp. larvae (yellow arrow) in the first filtration area between the retained residues (brown color). × 4.

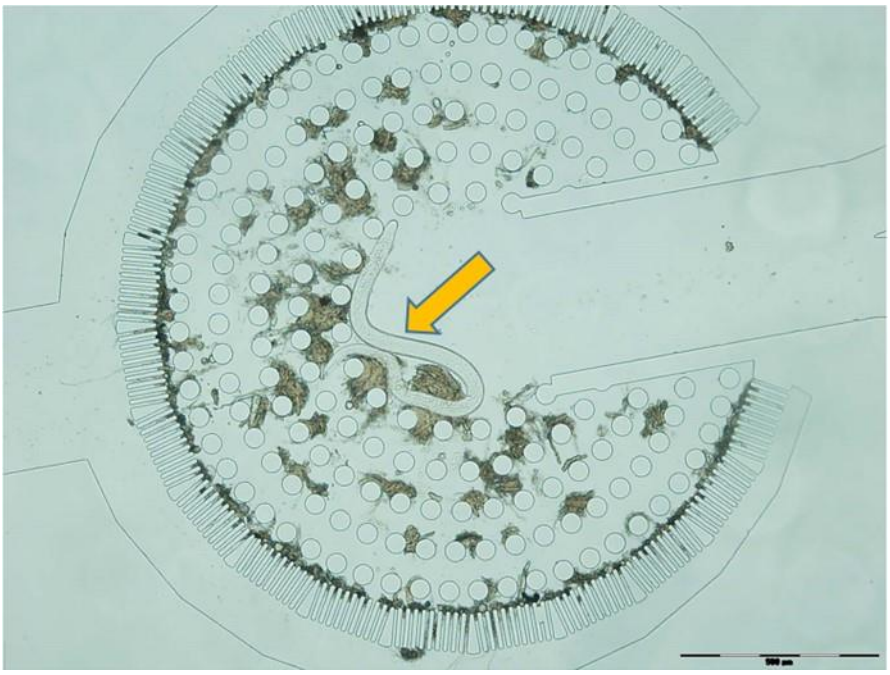

Figure 2. The second filtration area of the microfluidic device. The picture shows the trapped *Trichinella* spp. larvae (yellow arrow) in the second filtration area between the retained residues (brown color). × 4.

There are more and more studies regarding microfluidic devices used on different kinds of parasites (Holm et al., 2016; Szélig et al., 2019; Phuakrod et al., 2019). Unfortunately, data regarding the detection of *Trichinella* spp. with these types of devices are scarce. The current study results are based on a filtration rate of 50 ml/h, but a similar study conducted in Hungary used a filtration rate of 150 ml/h, indicating that both filtration rates are ideal for future experiments with *Trichinella* spp. (Szélig et al., 2019). Picco, in 2020, mentioned that a microfluidic system has to be capable of keeping a constant temperature of 37 °C for minimum 24 hours when it comes to *T. spiralis* examination. However, the ability of a microfluidic device to maintain a certain temperature (Picco, 2020) or increases in the filtration rate speed (Szélig et al., 2019) can improve the capacity of *Trichinella* larvae examination.

The capacity of microfluidic devices to detect specific parasitic infections was tested in several studies. A study conducted in Sweden tested a multitude of diseases like, leishmaniasis, Chagas disease, and malaria, and found that particles size, shape, and deformability can be leveraged for the enrichment of hard to find objects (Holm et al., 2016). Droplet microfluidics platform, which was tested on *Plasmodium* parasites has a high potential for adaptation to point-of-care setups suitable for low-resource settings. Potential adaptations of the presented setup for the detection of other microorganisms may form the basis for the development of a more generic platform for diagnosis, or other purposes within applied or basic science (Juul et al., 2012). A semi-automated microfluidic device was also designed to detect filarial parasites and it has the potential to be used for lymphatic filariasis diagnosis in human populations all over the world. This new device might facilitate rapid, higher-throughput detection and identification of infection with filariae in blood samples (Phuakrod et al., 2019). Another study showed that microfluidic chips are capable of electrophysiological recordings of other nematode species than *Caenorhabditis elegans*. This approach was optimized for trapping and recording smaller worms or worms with distinct anterior body shapes. The authors concluded that microfluidic devices could be applied to other species of nematodes that have economic or medical importance (Hu et al., 2014). Moreover, another experiment demonstrated the workability of the microfluidic platform on *Caenorhabditis elegans* and *Oesophagotomum dentatum* with levamisole as the test drug. The authors consider that the suggested drug screening on a microfluidic device could significantly improve the resolution, sensitivity, and data throughput of in vivo testing, but also offer new details on the transient and time-resolved exposure effects of new and existing anthelmintics (Carr et al., 2011). A microfluidic device was designed to cultivate adult worms of Schistosoma sp. The influence of the composition and physical characteristics of mass flow on the mobility, pairing and long-term survival of these worms were studied (Girod et al., 2021).

Conclusions

The microfluidic device is able to detect *Trichinella* larvae in swine and jackals, even in low degrees of parasitemia. However, artificial digestion is more efficient in detecting larvae of *Trichinella* spp. in both animal species than the microfluidic device. Based on the results, the microfluidic device has the same efficiency in detecting *Trichinella* larvae in both animal species included in the study, and therefore it can be considered for future studies on wild and domestic animals.

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