e-ISSN 1734-9168
http://www.isez.pan.krakow.pl/en/folia-biologica.html

Folia Biologica (Kraków), vol. 69 (2021), No 4
https://doi.org/10.3409/fb_69-4.18

Histological Changes in the Kidneys and Heart in Experimental Acanthamoebiasis in Immunocompetent and Immunosuppressed Hosts

Karolina KOT, Danuta KOSIK-BOGACKA, Natalia ŁANOCHA-ARENDARCZYK, Michał PTAK, Paulina ROSZKOWSKA, and Andrzej KRAM

Accepted November 29, 2021 *Published online December 20, 2021* *Issue online December 23, 2021*

Original article

KOT K., KOSIK-BOGACKA D., ŁANOCHA-ARENDARCZYK N., PTAK M., ROSZKOWSKA P., KRAM A. 2021. Histological changes in the kidneys and heart in experimental acanthamoebiasis in immunocompetent and immunosuppressed hosts. Folia Biologica (Kraków) **69**: 167-178.

The course of *Acanthamoeba* spp. infection depends on the age and immune status of the host, and the virulence of the *Acanthamoeba* spp. strain. Some strains of free-living amoebae exhibit organ specificity, during the course of infection, while others may cause changes in many organs or completely lose pathogenicity. The aim of the current study was to investigate the pathological properties of *Acanthamoeba* spp. isolated from a patient with acute myeloid leukemia and atypical pneumonia (AM22). Moreover, the objective was to investigate the histopathological changes in the kidneys and heart of immunocompetent and immunosuppressed mice infected with *Acanthamoeba* spp. Amoebae were re-isolated from both the kidneys and hearts of the inoculated mice, although no cysts or trophozoites of the amoebae were detected in microscopic slides of the fragments of these organs. *Acanthamoeba* spp. induced changes in the kidney and heart weight of infected mice. In immunocompetent and immunosuppressed *Acanthamoeba* spp. infected mice, we found some histopathological changes, including areas with less acidic cytoplasm and a relaxation of muscle fibers. In further studies, it is important to analyze changes in gene and protein expressions in the heart and kidneys of hosts with disseminated acanthamoebiasis to better understand the course of infection in these organs, because the results of histological analysis varied depending on the immune status and duration of infection.

Key words: *Acanthamoeba* spp., immunosuppressive drug, heart, histopathological changes, kidneys.

Karolina KOT, Natalia ŁANOCHA-ARENDARCZYK, Department of Biology and Medical Parasitology, Pomeranian Medical University in Szczecin, Poland.
Danuta KOSIK-BOGACKA✉, Independent Laboratory of Pharmaceutical Botany, Pomeranian Medical University, Powstanców Wielkopolskich 72, 70-111 Szczecin, Poland.
E-mail: danuta.kosik.bogacka@pum.edu.pl
Michał PTAK, Department of Nephrology, Transplantology, and Internal Medicine, Pomeranian Medical University in Szczecin, Poland.
Paulina ROSZKOWSKA, Department of Microbiology, Immunology, and Laboratory Medicine, Pomeranian Medical University in Szczecin, 70-204, Szczecin, Poland.
Andrzej KRAM, Department of Pathomorphology, West Pomeranian Oncology Center, Szczecin, Poland.

Acanthamoeba spp. are amphizoic and opportunistic free-living amoebae that have the potential to cause diseases in humans and other animals (KOT *et al*. 2018). Ubiquitous in the soil, air, and water, the severity of infection is related to the size of the inoculum, the immune status of a patient, and the virulence of the strain of the amoebas (WALOCHNIK *et al.* 2015). *Acanthamoeba* spp. exist in two forms: vegetative trophozoite and resistant double-walled cyst (ŁANOCHA-ARENDARCZYK *et al.* 2017), and can develop into granulomatous amebic encephalitis (GAE), *Acanthamoeba* keratitis (AK), skin ulcers, and upper respiratory tract infections (KHAN 2003; STAPLETON *et al.* 2009). Disseminated acanthamoebiasis with infection to other organs and tissues occurs principally in immunocompromised individuals, including HIV-infected patients, or following organ transplantation, such as kidney and heart grafts (STEINBERG *et al.* 2002; SATLIN *et al.* 2013; TAN *et al.* 2014; SALAMEH *et al.* 2015; BRONDFIELD *et al.* 2017;

WINSETT *et al.* 2017). The infection in immunosuppressed patients is violent and usually leads to death occurring within one month after the central nervous system is invaded (MARCIANO-CABRAL & CABRAL 2003). Mean time to infection by *Acanthamoeba* spp. following transplant was 18 months (ranging from three months to six years) and immunosuppressive regimens commonly include the use of mycophenolate, cyclosporine, tacrolimus, and/or prednisone (BRONDFIELD *et al.* 2017). The diagnosis of disseminated acanthamoebiasis is most often made *post mortem* by re-isolation and/or determination of developmental forms in histological studies from extracted organ fragments (VISVESVARA 2013). There are still no unambiguous methods of infection prevention, rapid diagnostic techniques, or treatment algorithms, due to the incomplete understanding of the pathogenesis and pathophysiology of *Acanthamoeba* spp. infections. Research on molecular biology, pathology, and immunology is often conducted on an animal model, in which animals are infected with *Acanthamoeba* spp. in nasal, intraperitoneal, intravenous, intramuscular, subcutaneous, or intracerebral areas. In laboratory animals, symptoms of infection with *Acanthamoeba* spp. appear a few days or weeks after infection, depending on the infecting dose and pathogenicity of the strain (CULBERTSON *et al.* 1959; SCHUSTER & VISVESVARA 2004). KASPRZAK (1974) found that some strains of free-living amoebae exhibit organ specificity, during the course of infection, while others may cause changes in many organs or completely lose pathogenicity. The aim of the current study was to investigate the pathological properties of *Acanthamoeba* spp. isolated from a patient with acute myeloid leukemia and atypical pneumonia (AM22). Moreover, the objective was to investigate the histopathological changes in the kidneys and heart of immunocompetent and immunosuppressed mice infected with *Acanthamoeba* spp.

Materials and Methods

Ethics statement

This study was approved by the Local Ethics Committee for Scientific Experiments on Animals in Szczecin (No. 29/2015) and Poznań (No. 64/2016), Poland. All animal experiments were performed in strict agreement with good animal practice with the recommendations in the Guide for Care and Use of Laboratory Animals.

Animals and *Acanthamoeba* spp.

The research was conducted on 96 mice. Adult male BALB/c mice (6-10 months old) were either infected with *Acanthamoeba* spp. using the method described in previous papers or not treated as controls (ŁANOCHA-ARENDARCZYK *et al.* 2018a). The duration of the experiment was 42 days, including two weeks for an acclimatization period. The mice were housed under controlled temperatures on a 12h:12h light/dark cycle and fed with Labofeed H (Morawski, Kcynia, Poland) and water *ad libitum.* The mice were divided into 4 groups:

– immunocompetent uninfected *Acanthamoeba* spp. mice (C; n=18);

– immunocompetent *Acanthamoeba* spp. infected mice (A; n=30);

– immunosuppressed *Acanthamoeba* spp. infected mice (AS; n=30);

– immunosuppressed uninfected *Acanthamoeba* spp. mice (CS; n=18).

Modification of the immune system

To suppress immunity, the selected mice were intraperitoneally (i.p.) dosed with 0.22 mg (10 mg/kg body weight) of methylprednisolone sodium succinate (MPS, Solu-Medrol, Pfizer, Europe MA EEIG) dissolved in 0.1 ml of 0.9% saline, daily for four days before inoculation with *Acanthamoeba* spp. (ŁANOCHA-ARENDARCZYK *et al.* 2018a; MARKOWITZ *et al.* 1978). Such an algorithm permitted the development of an experimental model similar to that of patients with low immunity response.

Intranasal inoculation and biological invasiveness test

Mice were infected by intranasal inoculation with 3 μl of suspension containing 10-20 thousand *Acanthamoeba* spp. strain AM22, isolated from a patient with acute myeloid leukemia (AML) and atypical pneumonia (ŁANOCHA *et al.* 2009). The amoebae were initially grown on agar plates (NN agar) covered with a suspension of deactivated *Escherichia coli* (deactivated at 110°C for 1 h) and incubated at 37°C for 72 h according to standard methods. Following that, the amoebas were washed three times with PBS to remove most of the extracellular bacteria. The residual bacteria were killed with ampicillin and streptomycin (each at a concentration of 100 μg/ml) over 24h followed by triple washing the amoebas with PBS. The final PBS wash was plated onto agar to ensure that any remaining extracellular bacteria had been killed. Afterward, the amoebas were rinsed from the agar surface with sterile saline (0.9% NaCl) and then centrifuged at 3000 x g for 10 minutes (ŁANOCHA-ARENDARCZYK *et al*. 2018a; KARAŚ *et al.* 2015). The non-infected mice were given the same volume of saline (3 μl 0.9% NaCl). The mice were euthanized with pentobarbital sodium (Euthasol vet, FATRO, Raamsdonksveer, the Netherlands) which was administered by intraperitoneal injection (2 ml/kg body weight). Then the mice were weighed, and their kidneys and hearts were re-

moved for analysis. Fragments (5 mm x 5 mm) of the kidneys and heart were put on NN agar and incubated at 41°C to assess the infection intensity level (GÓRNIK & KUŹNA-GRYGIEL 2005). The plates were monitored daily under microscope for 10 days at a low magnification. The virulence of the amoebae was determined based on the degree of infection (on a scale 0-2) and determined as a ratio of the number of infected animals (presence of amoebae in studied tissues) to the number of inoculated mice.

Histological examination

Six mice from each group were used for histological analysis. The collected fragments of their kidneys and hearts were also fixed in 4% buffered formalin solution (Avantor, Gliwice, Poland). After dehydration in alcohols with increasing concentrations, the organs were embedded in paraffin. Serial (5 μm) sections were stained using hematoxylin and eosin. The samples were then examined to evaluate histopathological changes in the kidneys and hearts of the mice. Additionally, the diameter of glomeruli in the kidneys were measured in 10 random fields per animal by two linear measurements in two different axes of each glomerulus. The results were expressed in μm. The examination was done by two independent pathologists.

Statistical analysis

The mean (AM), standard deviation (SD), median, and range (minimum and maximum values) were calculated for each group. Quantitative variables were compared between groups with a nonparametric Mann-Whitney U test, which was used because the number of mice was too small to reliably assess the normality of distribution. The statistical significance was set at $p<0.05$. Calculations were performed using Statistica Software 8.0.

Results

The pathogenicity test

Two immunocompetent animals died about 2 weeks post *Acanthamoeba* spp. infection. Immunocompetent (number of mice, n=8) and immunosuppressed (n=10) *Acanthamoeba* spp. infected mice showed changes in appearance and behavior, including emaciation, coat color changes, and hunched posture, as well as in changes in behavior such as aggression and circular marching.

The results of culturing organ samples on non-nutrient agar (NN agar) media previously coated with Gram-negative bacteria are shown in Table 1. In the immunocompetent *Acanthamoeba* spp. infected mice, amoebae were isolated from the heart (n=6) and kidney (n=7) samples at 8 days post *Acanthamoeba* spp. infection (dpi), 16 dpi (n=5 for both organs) and 24 dpi (n=6 for both organs). In the immunosuppressed *Acanthamoeba* spp. infected mice, amoebae were isolated at 8 dpi (n=4 for both organs), 16 dpi (from 6 kidneys and 4 heart samples), and 24 dpi (n=7 for both organs). Both cyst and trophozoites were found on the media (Fig. 1).

Kidneys and heart weight

We did not observe statistically significant differences in kidney weight between the immunocompetent uninfected and *Acanthamoeba* spp. infected immunocompetent mice. Significantly lower kidney weight was noted in immunocompetent infected mice at 8 and 16 dpi compared to the immunocompromised *Acanthamoeba* spp. infected mice (Mann-Whitney U test, $U=17.0$, $p<0.05$ and $U=9.0$, $p<0.05$, respectively) (Table 2).

We observed a significant lower heart weight in the immunocompetent mice at 16 dpi than in the uninfected mice (Mann-Whitney U test, $U=1.5$, $p<0.05$). The heart weight of the immunosuppressed mice at 24 dpi was significantly lower compared to the immunocompetent hosts (Mann-Whitney U test, $U=0.0$, $p<0.05$) (Table 2).

Histological evaluation of kidneys

In the immunocompetent and immunosuppressed uninfected mice, there were no changes observed in the kidneys. The border between the cortex and medulla was clearly visible in the uninfected and infected *Acanthamoeba* spp. mice. No pathomorphological changes were observed in the renal cortex, parenchyma of medulla, or renal pelvis in the immunocompetent *Acanthamoeba* spp. infected mice. At the periphery of the cortex however, there was an easily visible, darker stained zone with regularly arranged glomeruli. The diameter of the glomeruli in the immunocompetent *Acanthamoeba* spp. infected mice at 8 dpi was on average 48.84 μm, at 16 dpi – 49.65 μm, and at 24 dpi – 47.44 μm versus 49.36 μm in the immunocompetent uninfected mice; but the differences were not statistically significant. One kidney sample of an *Acanthamoeba* spp. infected immunocompetent mouse at 16 dpi showed atypical renal tubular epithelial cells. Elevated proliferation of cells in the proximal/distal tubule epithelium, abnormal nucleus to cytoplasm ratio, numerous vacuoles, mitotic figures (red arrow, Fig. 2), and single lymphocytes (black arrow, Fig. 2) were observed.

The renal morphology of immunosuppressed uninfected mice and immunosuppressed *Acanthamoeba* spp. infected mice was similar. The arrangement of individual elements in the kidney parenchyma was unchanged. However, at 16 days post *Acanthamoeba* spp.

Table 1

The pathogenic properties of *Acanthamoeba* spp. reisolated from the kidneys and heart of the immunocompetent and immunocompromised mice at 8, 16, and 24 days post infection (dpi): A – immunocompetent *Acanthamoeba* spp. infected mice; AS – immunosuppressed *Acanthamoeba* spp. infected mice; No. – consecutive mouse number; intensity of infection was expressed on a scale 0-2: absence (0 = -) and occurrence (1 = +; 2 = ++) of developmental stages of *Acanthamoeba* spp.)

No.	Organ	8 dpi		16 dpi		24 dpi	
		A	AS	A	AS	A	AS
1	Kidneys	+	–	+	+	–	+
	Heart	+	–	+	+	–	+
	Observations	–	–	agony, coat color changes emaciation, dehydration	agony, coat color changes emaciation, dehydration, hunched posture	–	agony
2	Kidneys	+	–	died	+	+	+
	Heart	+	–		–	–	+
	Observations	–	–		agony, coat color changes, emaciation dehydration, hunched posture	–	dehydration
3	Kidneys	+	–	–	–	+	+
	Heart	+	–	–	–	+	+
	Observations	–	–	–	–	coat color changes	emaciation, dehydration
4	Kidneys	–	+	–	+	+	–
	Heart	–	+	–	–	+	–
	Observations	–	coat color changes	–	–	aggression, coat color changes	–
5	Kidneys	+	+	+	–	–	+
	Heart	++	+	+	–	–	+
	Observations	–	coat color changes	–	–	–	–
6	Kidneys	–	+	+	–	+	–
	Heart	+	++	+	–	+	–
	Observations	agony, dehydration	–	–	–	–	–
7	Kidneys	+	–	++	+	++	+
	Heart	+	–	+	+	+	+
	Observations	circular marching, coat color changes, aggression	–	–	–	circular marching	–
8	Kidneys	–	+	+	–	+	–
	Heart	–	+	+	–	+	–
	Observations	–	coat color changes, dehydration	aggression, coat color changes, hunched posture	–	coat color changes	–
9	Kidneys	–	–	died	+	–	+
	Heart	–	–		+	–	+
	Observations	–	–		aggression	–	–
10	Kidneys	+	–	–	+	–	+
	Heart	+	–	–	+	+	+
	Observations	–	–	–	–	coat color changes	–

The table shows observations of each immunocompetent mouse at 8 (n=10), 16 (n=10), and 24 (n=10) dpi, as well as of each immunosuppressed mouse at 8 (n=10), 16 (n=10), and 24 (n=10) dpi.

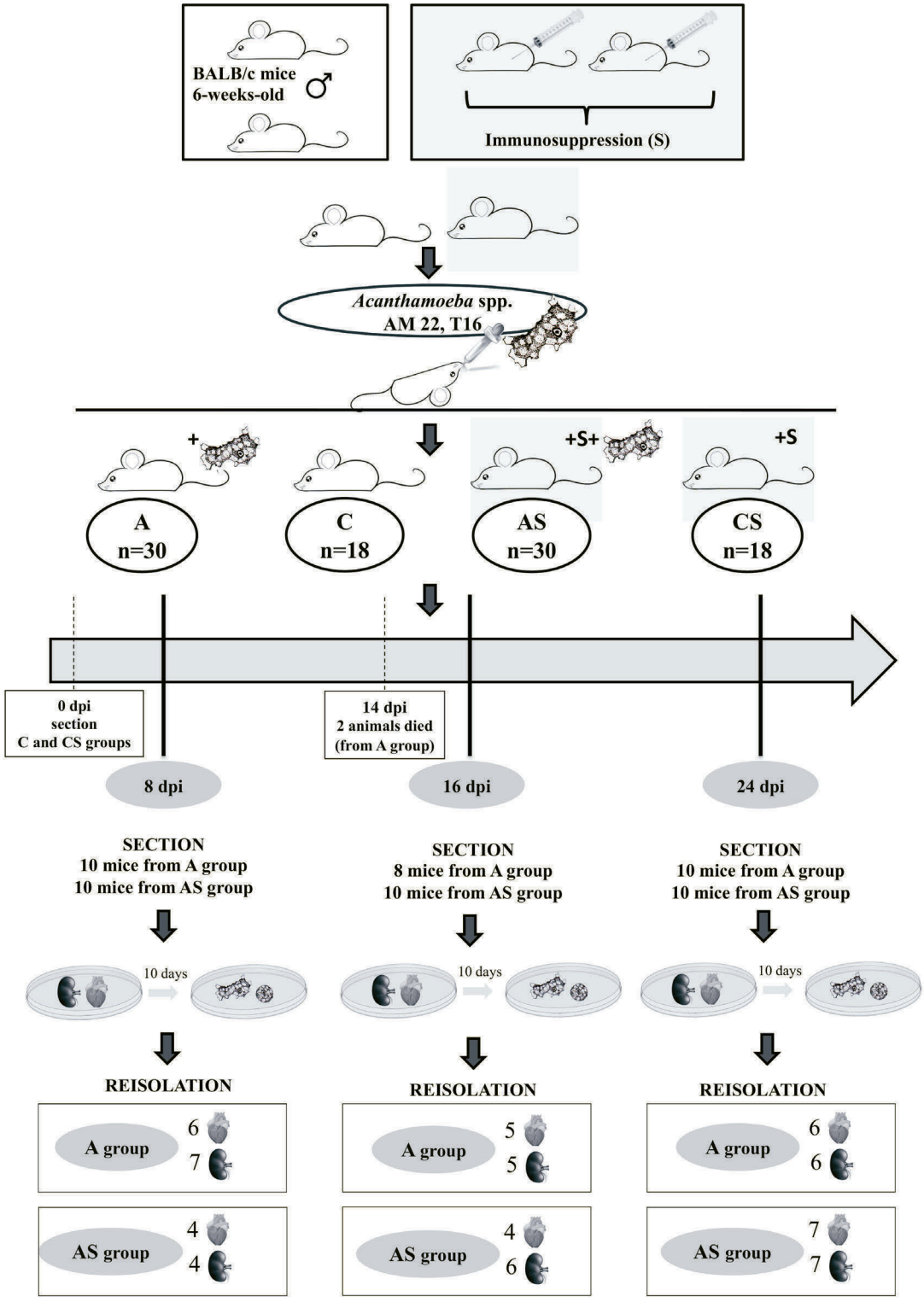

Fig. 1. The scheme of the experiment. At 0 dpi, 18 animals from the immunocompetent uninfected (C) and immunosuppressed uninfected (CS) groups were sacrificed. At 8 dpi, 10 mice from the immunocompetent *Acanthamoeba* spp. infected group (A) and immunosuppressed *Acanthamoeba* spp. infected group (AS) were sacrificed. The kidneys and heart were collected, and were then put on NN agar and incubated for 10 days. In the A group, amoebae were isolated from the hearts of 6 mice, and from the kidneys of 7 mice. In the AS group, amoebae were isolated from the heart and kidneys of 4 mice. At 14 dpi, two animals from A group died. At 16 dpi, 8 mice from A group and 10 mice from AS group were sacrificed. The kidneys and hearts were collected, and were then put on NN agar and incubated for 10 days. In the A group, amoebae were isolated from the heart and kidneys of 5 mice. In the AS group, amoebae were isolated from the hearts of 4 mice and from kidneys of 6 mice. At 24 dpi, 10 mice from the A and AS groups were sacrificed. The kidneys and heart were collected, and were then put on NN agar and incubated for 10 days. In the A group, amoebae were isolated from the heart and kidneys of 6 mice. In the AS group, amoebae were isolated from the heart and kidneys of 7 mice.

Table 2

Kidney and heart weight (g) of the uninfected and infected *Acanthamoeba* spp. immunocompetent and immunocompromised mice: C – immunocompetent uninfected mice; A – immunocompetent *Acanthamoeba* spp. infected mice; CS – immunosuppressed uninfected mice; AS – immunosuppressed *Acanthamoeba* spp. infected mice; n – number of animals; dpi – days post *Acanthamoeba* spp. infection; AM – arithmetic mean; SD – standard deviation of AM

Parameter/ group		C (n=18)	A (n=30)	CS (n=18)	AS (n=30)
		Kidney weight			
0 dpi	AM±SD	0.20±0.04		0.23±0.04	
	Median	0.21		0.20	
	Range	0.15-0.26		0.20-0.30	
8 dpi	AM±SD		0.18±0.05[a]		0.23±0.03 [a]
	Median		0.17		0.21
	Range		0.12-0.28		0.20-0.30
16 dpi	AM±SD		0.16±0.03[b]		0.22±0.05[b]
	Median		0.16		0.22
	Range		0.12-0.21		0.10-0.30
24 dpi	AM±SD		0.28±0.29		0.22 ± 0.05
	Median		0.19		0.22
	Range		0.13-1.10		0.10-0.30
		Heart weight			
0 dpi	AM±SD	0.15 ± 0.02[c]		0.16±0.05	
	Median	0.15		0.20	
	Range	0.12-0.16		0.10-0.20	
8 dpi	AM±SD		0.16±0.07		0.12±0.04
	Median		0.15		0.10
	Range		0.07-0.35		0.10-0.20
16 dpi	AM±SD		0.10±0.02 [c]		0.13±0.05
	Median		0.10		0.10
	Range		0.08-0.13		0.10-0.20
24 dpi	AM±SD		0.14±0.01[d]		0.10±0.00 [d]
	Median		0.015		0.1
	Range		0.13-0.16		0.1-0.1

[a]p=0.02 for the significance of difference A vs AS (Mann-Whitney U test, U=17.0)
[b]p=0.01 for the significance of difference A vs AS (Mann-Whitney U test, U=9.0)
[c]p=0.001 for the significance of difference C vs A (Mann-Whitney U test, U=1.5)
[d]p<0.001 for the significance of difference A vs AS (Mann-Whitney U-test, U=0.0)

infection the immunosuppressed mice had poorly visible Bowman's capsules and a lighter staining of the nuclei and cytoplasm of tubular cells. Lighter staining was also observed in the kidneys of the immunosuppressed mice at 24 dpi. The morphometric analysis of glomeruli diameter in the immunosuppressed mice was similar, at 8 dpi – 51.56 μm, at 16 dpi – 51.77 μm, and at 24 dpi – 53.40 μm versus the uninfected immunosuppressed mice – 54.83 μm; the differences were not statistically significant. In the immunocompetent and immunocompromised *Acanthamoeba* spp. infected mice kidneys, no developmental forms of the amoebae were found. However, amoeba-like cells were observed between the cell junction in one mouse from the A group at 16 dpi and in mice from the AS group at 16 and 24 dpi (data not shown).

Histological evaluation of hearts

There were no pathomorphological changes in the cardiac tissue of the immunocompetent and immunocompromised uninfected mice. At 8 days post *Acanthamoeba* spp. infection, the immunocompetent mice showed a relaxation of muscle fibers in the heart (Fig. 3). These changes were not observed in the

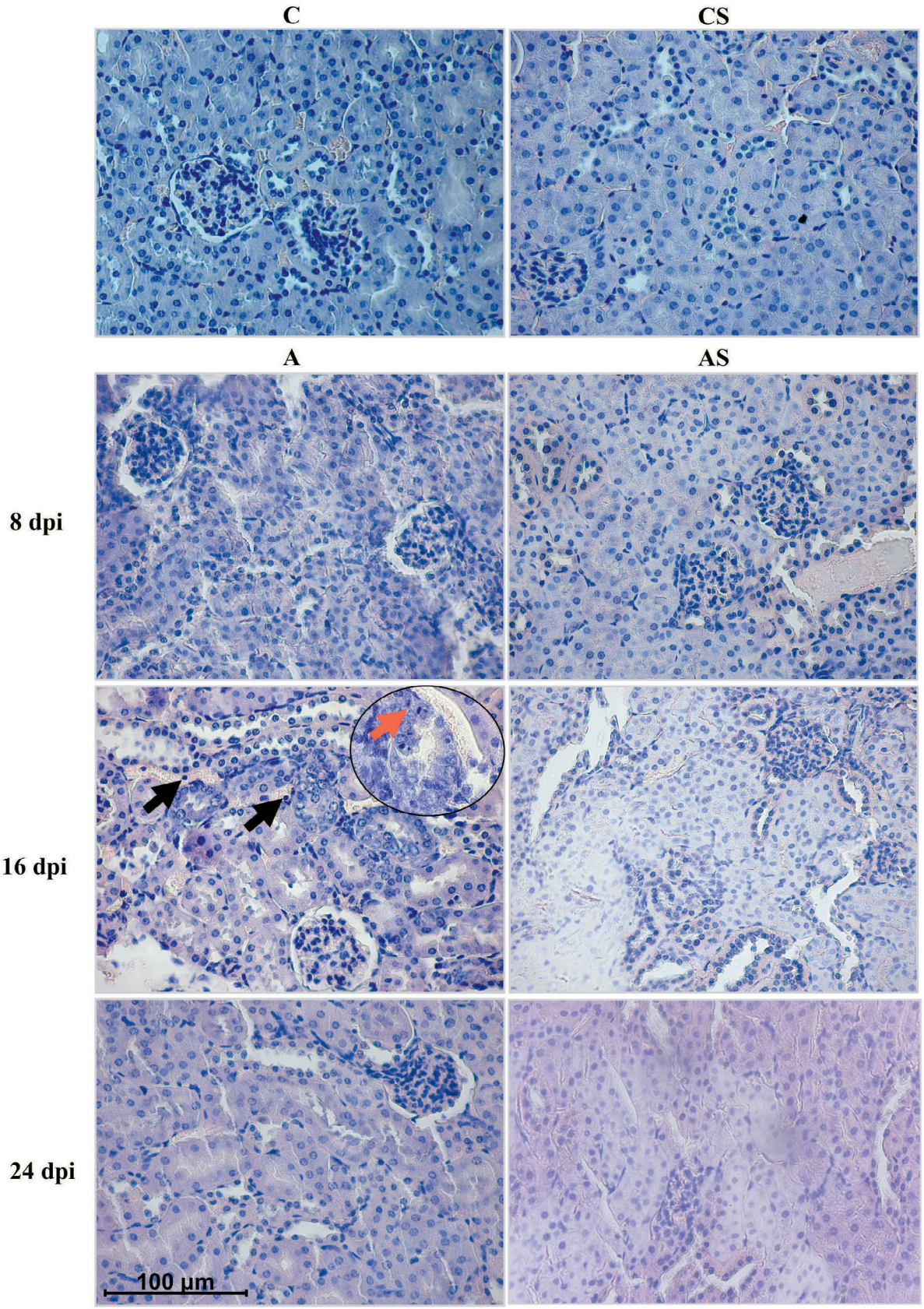

Fig. 2. Cross section of the kidneys of the uninfected and *Acanthamoeba* spp. infected immunocompetent and immunosuppressed mice (A – immunocompetent *Acanthamoeba* spp. infected mice; AS – immunosuppressed *Acanthamoeba* spp. infected mice; C – immunocompetent uninfected mice; CS – immunosuppressed uninfected mice; dpi – days post *Acanthamoeba* spp. infection; black arrow – single lymphocyte; red arrow – mitotic figure). Hematoxylin and eosin staining, magnification x400.

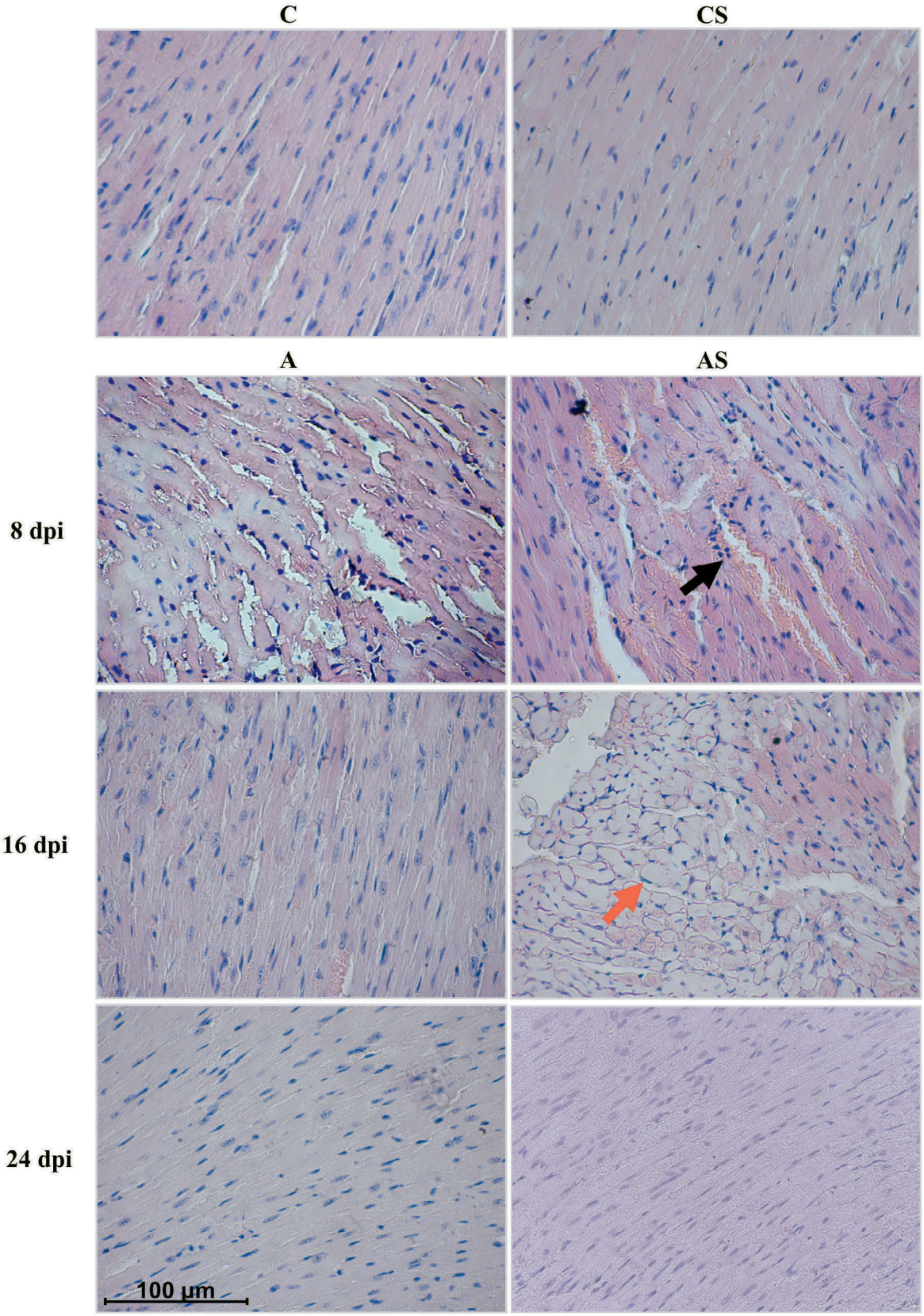

Fig. 3. Cross section of the hearts of the uninfected and *Acanthamoeba* spp. infected immunocompetent and immunosuppressed mice (A – immunocompetent *Acanthamoeba* spp. infected mice; AS – immunosuppressed *Acanthamoeba* spp. infected mice; C – immunocompetent uninfected mice; CS – immunosuppressed uninfected mice; dpi – days post *Acanthamoeba* spp. infection; black arrow – haemorrhage; red arrow – less acidophilic coloration area of muscle fibers). Hematoxylin and eosin staining, magnification x400.

hearts of the immunocompetent mice at 16 or 24 dpi. In one immunosuppressed mouse at 8 dpi, we detected an intramyocardial haemorrhage (black arrow, Fig. 3). At 16 dpi the immunosuppressed mice showed lesions in the muscle fibers, with the obliteration of striations and occurrence of areas with a less acidic cytoplasm were found (red arrow, Fig. 3). In the immunocompetent and immunosuppressed *Acanthamoeba* spp. infected mice, we found no developmental forms of *Acanthamoeba* spp. in the heart samples.

Discussion

The course of *Acanthamoeba* spp. infection has been shown to depend on the age and immune status of the host, the infecting dose, and the virulence of the *Acanthamoeba* spp. strain (MARCIANO-CABRAL & CABRAL 2003; FERRANTE 1991; GIERYNG & GIERYNG 1987; GÓRNIK & KUŹNA-GRYGIEL 2005; RUCKA 1974). In our study, the results of the pathogenicity test showed diverse courses of infection in animals infected with the amoebae. In the immunocompetent and immunosuppressed *Acanthamoeba* spp. infected mice, the amoebae were reisolated from 63% and 57% of infected animals, respectively. Similarly to our study, GIERYNG and GIERYNG (1987) and GIERYNG *et al.* (1993) reisolated *Acanthamoeba* spp. from about 70% of brain and 60% of lung samples taken from mice that had been nasally inoculated with *Acanthamoeba* spp.. Diverse courses of infection in human case reports have also been observed. STEINBERG *et al.* (2002) and BRONDFIELD *et al.* (2017) found that hematological and biochemical blood tests in renal or heart transplant patients with disseminated acanthamoebiasis were varied (increased, decreased, or within a normal range). RINGSTED *et al.* (1976), in a study on a Korean child with probable GAE and inflammatory lesions in the kidneys, found that a urine analysis disclosed no abnormality despite the fact that *Acanthamoeba* spp. were isolated from the kidneys of the patient. ŁANOCHA-ARENDARCZYK *et al.* (2018b) using mice from the same experimental model, did not reveal any significant difference in the levels of creatinine, urea, albumin, or total protein in the serum of *Acanthamoeba* spp. infected immunocompetent and immunosuppressed mice. Diverse course of infection in the hosts may be due to innate resistance to *Acanthamoeba* spp. (FERRANTE 1991). In humans, BRINDLEY *et al.* (2009) found the presence of natural IgG antibodies against *Acanthamoeba* spp. in the peripheral blood of over 80% of the studied population. The congenital resistance to *Acanthamoeba* spp. infection is also confirmed by studies that found the presence of developmental forms of *Acanthamoeba* spp. in the nasal and pharyngeal mucosa of healthy individuals (KRANJCIC-ZEC *et al.* 1990; RIVERA *et al.* 1991).

In the presented study, *Acanthamoeba* spp. re-isolation in the immunocompetent mice could be arranged in the following descending order: 8 dpi=24 dpi > 16 dpi; however, it is important to point out that two immunocompetent mice died about 2 weeks post *Acanthamoeba* spp. infection. No macroscopic or microscopic changes in the kidneys of the immunocompetent *Acanthamoeba* spp. infected mice were observed. We found no statistically significant differences in kidney weight or glomeruli diameter between the uninfected, immunocompetent, and immunosuppressed infected *Acanthamoeba* spp. mice. Only one immunocompetent mouse, at 16 day post *Acanthamoeba* spp. infection, showed an elevated proliferation of cells in the proximal/distal tubule epithelium, inappropriate nucleus to cytoplasm ratio, numerous vacuoles, mitotic figures, and single lymphocytes. The lack of changes in the histopathological picture of the kidneys as well as amoebas in the histological slides is consistent with molecular studies in which no elevated expression of TLRs, which are responsible for recognizing the amoebas, was found in the kidneys of immunocompetent mice infected with *Acanthamoeba* spp. (ALIZADEH *et al.* 2014; KOT *et al.* 2020). However, in available scientific literature, there is some data presenting histopathological changes in the kidneys of immunocompetent hosts. GÓRNIK and KUŹNA-GRYGIEL (2005) demonstrated various pathomorphological changes in the kidneys of *Acanthamoeba* spp. infected mice, depending on the mouse strain used in the experiment. Mice infected by strains isolated from swimming pools had haemorrhages and small inflammatory foci in their kidneys. Mice infected with a strain isolated in Goplana lake (Szczecin, northwest part of Poland) showed necrotic changes in renal tubules and Bowman's capsules. In the kidneys of *Acanthamoeba* spp. infected mice from which the amoebas were not reisolated *in vitro*, GÓRNIK and KUŹNA-GRYGIEL (2005) found only small inflammatory foci in the renal cortex. In dogs with a disseminated acanthamoebiasis, macroscopic and microscopic kidney lesions were observed (FRADE *et al.* 2015). The authors observed kidney enlargement with the presence of red and yellow nodules, distributed randomly beneath the subcapsular surface, and in microscopic slides, trophozoites of *Acanthamoeba* spp. within the periglomerular region. DUBEY *et al.* (2005) observed multifocal coalescing infiltrates of macrophages, lymphocytes, and neutrophils, with occasional intralesional amoebae in the kidneys of *Acanthamoeba* spp. infected dogs. Differences in the effects in the host kidney may be related to the strain of *Acanthamoeba* spp. OMAÑA-MOLINA *et al.* (2017) found that *Acanthamoeba castellani* trophozoites migrate to the kidneys of mice, while *Acanthamoeba culbertsoni* trophozoites do not migrate to the host kidney. The exact species of amoebae used in the study by GÓRNIK and KUŹNA-GRYGIEL (2005), FRADE *et al.* (2015), and DUBEY *et al.* (2005)

are not known. The aforementioned authors mentioned only that they used environmental strains.

In the immunosuppressed mice, we did not observe statistically significant differences in kidney weight and glomeruli diameter between the infected and uninfected hosts. However, at 16 and 24 days post *Acanthamoeba* spp. infection, the immunosuppressed mice had a lighter staining of the nuclei and cytoplasm of their tubular cells. Inflammatory cells, e.g. T lymphocytes, may induce expression of transforming growth factor β (TGF-β), which induced the transformation of kidney tubule cells to proliferating fibroblasts, causing fibrous changes in the kidney parenchyma (ROBERTSON *et al.* 2004). Such changes may be visible in a histological preparation in the form of a lighter color of the kidney parenchyma. Further analyses, including inflammatory markers and TGF-β, need to be performed to check whether lighter staining in the kidneys were results of inflammatory processes or the use of an immunosuppressive drug. In the immunosuppressed mice at 16 and 24 dpi, we also found amoeba-like cells between cell junctions. We did not classify them as amoebas but in future studies it is important to perform immunohistochemical of immunofluorescence staining to determine with certainty the presence or absence of amoebas in the evaluated tissue. In particular because OMAÑA-MOLINA *et al.* (2017) found *A. castellanii* trophozoites between cell junctions using immunohistochemical staining. The authors observed that *A. castellanii* trophozoites penetrated and invaded renal tissue through renal tubules, but they did not observe tissue destruction. Further studies are also needed because elevated TLR2 and TLR4 expression was observed in the kidneys of immunosuppressed mice at 16 and 24 days post *Acanthamoeba* spp. infection (KOT *et al.* 2020), which may suggest the presence of amoebas in the studied tissue.

In the presented study, we observed higher kidney weight between the *Acanthamoeba* spp. infected immunocompetent and immunosuppressed mice, which may be caused by the immunosuppressive drug. KABAT-KOPERSKA *et al.* (2017) who analyzed the influence of immunosuppressive drugs on rat organs observed changes in kidney weight.

The current literature presents little information about the influence of *Acanthamoeba* spp. on the cardiac muscle. ŁANOCHA-ARENDARCZYK *et al.* (2018b) examined the biochemical profile of mice infected with *Acanthamoeba* spp. and observed that serum aspartate aminotransferase (AST) levels were two times higher in immunocompetent infected mice than in immunocompetent uninfected animals. Similarly, *Acanthamoeba* spp. infected immunosuppressed mice had higher serum AST levels than the immunosuppressed uninfected mice, although the difference was not statistically significant. AST is a widely known indicator enzyme used in the diagnosis of liver disease, heart disease, and the inflammation of various organs. AST is involved in many important metabolic processes in the body, such as the urea cycle in the liver and the malate-aspartate shuttle in the heart muscle (OTTO-ŚLUSARCZYK *et al.* 2016). Since AST is used in the diagnosis of many diseases, it would be essential to determine the activity of creatine kinase to confirm cardiovascular disorders. However, in the aforementioned study by ŁANOCHA-ARENDARCZYK *et al.* (2018b), activity of creatine kinase was not determined.

In the presented study, no macroscopic changes were found in the hearts of *Acanthamoeba* spp. infected immunocompetent and immunosuppressed mice. In the hearts of all the immunocompetent 8 days post *Acanthamoeba* spp. infected mice, a relaxation of muscled fibers was observed. Despite the fact that we observed a lower heart weight in the immunocompetent mice at 16 days post *Acanthamoeba* spp. infection compared to the uninfected mice, no microscopic changes in the structure of cardiomyocytes were observed. It is worth noting that changes in the expression of immune response receptors were also found in the immunocompetent mice only on 8 days post *Acanthamoeba* spp. infection. However, at the beginning of the infection, in the heart of the immunosuppressed *Acanthamoeba* spp. infected mice, a discontinuity of the vascular walls and slight bleeding outages was observed, while in all mice at 16 dpi the amount of endomysium increased and the color of cardiomyocytes changed. When investigating the effect of immunosuppressive drugs on pathomorphological changes in rat organs KABAT-KOPERSKA *et al.* (2017), observed similar cells with a lighter colored cytoplasm called vacuolated cardiac muscles cells. Therefore, it is difficult to determine whether the observed changes in the muscle fibers of the *Acanthamoeba* spp. infected mice were caused by the parasitic infection or administration of immunosuppressive drugs. In other studies on changes in the heart of *Acanthamoeba* spp. infected mice, a bleeding stroke was also observed as a consequence of the discontinuity of vessel walls (GÓRNIK & KUŹNA-GRYGIEL 2005). Vessel damage by amoeba present in the wall and perivascular zone was also observed by MARTINEZ (1991) and GIERYNG *et al.* (1993). FRADE *et al.* (2015) found macroscopically multifocal red, irregular, centrally yellowish and slightly prominent areas on the epicardial surface of the right and left ventricles and the left atrium in dogs with disseminated acanthamoebiasis. Analysis of microscopic slides showed inflammatory foci and developmental forms of amoebae. The changes observed by FRADE *et al.* (2015) within the heart may result from a significantly longer duration of the infection compared to the present studies and/or the type of pathogenic *Acanthamoeba* spp. strain.

In the presented histological examination of hearts, we found no trophozoites or cysts of *Acanthamoeba* spp.,

that were in agreement with case reports concerning the histological preparations of hearts of patients with acanthamoebiasis (BRONDFIELD *et al.* 2017; BARETE *et al.* 2007; TAN *et al*. 2014). Despite, no developmental forms of amoebas in the histological preparations, we reisolated *Acanthamoeba* spp. from cardiac fragments using NN agar. It should be taken into account that amoebas may have been re-isolated from blood residue in the heart, as they spread through the blood of the host, not from a fragment of heart tissue.

Conclusions

Acanthamoeba spp. (strain AM22) retained their pathogenic properties after 10 years of first re-isolation. The amoebae showed varying degrees of virulence in inoculated mice, and exhibited not only pneumotropic properties but also affected organs distant from the original site of infection. Some changes in the heart and kidneys were observed even though no developmental forms of *Acanthamoeba* spp. were found in the organs of the infected mice. However, in future studies, it is important to perform immunohistochemical immunofluorescence staining to determine with certainty the presence of amoebas in the evaluated tissue. Additionally, it will be important to analyze changes in gene and protein expressions in the heart and kidneys of hosts with disseminated acanthamoebiasis to better understand the influence of *Acanthamoeba* spp. on organs which are not the main biotype of these parasites.

Author Contribution

Research concept and design: K.K., D.K.-B., N.Ł.-A.; Collection and/or assembly of data: K.K., D.K.-B., N.Ł.-A.; Data analysis and interpretation: K.K., M.P., A.K.; Writing of the article: K.K., M.P., P.R.; Critical revision: D.K.-B.; N.Ł.-A., A.K.; Final approval of article: K.K., D.K.-B., N.Ł.-A., M.P., P.R., A.K.

Conflict of interest

The authors declare no conflict of interest.

References

ALIZADEH H., TRIPATHI T., ABDI M., SMITH A.D. 2014. Pathogenic strains of *Acanthamoeba* are recognized by TLR4 and indicated inflammatory responses in the cornea. PLoS One **9**: e92375. https://doi.org/10.1371/journal.pone.0092375

BARETE S., COMBES A., DE JONCKHEERE J.F., DATRY A., VARNOUS S., MARTINEZ V., PTACEK S.G., CAUMES E., CAPRON F., FRANCČS C., GIBERT C., CHOSIDOW O. 2007. Fatal disseminated *Acanthamoeba lenticulata* infection in a heart transplant patient. Emerg. Infect. Dis. **13**: 736-738. https://doi.org/10.3201/eid1305.061347

BRINDLEY N., MATIN A., KHAN N.A. 2009. *Acanthamoeba castellanii*: high antibody prevalence in racially and ethnically diverse populations. Exp. Parasitol. **121**: 254-256. https://doi.org/10.1016/j.exppara.2008.11.009

BRONDFIELD M.N., REID M.J., RUTISHAUSER R.L., COPE J.R., TANG J., RITTER J.M., MATANOCK A., ALI I., DOERNBERG S.B., HILTS-HORECZKO A., DEMARCO T., KLEIN L., BABIK J.M. 2017. Disseminated *Acanthamoeba* infection in a heart transplant recipient treated successfully with a miltefosine-containing regimen: Case report and review of the literature. Transpl. Infect. Dis. **19**: e12661. https://doi.org/10.1111/tid.12661

CULBERTSON C.G., SMITH J.W., COHEN H.K., MINNER J.R. 1959. Experimental infection of mice and monkeys by *Acanthamoeba*. Am. J. Pathol. **35**: 185-197.

DUBEY J.P., BENSON J.E., BLAKELEY K.T., BOOTON G.C., VISVESVARA G.S. 2005. Disseminated *Acanthamoeba* sp. infection in a dog. Vet. Parasitol. **128**: 183-187. https://doi.org/10.1016/j.vetpar.2004.11.022

FERRANTE A. 1991. Free living amoebae: pathogenicity and immunity. Parasite Immunol. **13**: 31-47.

FRADE M.T.S., DE MELO L.F., PESSOA C.R.M., DE ARAÚJO J.L., FIGHERA R.A., SOUZA A.P., UZAL F., DANTAS A.F.M. 2015. Systemic acanthamoebiasis associated with canine distemper in dogs in the semiarid region of Paraíba, Brazil. Pesq. Vet. Bras. **35**: 160-164. https://doi.org/10.1590/S0100-736X2015000200011

GIERYNG H., GIERYNG R. 1987. Histopathological changes in mice brain caused by experimental infection with limax amoebas. Ann. Univ. Mariae Curie-Skłodowska. **16**: 103-109.

GIERYNG H., GIERYNG R., PIRÓG Z. 1993. Histopathological changes in mice lungs caused by experimental infection with limax amoebas. Ann. Parasitol. **39**: 367-372.

GÓRNIK K., KUŹNA-GRYGIEL W. 2005. Histological studies of selected organs of mice experimentally infected with *Acanthamoeba* spp. Folia Morphol. **64**: 161-167.

KABAT-KOPERSKA J., KOLASA-WOŁOSIUK A., PILUTIN A., SAFRANOW K., KOSIK- BOGACKA D., BARANOWSKA-BOSIACKA I., GOŁEMBIEWSKA E., KĘDZIERSKA K., DOMAŃSKI L., CIECHANOWSKI K. 2017. Birth defects in juvenile Wistar rats after exposure to immunosuppressive drugs during pregnancy. Histol. Histopathol. **32**: 43-55. https://doi.org/10.14670/HH-11-769

KARAŚ M.A., TURSKA-SZEWCZUK A., TRAPSKA D., URBANIK-SYPNIEWSKA T. 2015. Growth and Survival of *Mesorhizobium loti* Inside *Acanthamoeba* Enhanced Its Ability to Develop More Nodules on *Lotus corniculatus*. Microb. Ecol. **70**: 566-575. https://doi.org/10.1007/s00248-015-0587-6

KASPRZAK W. 1974. Free-living, potentially pathogenic amoebae in natural environments. Wiad. Parazytol. **20**: 233-246.

KHAN N.A. 2003. Pathogenesis of *Acanthamoeba* infections. Microb. pathog. **34**: 277-285. https://doi.org/10.1016/s0882-4010(03)00061-5

KOT K., KOSIK-BOGACKA D., WOJTKOWIAK-GIERA A., KOLASA-WOŁOSIUK A., ŁANOCHA-ARENDARCZYK N. 2020. The expression of TLR2 and TLR4 in the kidneys and heart of mice infected with *Acanthamoeba* spp. Parasit. Vectors. **13**: 480. https://doi.org/10.1186/s13071-020-04351-4

KOT K., ŁANOCHA-ARENDARCZYK N., KOSIK-BOGACKA D.I. 2018. Amoebas from the genera *Acanthamoeba* and their pathogenic properties. Ann. Parasitol. **64**: 299-308. https://pubmed.ncbi.nlm.nih.gov/30720249/

KRANJCIC-ZEC I., SCEPAN L., MITROVIC S., ARSIC V. 1990. Free-living amebe *Acanthamoeba* sp. in throat's and nose's swabs of young-isolation and testing of the virulence. Bull. Soc. Fr. Parasitol. Suppl **1**: 357.

ŁANOCHA-ARENDARCZYK N., BARANOWSKA-BOSIACKA I., KOT K., GUTOWSKA I., KOLASA-WOŁOSIUK A., CHLUBEK D., KOSIK-BOGACKA D. 2018a. Expression and activity of COX-1 and COX-2 in *Acanthamoeba* sp. – infected lungs according to

the host immunological status. Int. J. Mol. Sci. **19**: E121. https://doi.org/10.3390/ijms19010121

ŁANOCHA-ARENDARCZYK N., BARANOWSKA-BOSIACKA I., KOT K., PILARCZYK B., TOMZA-MARCINIAK A., KABAT-KOPERSKA J., KOSIK-BOGACKA D. 2018b. Biochemical profile, liver and kidney selenium (Se) status during acanthamoebiasis in a mouse model. Folia Biol. **66**: 33-40. https://doi.org/10.3409/fb_66-1.04

ŁANOCHA-ARENDARCZYK N., KOSIK-BOGACKA D., GALANT K., ZAORSKI W., KOT K., ŁANOCHA A. 2017. Pathogenic free-living amoeba. Postep. Mikrobiol. **56**: 106-112. https://doi.org/10.21307/PM-2017.56.1.106

ŁANOCHA N., KOSIK-BOGACKA D., MACIEJEWSKA A., SAWCZUK M., WILK A., KUŹNA-GRYGIEL W. 2009. The occurence *Acanthamoeba* (Free Living Amoeba) in environmental and respiratory samples in Poland. Acta Protozool. **48**: 271-279.

MARCIANO-CABRAL F., CABRAL G., 2003. *Acanthamoeba* spp. as agents of disease in humans. Clin. Microbiol. Rev. **16**: 273-307. https://doi.org/10.1128/CMR.16.2.273-307.2003

MARKOWITZ S.M., SOBIESKI T., MARTINEZ A.J., DUMA R.J. 1978. Experimental *Acanthamoeba* infections in mice pretreated with methylprednisolone or tetracycline. Am. J. Pathol. **92**: 733-744.

MARTINEZ A.J. 1991. Infection of the central nervous system due to *Acanthamoeba*. Rev. Infect. Dis. **13**: 399-402.

OMAÑA-MOLINA M., HERNANDEZ-MARTINEZ D., SANCHEZ-ROCHA R., CARDENAS-LEMUS U., SALINAS-LARA C., MENDEZ-CRUZ A.R., COLIN-BARENQUE L., ALEY-MEDINA P., ESPINOSA-VILLANUEVA J., MORENO-FIERROS L., LORENZO-MORALES J. 2017. *In vivo* CNS infection model of *Acanthamoeba* genotype T4: the early stages of infection lack presence of host inflammatory response and are a slow and contact-dependent process. Parasitol. Res. **116**: 725-733.

OTTO-ŚLUSARCZYK D., GRABOŃ W., MIELCZAREK-PUTA M. 2016. Aspartate aminotransferase- key enzyme in the human systemic metabolism. Postępy Hig. Med. Dosw. **70**: 219-230. https://doi.org/10.5604/17322693.1197373

RINGSTED J., VAL JAGER B., SUK D.S., VISVESVARA G.S. 1976. Probable *Acanthamoeba* meningoencephalitis in a Korean child. Am. J. Clin. Pathol. **66**: 723-730.

RIVERA F., LARES F., RAMIREZ E., BONILLA P., RODRIGUEZ S., LABASTIDA A., ORTIZ R., HERNANDEZ D. 1991. Pathogenic *Acanthamoeba* isolated during an atmospheric survey in Mexico City. Rev. Infect. Dis. **13**: 388-389.

ROBERTSON H., ALI S., MCDONNELL B.J., BURT A.D., KIRBY J.A. 2004. Chronic renal allograft dysfunction: the role of T cell-mediated tubular epithelial to mesenchymal cell transition. J. Am. Soc. Nephrol. **15**: 390-397. https://doi.org/10.1097/01.asn.0000108521.39082.e1

RUCKA A. 1974. Histopathological changes in the brain and lungs of mice infected with pathogenic free-living amoebas. Wiad. Parazytol. **2-3**: 247-250.

SALAMEH A., BELLO N., BECKER J., ZANGENEH T. 2015. Fatal granulomatous amoebic encephalitis caused by *Acanthamoeba* in a patient with kidney transplant: a case report. Open Forum Infect. Dis. **2**: ofv104. https://doi.org/10.1093/ofid/ofv104

SATLIN M.J., GRAHAM J.K., VISVESVARA G.S., MENA H., MARKS K.M., SAAL S.D., SOAVE R. 2013. Fulminant and fatal encephalitis caused by *Acanthamoeba* in a kidney transplant recipient: case report and literature review. Transpl. Infect. Dis. **15**: 619-626. https://doi.org/10.1111/tid.12131

SCHUSTER F.L., VISVESVARA G.S. 2004. Free-living amoebae as opportunistic and non-opportunistic pathogens of humans and animals. Int. J. Parasitol. **34**: 1001-1027. https://doi.org/10.1016/j.ijpara.2004.06.004

STAPLETON F., OZKAN J., JALBERT I., HOLDEN B., PETSOGLOU C., MCCLELLAN K. 2009. Contact lens-related *Acanthamoeba* keratitis. Optom. Vis. Sci. **86**: e1196-e1201. https://doi.org/10.1097/OPX.0b013e3181baae11

STEINBERG J.P., GALINDO R.L., KRAUS E.S., GHANEM K.G. 2002. Disseminated acanthamebiasis in a renal transplant recipient with osteomyelitis and cutaneous lesions: case report and literature review. Clin. Infect. Dis. **35**: e43-e49. https://doi.org/10.1086/341973

TAN S.K., GAJUREL K., TUNG C., ALBERS G., DERESINSKI S., MONTOYA J.G., SHEIKH A.Y., BANERJEE D., HA R. 2014. Fatal *Acanthamoeba* encephalitis in a patient with a total artificial heart (syncardia) device. Open Forum Infect. Dis. **1**: ofu057. https://doi.org/10.1093/ofid/ofu057

VISVESVARA G.S. 2013. Infections with free-living amoebae. In: GARCIA H.H., TANOWITZ H.B., DEL BRUTTO O.H. (eds.) Handbook of Clinical Neurology, **114**: 153-168. Elsevier. https://doi.org/10.1016/B978-0-444-53490-3.00010-8

WALOCHNIK J., SCHEIKL U., HALLER-SCHOBER E.M. 2015. Twenty years of *Acanthamoeba* diagnostics in Austria. J. Eukaryot. Microbiol. **62**: 3-11. https://doi.org/10.1111/jeu.12149

WINSETT F., DIETERT J., TSCHEN J., SWABY M., BANGERT C.A. 2017. A rare case of cutaneous acanthamoebiasis in a renal transplant patient. Dermatol. Online J. **23**: 13030.