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Original article

Detection of experimental swine trichinellosis using commercial ELISA test

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Abstract

The aim of the study carried out on ten young (10-week old) pigs of the native Polish Large White breed experimentally infected with a low dose of 300 invasive muscle larvae (ML) of *Trichinella spiralis* was intravital detection of trichinellosis using the E-S ELISA test, determination of a variation level of IgG antibodies against excretory-secretory (E-S) antigens of *T. spiralis* muscle larvae and finally, describing the intensity of *T. spiralis* larvae infection in selected muscles. The pig sera were collected at 7 and 9 days prior to the experimental infection with *T. spiralis* and at 9, 14, 20, 23, 25, 27, 30, 33, 37, 41, 46 days post-infection (d.p.i.). The anti-*T. spiralis* IgG antibodies were detected by a commercial E-S ELISA test (PrioCHECK Trichinella Ab). Average intensity of the *T. spiralis* infection in the examined muscles of pigs ranged from 1.52 up to 43.09 larvae/g. The studies revealed that the E-S antigen in the ELISA test did not show cross-reaction with the sera of pigs infected with *Oesophagostomum* spp. The ELISA assay did not recognize trichinellosis in pigs until 27 days after the *T. spiralis* infection. The anti-*T. spiralis* IgG antibodies were first detected on day 30 post-infection. A statistically significant increase of IgG antibodies against *T. spiralis* ML E-S antigens was first observed between days 27-30 ($p<0.01$) post-infection, and a further significant rise in the antibody level occurred between days 27 and 33 ($p<0.01$); 30 and 33 ($p<0.01$); 33 and 37 ($p<0.05$) following infection.

Key words: *Trichinella spiralis*, pig, E-S ELISA, experimental infection, intensity of infection

Introduction

Trichinellosis, a parasitic zoonosis caused by nematodes of the genus *Trichinella*, is a serious risk to human health and life. Infection occurs by consumption of raw or undercooked meat infected with invasive muscle larvae (ML) of *Trichinella* at the first stage (L1). *Trichinella* spp. circulate in the domestic (pigs, horses, nutria) and companion animals of the synanthropic environment as well as in the wild carnivores and omnivores of the sylvatic transmission cycle (Campbell 1983). Trichinellosis of pigs is closely related with the synanthropic cycle of *Trichinella* circulation and the most common species inducing infec-

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tion is *Trichinella spiralis* (Pozio 2000). While among the sylvatic hosts, wild boars are the primary reservoir of the parasite. A progressive increase in wild boar population increases the biomass of *Trichinella* circulating in the environment and in turn, enhances the risk of its transmission to domestic pigs maintained under the free-range and ecological system (Pozio et al. 2009). Therefore, pigs from these farming systems are currently considered the most serious public health threat in Poland and other European countries (Pozio 1998, EFSA 2005, Pozio et al. 2009). In 2003-2015 in Poland, trichinellosis was diagnosed in 423 pigs with a mean prevalence of 0.00016%, varying between 0.00001% in 2014 up to 0.00035% in 2003 and 2013 (General Veterinary Inspectorate 2016).

The World Organisation for Animal Health recommends direct and indirect tests for detecting *Trichinella* spp. The direct tests, i.e. trichinoscopy (tissue compression) and artificial digestion method directly demonstrate encysted *Trichinella* larvae in muscle tissue through microscopy or visualize larvae released from muscle during digestion procedure. According to the Commission Regulation (EU) 2015/1375 of 10 August 2015, the magnetic stirrer method for pooled-sample digestion is indicated as the reference method applied for official control of meat intended for human consumption from swine, other species of slaughter animals and wild boars for *Trichinella*.

Indirect methods are based on immunological tests that detect specific circulating antibodies against *Trichinella* (OIE 2012). The most commonly used method is ELISA (enzyme-linked immunosorbent assay) based on excretory-secretory (E-S) antigens of *Trichinella* ML. It is also the only one recommended by the International Commission on Trichinellosis for the detection of *Trichinella* infection in pigs (Gamble et al. 2004). Although serological methods are not considered suitable for routine inspection of meat for *Trichinella,* E-S ELISA has been recommended for practical use in herd surveillance in pigs and wildlife to determine the epizootic and epidemiological status of trichinellosis in the given area (Gamble et al. 2004, Gajadhar et al. 2009, Gómez-Morales et al. 2009, Gómez-Morales et al. 2014, Commission Implementing Regulation 2015). IgG class antibodies are most commonly used for trichinellosis diagnostics in swine. The time needed to develop a specific IgG antibody response in pigs infected with *Trichinella* is correlated to the infection dose and the intensity of *Trichinella* infection in the muscles (Nöckler and Kapel 2007, Gottstein et al. 2009). In addition, the *Trichinella* species triggering the infection, the species and breed of the host and its individual features also play a role (Smith 1987, Smith 1988, Smith and Snowdon 1989, Nöckler et al. 2000, Nöckler et al. 2005). All the steps of the ELISA, including production of *Trichinella* E-S antigens, can be carried out in a laboratory, yet it requires experienced and qualified staff, high-tech laboratory equipment, maintenance of *Trichinella* strains for diagnostic purposes and sufficiently long time for laboratory analyses. Hence, several manufacturers of veterinary products for diagnosis of trichinellosis in swine offer commercial E-S ELISA kits which are easy to perform and conveniently automated and facilitate a great number of pigs to be efficiently tested in short time.

Literature data confirm the value of E-S ELISA for the detection of trichinellosis in pigs (Gamble et al. 1983, Smith 1987, Smith 1988, Smith and Snowdon 1989, van der Leek et al. 1992, Nöckler et al. 1995, Gamble 1996, Gamble 1998, Kapel et al. 1998, Reiterová et al. 1999, Kapel and Gamble 2000, Nöckler et al. 2005, Kořínková et al. 2008). The authors of the studies cited above applied the E-S ELISA to detect experimental swine trichinellosis induced by varying infection doses of *T. spiralis* larvae (50, 100, 200, 500, 1000, 1500, 3000, 5000, 8000, 10 000, 15 400, 20 000, 64 000). However, no studies have been conducted in Poland on the use of E-S ELISA for trichinellosis detection in pigs of indigenous breeds inoculated experimentally with *T. spiralis*. Therefore, the aim of the study carried out on ten young (10-week old) pigs of the native Polish Large White breed experimentally infected with a low dose of 300 invasive muscle larvae (ML) of *Trichinella spiralis* was intravital detection of trichinellosis using the E-S ELISA test, determination of a variation level of IgG antibodies against excretory-secretory antigens of *T. spiralis* muscle larvae and finally, describing the intensity of *T. spiralis* infection in selected muscles.

Materials and Methods

Ethics statement

Permission for the study was granted by II Local Ethics Committee for Animal Experimentation at the University of Life Sciences in Lublin – Resolution No. 34/2013.

Hosts and parasite material

The studies were carried out on 10 young healthy Polish Large White pigs (aged 10 weeks, average body weight 20 kg). Prior to the experimental infection with *T. spiralis*, swine fecal samples were screened for parasite eggs using flotation with a solution-saturated

Table 1. Distribution and intensity of *T. spiralis* larvae infection in muscles and organs of pigs experimentally infected with *T. spiralis*.

Muscle	Numbers of *T. spiralis* larvae/g muscle (LPG)										Mean weight of digested muscle (g)
	Pig/Number										
	1	2	3	4	5	6	7	8	9	10	
Diaphragma (*pars lumbalis,costalis et sternalis*)	4.26	24.32	26.35	43.27	49.64	44.92	70.86	71.49	114.75	11.94	96.00
Mm. colli	2.36	11.89	18.06	29.67	28.56	27.17	47.76	38.40	50.93	5.79	95.63
Lingua	3.83	13.86	17.49	26.31	29.47	23.67	24.68	40.34	58.62	4.77	67.66
Mm. abdominis	1.79	9.92	9.95	24.63	22.38	17.40	27.04	36.65	59.33	4.55	164.35
Mm. extensores antebrachii	1.32	4.42	11.32	23.12	24.07	22.16	38.31	38.04	39.12	4.62	60.86
Mm. extensores cruris	1.21	6.34	8.75	24.95	22.88	16.15	35.79	37.81	45.18	6.29	54.98
M. masseter	0.86	14.31	12.81	18.42	14.00	8.62	35.24	21.93	64.93	1.32	48.90
M. psoas major et minor	0.90	5.18	12.67	19.31	19.34	18.12	34.29	29.89	39.50	4.56	97.62
M. pterygoideus lateralis et medialis	1.58	9.32	11.45	22.06	21.55	19.64	28.13	25.74	35.85	2.85	22.45
Mm. flexores cruris	1.03	6.29	8.26	20.38	19.45	11.39	29.51	26.38	34.35	3.76	88.03
M. triceps brachii	1.19	6.91	8.56	18.01	16.71	11.75	23.03	30.27	38.30	3.76	200.00
Mm. flexores antebrachii	0.94	3.41	7.92	18.60	20.23	13.06	31.71	30.32	18.47	4.07	73.67
Mm. intercostales	1.10	5.55	8.94	12.27	13.74	11.82	14.73	22.97	29.27	1.79	109.00
M. biceps femoris	0.82	1.98	5.06	16.00	14.34	7.47	16.30	20.23	29.99	2.58	200.00
Oesophagus	0.78	2.22	3.94	2.51	5.68	3.02	10.99	10.65	21.93	0.32	15.77
M. longissimus thoracis	0.32	2.83	2.94	6.04	6.97	3.80	9.39	10.64	8.87	0.99	200.00
Mean from 16 muscles	**1.52**	**8.05**	**10.90**	**20.35**	**20.56**	**16.26**	**29.86**	**30.73**	**43.09**	**4.00**	**1594.92**

NaCl and decantation. Morphometric study of parasite eggs was performed with a light microscope Olympus BC51 with differential interference contrast (DIC Nomarski), while image analysis by the camera integrated with Olympus cell software. Ten pigs were experimentally infected with *T. spiralis* muscle larvae isolated by the digestion procedure from a naturally infected swine. *T. spiralis* larvae were identified at the species level by multiplex polymerase chain reaction (multiplex PCR) according to Zarlenga et al. (1999). The counted *T. spiralis* ML exhibiting motility were suspended in 20% gelatin blocks. Pigs were infected by administering *per os* a single dose of 300 *T. spiralis* muscle larvae/pig. Pigs were slaughtered at various times after *T. spiralis* infection, i.e. at days: 21 (pig no. 1), 33 (pigs no. 2 and 3), 37 (pig no. 4), 41 (pig no. 5), 46 (pigs no. 6 and 7), 47 (pigs no. 8 and 9), 48 (pig no. 10). Average post-slaughter body weight of pigs was 25.9 kg, ranging from 21.1 kg to 34.8 kg.

Digestion method for detection of *T. spiralis* muscle larvae in infected pigs

Distribution and intensity of the *T. spiralis* larvae infection in the pig muscles were determined by digestion method according to Commission Regulation (EU) 2015/1375. Sixteen different muscles were collected from the left and right halves of the carcass. Table 1 summarizes the muscles and their average mass collected for the digestion assay. The average weight of muscle samples taken from each pig for digestion procedure was 1600 g. The intensity of the *T. spiralis* infection in the muscles was calculated as the number of larvae per gram (LPG) of muscle tissue.

Intravital detection of swine trichinellosis using E-S ELISA test

To detect trichinellosis by E-S ELISA, 10 ml of blood was taken from the swine anterior vena cava 9 and 7 days prior to *T. spiralis* infection and 9, 14, 20, 23, 25, 27, 30, 33, 37, 41, 46 and 47 days post-infection (d.p.i.). The blood samples were centrifuged for 10 minutes at 2000 x g to obtain serum which was put into 1.5 ml Eppendorf tubes and frozen at -20°C. The serum level of specific IgG antibodies against *T. spiralis* ML E-S antigens was determined using a commercial diagnostic E-S ELISA kit (PrioCHECK Trichinella Ab ELISA; Prionics AG, Schlieren-Zurich, Switzerland) according to the manufacturer's instructions. Briefly, the control sera from the assay kit (positive, weak positive, negative) and test sera from the infected pigs drawn prior to and after infection were diluted to 1:50 and incubated in microtiter plate wells coated with *Trichinella* E-S antigen for 30 min at 22°C. After incubation, the microtiter plates were washed four times with wash solution (300 µl/well) using an Immuno Wash 1575 Microplate Washer (Bio-Rad, USA). Then, 100 µl of diluted conjugate (peroxidase-labelled anti-swine IgG antibody) was added to each well and the microtiter plates

incubated for 30 min at 22°C. Afterwards, the plates were washed four times. To develop the color reaction, 100 µl TMB substrate (3,3',5,5'-tetramethylobenzidine) was applied to each well and the microtiter plates incubated for 15 min at 22°C. The color reaction was halted by adding 100 µl of stop solution to each well. The plates were read at 450 nm (Elx 800, Bio-Tek Instruments, USA). The sera from *T. spiralis* infected pigs and controls were tested in duplicate; the final result was the mean of two measurements of optical density (OD) for each serum analyzed. The ELISA kit met the validation acceptance criteria. The results were estimated by calculating P/P% value according to the formula:

$$P/P\% = \frac{\text{OD Sample}}{\text{OD Positive Control}} \text{ x } 100\%$$

The serum with P/P% value equal to, or over, 15% (cut-off) was classified as positive (trichinellosis detected), while those with P/P% values lower than 15% were regarded as negative (trichinellosis not detected).

Statistical analysis

As the pigs were slaughtered at different times after *T. spiralis* infection, the results obtained by E-S ELISA i.e. level of anti-*Trichinella* IgG antibodies against ML E-S antigens (OD value) and the results of muscle examination for *T. spiralis* by digestion method (*T. spiralis* ML infection intensity) were presented as mean values of the results from the following: 10 pigs (pigs no. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10) up to 20 d.p.i.; 9 pigs (pigs no. 2, 3, 4, 5, 6, 7, 8, 9, 10) up to 33 d.p.i.; 7 pigs (pigs no. 4, 5, 6, 7, 8, 9, 10) up to 37 d.p.i.; 6 pigs (pigs no. 5, 6, 7, 8, 9, 10) up to 41 d.p.i.; 5 pigs (pigs no. 6, 7, 8, 9, 10) up to 46 d.p.i.

The results obtained by E-S ELISA and the results of muscle tissue examination for *T. spiralis* by digestion technique were analyzed statistically by calculating the means and standard deviation (SD). Significance of differences between the obtained results for the anti-*T. spiralis* IgG antibodies level (OD value) was computed using the Student's t-test for dependent measures at $p<0.01$ or $p<0.05$. The statistical calculations were performed with Statistica 9.1 software (StatSoft, Poland).

Results

Parasitological examination of swine fecal samples

The parasitological examination of the fecal samples from pigs no. 1, 3, 6, 9, 10 showed the presence of gastrointestinal nematodes from the *Oesophagostomum* genus, whereas no parasite eggs were detected in pigs no. 2, 4, 5, 7 and 8.

Distribution and intensity of *T. spiralis* larvae infection in muscles and organs of pigs experimentally infected with *T. spiralis*

Distribution and intensity of *T. spiralis* larvae infection in muscles and organs of 10 pigs experimentally infected with *T. spiralis* are presented in Table 1. The presence of *T. spiralis* larvae was observed in all the analyzed muscles of the infected pigs. The highest intensity of *T. spiralis* infection was reported in the diaphragm for all pigs. The average *T. spiralis* infection intensity was analyzed in 16 muscles and determined for each pig (in order from no. 1 to 10) as follows: pig no. 1 – 1.52 LPG; pig no. 2 – 8.05 LPG; pig no. 3 – 10.90 LPG; pig no. 4 – 20.35 LPG; pig no. 5 – 20.56 LPG; pig no. 6 – 16.26 LPG; pig no. 7 – 29.86 LPG; pig no. 8 – 30.73 LPG; pig no. 9 – 43.09 LPG; pig no. 10 – 4.0 LPG, whereas for 10 pigs it was 18.53 LPG; 9 pigs – 20.42 LPG; 7 pigs – 23.55 LPG; 6 pigs – 24.08 LPG; 5 pigs – 24.79 LPG.

Intravital detection of trichinellosis in pigs using E-S ELISA test; variation in the level of IgG antibodies against *T. spiralis* ML E-S antigens in serum of pigs infected with *T. spiralis*

IgG antibodies against *T. spiralis* ML E-S antigens were not detected in serum samples derived from ten pigs (no. 1 to 10) at 9 and 7 days prior to *T. spiralis* experimental infection. Thus, swine trichinellosis was not diagnosed and the E-S antigen in the ELISA did not show cross-reaction with the sera of pigs infected with *Oesophagostomum* spp. and no false positive diagnostic results were observed (Fig. 1, Table 2). The ELISA test performed on sera from all the pigs did not show the presence of IgG class antibodies against *T. spiralis* ML E-S antigens (cut-off – P/P% value < 15%) at 9, 14, 20, 23, 25 and 27 days after *T. spiralis* infection (Table 2 and 3). However, in the serum of individual pigs, anti-*T. spiralis* IgG antibodies were not detectable until 20 d.p.i. (pig no. 1), 25 d.p.i. (pigs no. 8 and 9), day 27 (pigs no. 3, 4, 5, 6 and 7), day 30 (pig no. 2) or day 37 (pig no. 10) (Fig. 1).

The ELISA first detected IgG antibodies against *T. spiralis* ML E-S antigens and recognized trichinellosis (cut-off – P/P% value ≥ 15%) on day 30, then 33, 37, 41 and 46 days after *T. spiralis* infection (Table 3 and 4). However, in the serum samples of individual pigs infected with *T. spiralis,* ELISA first

Table 2. Level of IgG antibodies against E-S antigens of *T. spiralis* muscle larvae (OD and P/P%) in serum of pigs experimentally infected with *T. spiralis* (n=10). Analysis performed with ELISA PrioCHECK Trichinella Ab.

Day before/after infection	Serum analyzed					ELISA test result
	OD value (mean ± SD)	1	2	3	P/P% value (mean)	
-9	0.061 ± 0.003	a	–	–	2.91%	negative
-7	0.063 ± 0.008	a	–	–	3.01%	negative
9	0.070 ± 0.022	a	a	–	3.34%	negative
14	0.067 ± 0.012	a	a	a	3.20%	negative
20	0.073 ± 0.019	a	a	a	3.18%	negative

Cut-off value P/P% = 15% (P/P% ≥ 15% – positive result; P/P% < 15% – negative result)
1, 2, 3 – means of OD are compared in column:
1 – a – means of OD do not differ significantly compared to day -9 at $p<0.05$ (t – test)
2 – a – means of OD do not differ significantly compared to day 9 at $p<0.05$ (t – test)
3 – a – means of OD do not differ significantly compared to day 14 at $p<0.05$ (t – test)
Average infection intensity of *T. spiralis* in 16 swine muscles analyzed (n=10) was 18.53 larvae of *T. spiralis*/g muscle

Table 3. Variation in the level of IgG antibodies against E-S antigens of *T. spiralis* muscle larvae (OD and P/P%) in serum of pigs experimentally infected with *T. spiralis* (n=9). Analysis performed with ELISA PrioCHECK Trichinella Ab.

Day before/after infection	Serum analyzed							ELISA test result
	OD value (mean ± SD)	1	2	3	4	5	6	P/P% value (mean)	
-9	0.061 ± 0.003	a	–	–	–	–	–	2.91%	negative
20	0.074 ± 0.019	a	a	–	–	–	–	3.23%	negative
23	0.074 ± 0.018	a	a	a	–	–	–	2.97%	negative
25	0.091 ± 0.042	a	a	a	a	–	–	3.65%	negative
27	0.183 ± 0.173	a	a	a	a	a	–	7.35%	negative
30	0.637 ± 0.432	b	b	b	b	b	a	25.58%	positive
33	0.895 ± 0.503	b	b	b	b	b	b	36.06%	positive

Cut-off value P/P% = 15% (P/P% ≥ 15% – positive result; P/P% < 15% – negative result)
1, 2, 3, 4, 5, 6 – means of OD are compared in column:
1 – a – mean values of OD do not differ significantly compared to day -9 at $p<0.05$ (t – test)
1 – a,b – mean values of OD differ significantly compared to day -9 at $p<0.01$ (t – test)
2 – a – mean values of OD do not differ significantly compared to day 20 at $p<0.05$ (t – test)
2 – a,b – mean values of OD differ significantly compared to day 20 at $p<0.01$ (t – test)
3 – a – mean values of OD do not differ significantly compared to day 23 at $p<0.05$ (t – test)
3 – a,b – mean values of OD differ significantly compared to day 23 at $p<0.01$ (t – test)
4 – a – mean values of OD do not differ significantly compared to day 25 at $p<0.05$ (t – test)
4 – a,b – mean values of OD differ significantly compared to day 25 at $p<0.01$ (t – test)
5 – a,b – mean values of OD differ significantly compared to day 27 at $p<0.01$ (t – test)
6 – a,b – mean values of OD differ significantly compared to day 30 at $p<0.01$ (t – test)
Average infection intensity of *T. spiralis* in 16 swine muscles analyzed (n=9) was 20.42 larvae of *T. spiralis*/g muscle

detected anti-*Trichinella* IgG antibodies on the following days after infection: 27 d.p.i. (pigs no. 8 and 9), 30 d.p.i. (pigs no. 3, 4, 5, 6 and 7), 33 d.p.i. (pig no. 2) and 41 d.p.i. (pig no. 10) (Fig. 1).

No statistically significant differences were found between the level of anti-*T. spiralis* IgG antibodies on days 9, 14, 20, 23, 25, 27 after the *T. spiralis* infection and the level of the same antibodies in pigs on day 9 before the infection (Table 2 and 3). However, a statistically significant increase of the level of IgG antibodies against *T. spiralis* ML E-S antigens compared to day 9 prior to infection was first observed on day 30 ($p<0.01$) and then on day 33 after experimental infection ($p<0.01$) (Table 3). No significant differences in the level of anti-*T. spiralis* IgG antibodies were observed between the days 9 and 14; 9 and 20; 14 and 20; 20 and 23; 23 and 25; 25 and 27 after *T. spiralis* infection (Table 2 and 3). Notably, the first significant rise in the level of IgG antibodies against *T. spiralis* ML E-S antigens was observed between the days 27 and

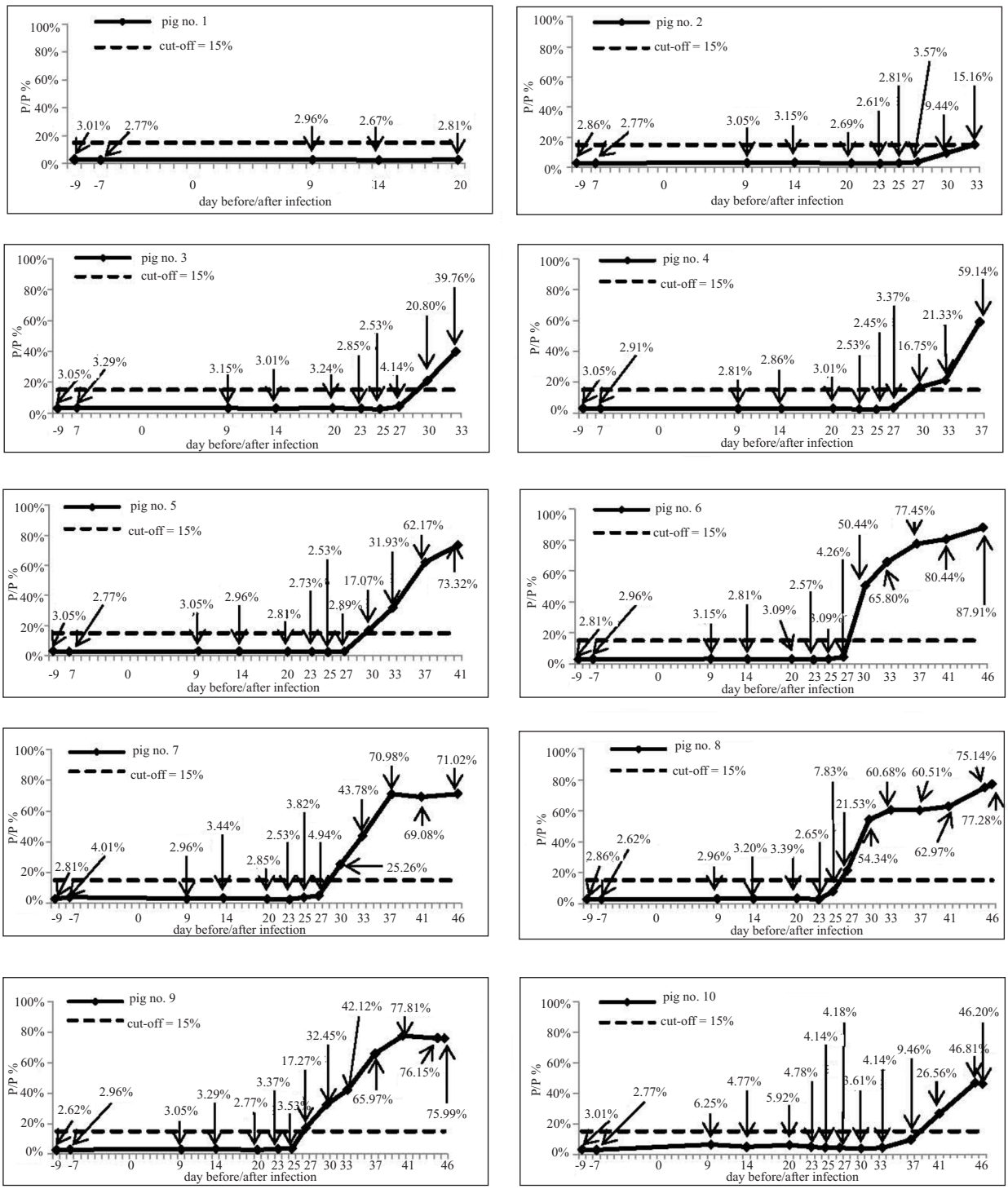

Fig. 1. Level of IgG antibodies against E-S antigens of *T. spiralis* muscle larva (P/P%) in serum of individual pigs (no. 1 to 10) experimentally infected with *T. spiralis*. Analysis done with ELISA test PrioCHECK Trichinella Ab.

30 ($p<0.01$), followed by further increases between 27 and 33 ($p<0.01$); 30 and 33 ($p<0.01$); 33 and 37 ($p<0.05$); 33 and 41 ($p<0.05$) d.p.i. with *T. spiralis* (Table 3 and 4). However, no significant differences were found between the days 37 and 41; 37 and 46; 41 and 46 after the infection (Table 4). The highest level of IgG antibodies against *T. spiralis* muscle larvae E-S antigens was observed at 37, 41 and 46 d.p.i. (Table 4).

Table 4. Variation in the level of IgG antibodies against E-S antigens of *T. spiralis* muscle larvae (OD and P/P%) in serum of pigs infected experimentally with *T. spiralis* (n=7), (n=6), (n=5). Analysis performed with ELISA PrioCHECK Trichinella Ab.

Day after infection	Serum analyzed				ELISA test result	The number of pigs
	OD value (mean ± SD)	1	2	P/P% value (mean)		
33	0.956 ± 0.535	a*	–	38.52%	positive	7
37	1.434 ± 0.552	b*	–	57.96%	positive	7
33	1.027 ± 0.549	a**	–	41.38%	positive	6
37	1.429 ± 0.605	b**	a**	57.76%	positive	6
41	1.609 ± 0.491	b**	a**	65.04%	positive	6
37	1.407 ± 0.674	a***	–	56.87%	positive	5
41	1.568 ± 0.537	a***	a***	63.38%	positive	5
46	1.767 ± 0.374	a***	a***	71.42%	positive	5

Cut-off value P/P% = 15% (P/P% ≥ 15% – positive result; P/P% < 15% – negative result)
1, 2 – means of OD are compared in column:
1 – a,b* – mean values of OD on days 33 and 37 differ significantly at $p<0.05$ (t – test)
1 – a,b** – mean values of OD differ significantly compared to day 33 at $p<0.05$ (t – test)
2 – a** – mean values of OD on days 37 and 41 do not differ significantly at $p<0.05$ (t – test)
1 – a*** – mean values of OD do not differ significantly compared to day 37 at $p<0.05$ (t – test)
2 – a*** – mean values of OD on days 41 and 46 do not differ significantly at $p<0.05$ (t – test)
Average infection intensity of *T. spiralis* (the number of *Trichinella* larvae/g muscle) in 16 muscle analyzed was for pigs (n=7) – 23.55; (n=6) – 24.08; (n=5) – 24.79

Discussion

In the present study, a commercial ELISA kit based on the *Trichinella* E-S antigen was used for intravital detection of trichinellosis in young (10-week old) Polish Large White pigs experimentally infected with a low dose of 300 muscle larvae of *T. spiralis*. It was shown that the average intensity of *T. spiralis* infection in 16 muscles of each pig ranged between 1.52 and 43.09 larvae of *T. spiralis*/g muscle. The differences in the *Trichinella* infection intensity in the muscles of pigs can be associated with individual features of animals. Significant differences in the muscle larval burden in pigs infected with the same inoculum dose have been highlighted by van der Leek et al. (1992) who attributed these differences to individual variations in the maturity of the immune system. Our findings indicate that the highest intensity of *T. spiralis* infection occurred in the diaphragm. A large number of authors have identified swine diaphragm and tongue as equally valuable research material for *T. spiralis* muscle larvae detection (Kotula et al. 1984, Gamble 1996, Forbes and Gajadhar 1999, Serrano et al. 1999, Kapel et al. 2005). In the present study *T. spiralis* infection intensity averaged to 24.30 larvae/g in the tongue, which was significantly lower (53%) than in the diaphragm (46.18 larvae/g = 100%). The results obtained in this research are comparable to those reported by Prost and Nowakowski (1990) who showed that the mean intensity of *T. spiralis* infection in the tongue of 10 *T. spiralis* infected pigs was only 52% of that observed in the diaphragm (2.40 larvae/g = 100%). Our results also indicate that the most common predilection site was the diaphragm, followed by the neck muscles; these findings are in accordance with those given by Kapel et al. (1998).

No cross-reactivity was found between the E-S antigen of *Trichinella* used in the ELISA PrioCHECK Trichinella Ab and the sera of the pigs uninfected with *T. spiralis* but naturally infected with *Oesophagostomum* spp. No false positive diagnostic results were observed and the obtained findings are consistent with those presented by Reiterová et al. (1999). The ELISA used in the present research did not detect trichinellosis of pigs until 27 days after *T. spiralis* infection and the test gave false negative results on these days. The E-S ELISA assay applied to a group of nine pigs first detected IgG antibodies against E-S antigens of *T. spiralis* ML and hence first recognized trichinellosis as late as 30 d.p.i. Following this, anti-*T. spiralis* IgG antibodies were detected until the end of the experiment, i.e. until 46 d.p.i. A statistically significant increase of IgG antibodies against E-S antigens of *T. spiralis* ML was first noted between days 27 and 30 post-infection followed by further significant rises between 27 and 33; 30 and 33; 33 and 37; 33 and 41 d.p.i. However, from 37 until 46 days after *T. spiralis* infection, these antibodies persisted at a constant high level. No statistically significant differences were recorded in the antibody level between the days 37 and 41; 37 and 46; 41 and 46 post-infection.

It is challenging to compare the findings of the

present study with those of other studies due to differences in inoculum doses of *T. spiralis* larvae (50 – 64 000), pig breeds and ELISA protocol. Moreover, pigs infected with the same or similar doses of *T. spiralis* demonstrated marked differences in the muscle larvae infection intensity on several occasions. A number of studies compare the anti-*T. spiralis* IgG antibody level (seroconversion) with regard to the infective dose or intensity of *T. spiralis* infection in pig muscles, most frequently in the diaphragm or only in the predilection muscles, whereas in the present study the *T. spiralis* infection intensity was presented as the mean from 16 different muscles of each pigs. The time of seroconversion is also dependent on a *Trichinella* species that triggers infection, infected animal species and breed as well as its individual features (Smith 1987, Smith 1988, Smith and Snowdon 1989, Nöckler et al. 2000, Nöckler et al. 2005). Van der Leek et al. (1992) showed that in three groups of pigs, 3 animals per group, inoculated with 5000, 500 and 50 larvae of *T. spiralis*, specific anti-*T. spiralis* IgG antibodies were detected in individual pigs by ELISA in respectively 4-5, 5-7 and 6-8 weeks post-infection; *T. spiralis* infection intensity in the diaphragm and tongue of each pig in the three groups ranged from 93.33 to 933.33; 11.07 – 303.33 and 0.87 – 13.57 *T. spiralis*/g of muscle according to the three groups, respectively. Similarly, Gamble (1996) reported that in three groups of pigs (3 pigs per group) experimentally infected with 2500, 500 and 100 *T. spiralis,* specific IgG antibodies were first detected by E-S ELISA on days 28, 28-35, 35-49 after infection and the mean *T. spiralis* infection intensity in the diaphragm was 105.8; 26.8 and 3.3 *T. spiralis*/g of muscle, respectively. To determine the correlation between the intensity of *T. spiralis* infection in the muscles and the time of seroconversion, Gamble (1998) used 47 pigs that were experimentally inoculated with 25, 50, 100, 250 or 500 *T. spiralis* larvae. The author obtained various intensity of *T. spiralis* infection in the infected animals ranging from 0.01 up to 248.80 *T. spiralis* larvae/g of the diaphragm and tongue (mixed together). The E-S ELISA first detected anti-*Trichinella* IgG on 37.6 (from 35 to 49), 38 (from 28 to 49), 42 (from 35 to 56) and 32.1 (from 28 to 56) d.p.i. in the respective pig group; average *T. spiralis* invasion intensity in the diaphragm and tongue (together) was, respectively, 0.02-1, 1-3, 3-10 and over 10 (i.e.: 12.4 – 29.6 in nine pigs; 32 – 68.8 in eight pigs; 87.2 – 108.8 in four pigs; 146.4 – 248.8 in three pigs) *Trichinella* larvae/g. Kapel et al. (1998) found that the E-S ELISA first detected anti-*T. spiralis* IgG antibodies 21 d.p.i. in four pigs given a high dose of 10 000 *T. spiralis* larvae. The average *Trichinella* infection intensity in 18 analyzed muscles was also high and reached 190 larvae/g muscle. In other studies conducted by Kapel and Gamble (2000), 14 pigs were experimentally infected with 10 000 *T. spiralis* larvae. The authors used the E-S ELISA assay and first detected specific anti-*T. spiralis* IgG 3 weeks post-infection. A rapid increase in the IgG level was noted between three and five weeks following experimental infection and the antibodies persisted at a constant level for 40 weeks after infection. The mean *Trichinella* infection intensity in 18 muscles of the infected pigs was as follows: 427.4 larvae/g muscle five weeks after infection, 171.5 larvae/g 10 weeks after infection, 196.3 larvae/g 20 weeks after infection, 103.5 *T. spiralis* larvae/g 40 weeks after infection. Nöckler et al. (2005) reported that seroconversion in three groups of pigs inoculated with 200, 1000 and 20 000 muscle larvae of *T. spiralis* was observed on day 40, 40 and 25 post-infection, respectively, whereas the average *Trichinella* infection intensity in nine muscles was 3, 43.1 and 538.8 *T. spiralis*/g muscle, depending on the dose. In a study of three pigs experimentally infected with 600 *T. spiralis* larvae, Reiterová et al. (1999) found with E-S ELISA specific anti-*T. spiralis* IgG on day 28 following infection. Average intensity of *T. spiralis* infection in the group of three pigs was 68 larvae/g in the diaphragm, 40.8 larvae/g in the tongue and 47.7 larvae/g in the masseter muscles. Kořínková et al. (2008) detected experimental trichinellosis using the E-S ELISA assay in eight pigs inoculated with 500 larvae of *T. spiralis*. Specific anti-*T. spiralis* IgG were determined in four pigs as early as on day 21, in two pigs on day 24, in one pig on day 28 and in one pig on day 31 after infection. The intensity of *T. spiralis* infection in the diaphragm of infected pigs was in the order: 96, 76.8, 44, 40.8, 28, 22.8, 28.2 and 12.8 larvae/g.

It is assumed that *T. spiralis* muscle larvae become invasive on day 17 after the moment of animal infection (Kocięcka et al. 2003). In the present study involving E-S ELISA use, it was found that anti-*T. spiralis* IgG antibodies were detected in all infected pigs as late as day 30; that is 13 days later than the period of time needed by *Trichinella* to become infective for the next host. The finding is confirmed by the test results of pig no. 1, in which the ELISA applied on 20 d.p.i. did not show the presence of anti-*T. spiralis* IgG but the digestion testing did detect trichinellosis of the infection intensity posing a health threat to consumers. The late (i.e. 30 day after inoculation) occurrence of IgG antibodies against E-S antigens of *T. spiralis* ML and, consequently, their late detection by ELISA is consistent with the research results presented by other authors (Gamble et al. 1983, Smith 1987, Smith 1988, van der Leek et al. 1992, Gamble 1996, Gamble 1998, Kapel et al. 1998, Reiterová et al. 1999, Kapel and Gamble 2000, Nöckler et al. 2005, Kořínková et al. 2008). Our findings show that the commercial diagnostic E-S ELISA kit is a suitable tool only for surveillance or verification

of *Trichinella*-free herds of pigs as well as for control of hazards related to trichinellosis occurrence in wildlife, which is in accordance with the International Commission on Trichinellosis guidelines and the Regulation (EU) 2015/1375. The intravital examination of swine sera for trichinellosis caused by *Trichinella spiralis* by E-S ELISA should be performed twice, at an interval of at least 30 days.

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