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# HAEMATOLOGICAL AND MORPHOLOGICAL CHANGES IN EXPERIMENTAL ANISAKIOSIS IN PIGS

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## HEMATOLOGICZNE I MORFOLOGICZNE ZMIANY W EKSPERYMENTALNEJ ANISAKIOZIE ŚWIŃ

Abstract. The aim of this paper is to establish the haematological and organic changes in halothane-sensitive and halothane-resistant pigs in the course of experimental anisakiosis. Experiments were carried out on two groups of pigs (3 animals each). The pigs from the first group were given fifty *A. simplex* B larvae, the pigs from the second one received ten larvae and then again fifteen larvae each after the 5th and 6th days. The number of leucocytes, neutrophiles, lymphocytes, monocytes and eosinophiles was greater — different in both series, but similar in halothane (stress)—resistant and —sensitive pigs. In the case of sensitive pigs much greater reactive changes were found in the stomach submucosa than in that of resistant pigs. In this group of pigs nematode larvae have also been traced in the submucosa of the same organ.

## INTRODUCTION

The course of anisakiosis does not give a specific image of the illness, due to the varied behaviour of larvae in the alimentary canal of the random hosts, which are terrestrial animals, including man (GIBSON 1970, BHAIBULAI and STITYATHAI 1982). Anisakiosis in man can easily be confused with other illnesses (OSHIMA 1972, WILLIAMS and JONES 1976, MARGOLIS 1977). In order to avoid gastroscopy or surgery, other, non-invasive, diagnostic methods are sought. Research has not been successful so far. The aim of this paper is thus to examine morphological changes of internal organs during the course of anisakiosis, particularly mucosa and submucosa in the alimentary canal of pigs with genetically determined sensitivity or resistance to stress, changes in blood morphology in both groups of pigs examined, according to the doses of *Anisakis simplex* B larvae, the behaviour of larvae in the pigs stomachs depending upon the time of administration.

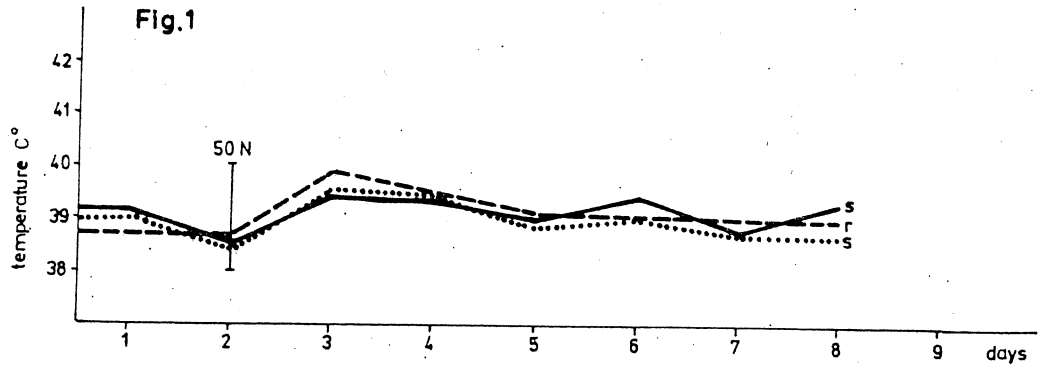
## Materials and methods

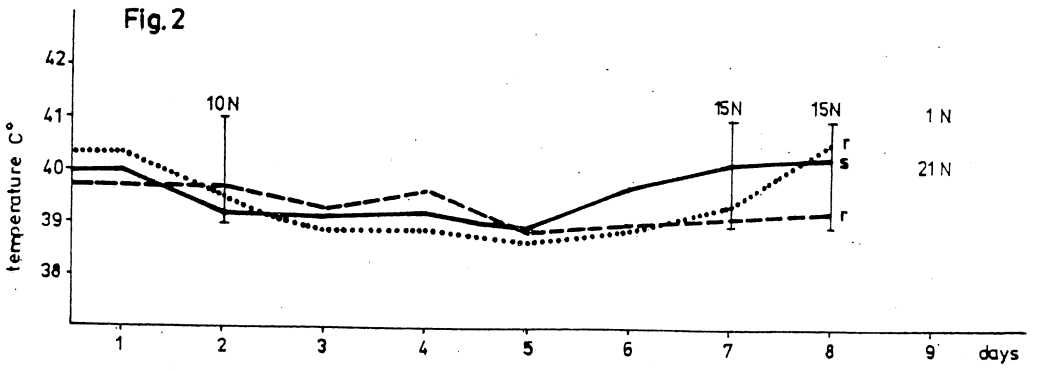
All experiments were carried out on 6 Belgian Landrace pigs of both sexes, about 8-10 weeks of age and average body weight of 20 kg. Animals were taken from the Central Hybridization Station, Institute of Zootechnics of the Polish Academy of Sciences in Cracow (Poland). The pigs had been classified as stress-resistant or stress-susceptible by the standard halothane test (BORMAN et al. 1985a, 1985b).

Blood for testing was sampled via the catheter inserted into the *vena cava exterior* 3 days before experiments. In each sample a total number of leucocytes and their populations (neutrophiles, lymphocytes, monocytes, eosinophiles) were estimated.

The pigs were infected by previously determined (MATTIUCCI et al. 1989) L III *Anisakis simplex* B, taken from herring, *Clupea harengus* from the southern Baltic. Before the larvae were given to the pigs they had been kept in Ringer solution for 12-24 hours.

The pigs were divided into two experimental groups. The first group consisted of two sensitive and one resistant pig (the first experimental series, Fig. 1). They were infected by fifty larvae and put to sleep by means of penthabarbiturate after seven days. The second group consisted of two resistant and one sensitive pig (the second experimental series, Fig. 2). The animals were initially infected with ten larvae, and further with fifteen on the fifth and sixth days, then were also put to sleep after seven days.




Explanations for figs 1-12 in the text.
Legends for figs 1 and 2: N — nematode, r — pig resistant, s — pig susceptible

In the course of the experiment the pigs' temperatures were taken every day, with blood tests being carried out on specific days (1, 2, 4, 5, 8, 9), (Fig. 3). After the pigs had been put to sleep their organs were checked for the presence of nematode larvae and pathological changes.

**Results**

Observations of stress-sensitive and stress-resistant pigs after their being infected with *Anisakis simplex* B larvae proved their visible reactions to have remained unchanged. They were fit and ate well during the experiment. There were no noticeable temperature rises in either sensitive or resistant pigs. After infection with the fifty larvae, however, the body temperature rose by 1.5°C in two sensitive and one resistant pig. On the following day the temperature fell and on the third day returned to normal (Figs 1, 2).

After infection, all pigs tested showed a rise in the leucocyte number in the peripheral blood (Figs 3, 4). Marked leucocytosis occurred in the stress-resistant pigs which received both fifty and ten parasites. The increase in leucocyte number appeared on the second day after infection and remained until the end of the experiment i.e. till the seventh day after the parasites had been introduced.

No substantial statistical differences were noted in the number of neutrophilic granulocytes between the stress-sensitive and stress-resistant pigs in either series. An increase in the number of such cells did, however, occur in the second experimental series in the animals which received first ten and then fifteen larvae on the fifth and sixth days, respectively (Figs 5, 6).

All animals had considerable lymphocytosis (Figs 7, 8). The increase in lymphocyte number occurred in both sensitive pigs which, in addition, suffered from tissue reaction in the form of mucosa and submucosa congestion (apart from the nematodes presence in the stomach walls) and in stress-resistant pigs, in the case of which there was generally very little change in the stomach walls.

Apart from the lymphocytosis all the pigs tested indicated a monocytic reaction (Figs 9, 10). On the second and third day after infection with the parasite, the number of monocytes rose irrespective of the number of nematodes given or sensitive resistant category.

Five pigs out of six in both experimenal series showed an increase in the eosinophilic granulocyte number (Figs 11, 12). This occurred on the second day after administering the nematode and showed no tendency to stay that way. In subsequent experiments carried out on the sixth and seventh days after infection a drop in the eosinophilic granulocyte number was noted.

Of the internal organs only the stomach walls showed changes resulting from the *A. simplex* B lavae infection. In the resistant pigs the fundus of the stomach was slightly congested. In one pig in the second series of the experiment only one nematode was found with its head stuck in the submucose

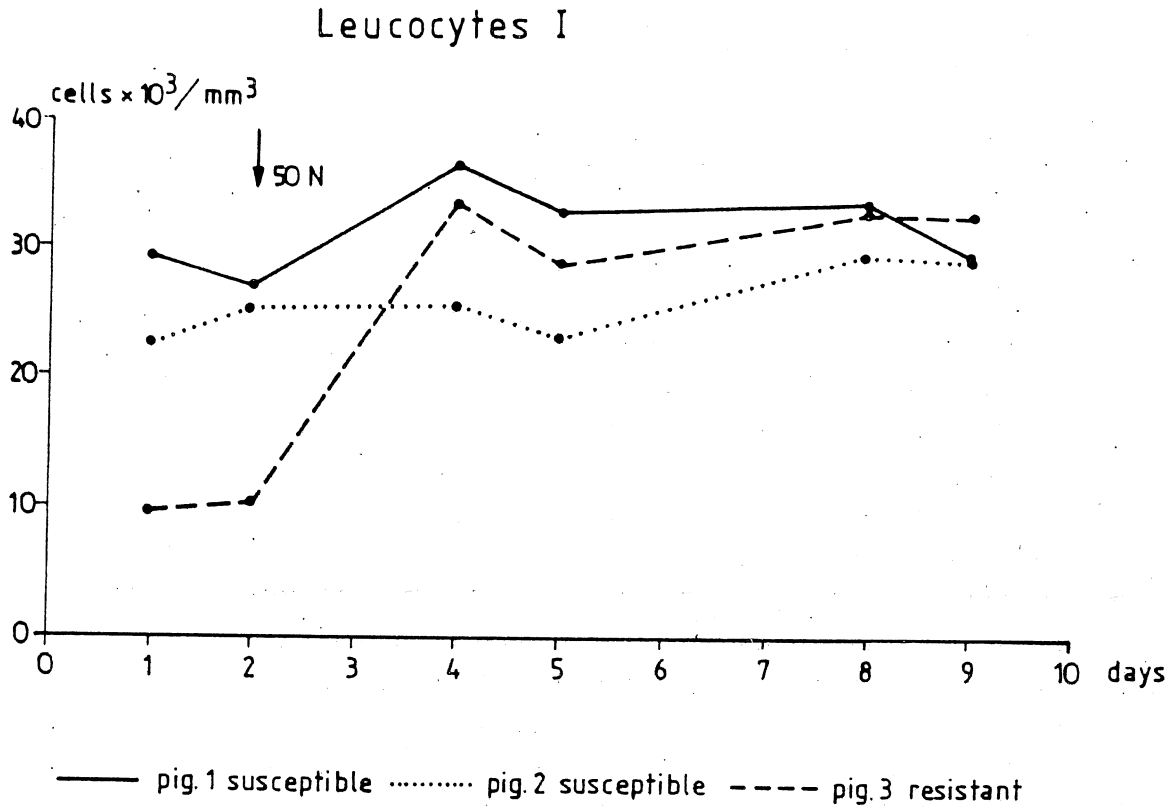

Leucocytes I
cells × 10³/ mm³
50 N
40
30
20
10
0
0 1 2 3 4 5 6 7 8 9 10 days
pig. 1 susceptible
pig. 2 susceptible
pig. 3 resistant

Fig. 3.

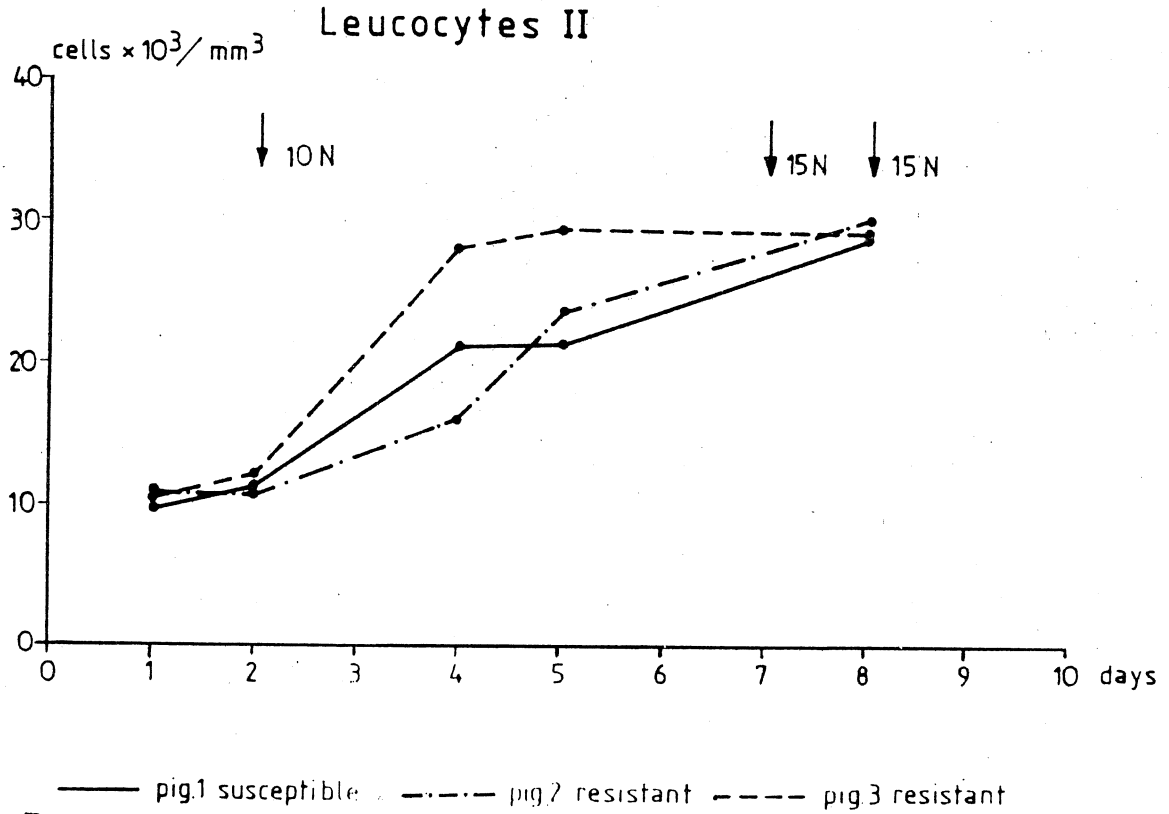

Leucocytes II
cells × 10³/ mm³
10 N
15 N
15 N
40
30
20
10
0
0 1 2 3 4 5 6 7 8 9 10 days
pig.1 susceptible
pig.2 resistant
pig.3 resistant

Fig. 4.

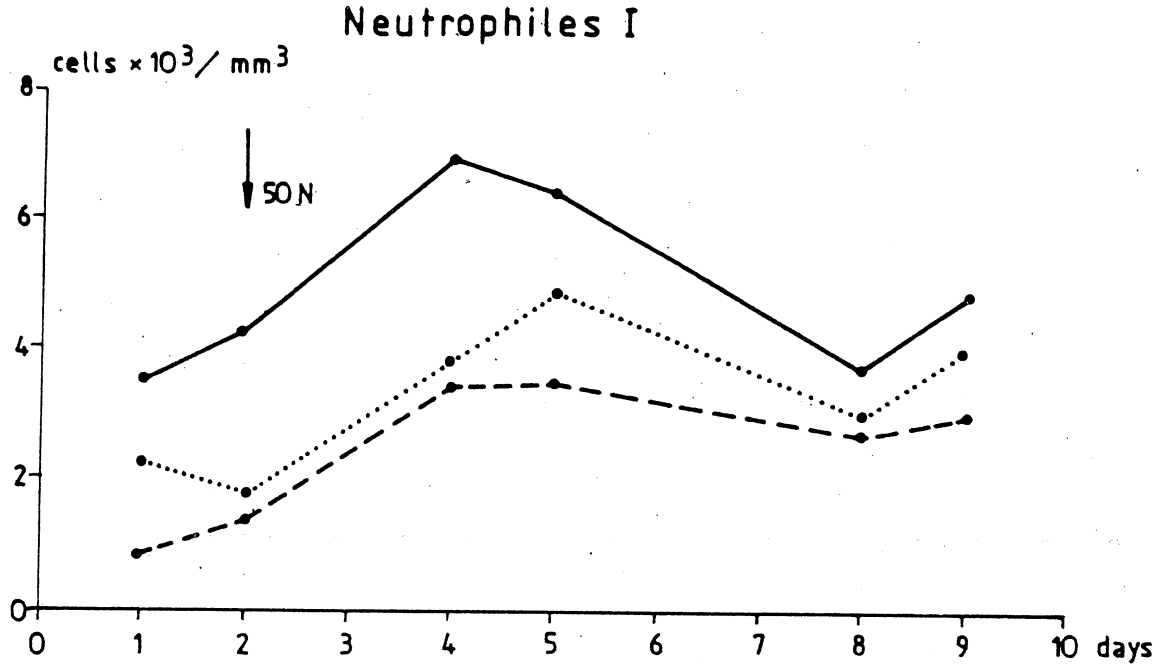

Neutrophiles I
cells × 10³/ mm³
50 N
0
2
4
6
8
0 1 2 3 4 5 6 7 8 9 10 days
pig.1 susceptible
pig.2 susceptible
pig.3 resistant

Fig. 5.

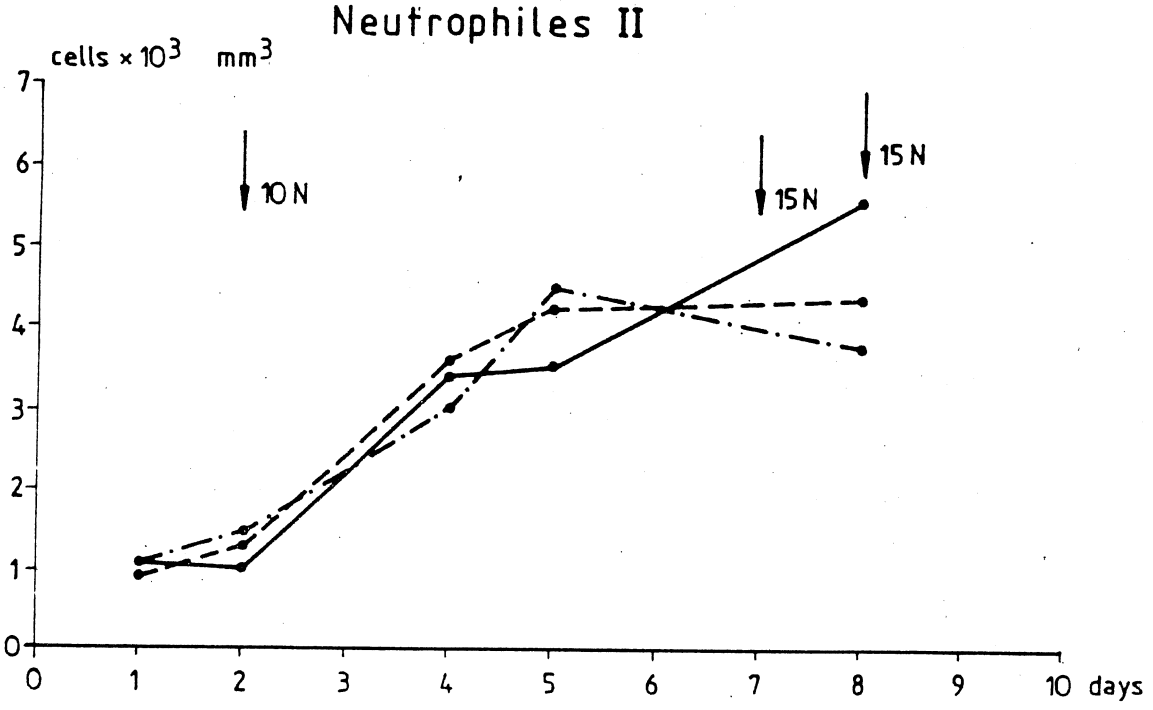

Neutrophiles II
cells × 10³ mm³
10 N
15 N
15 N
0
1
2
3
4
5
6
7
0 1 2 3 4 5 6 7 8 9 10 days
pig.1 susceptible
pig.2 resistant
pig.3 resistant

Fig. 6.

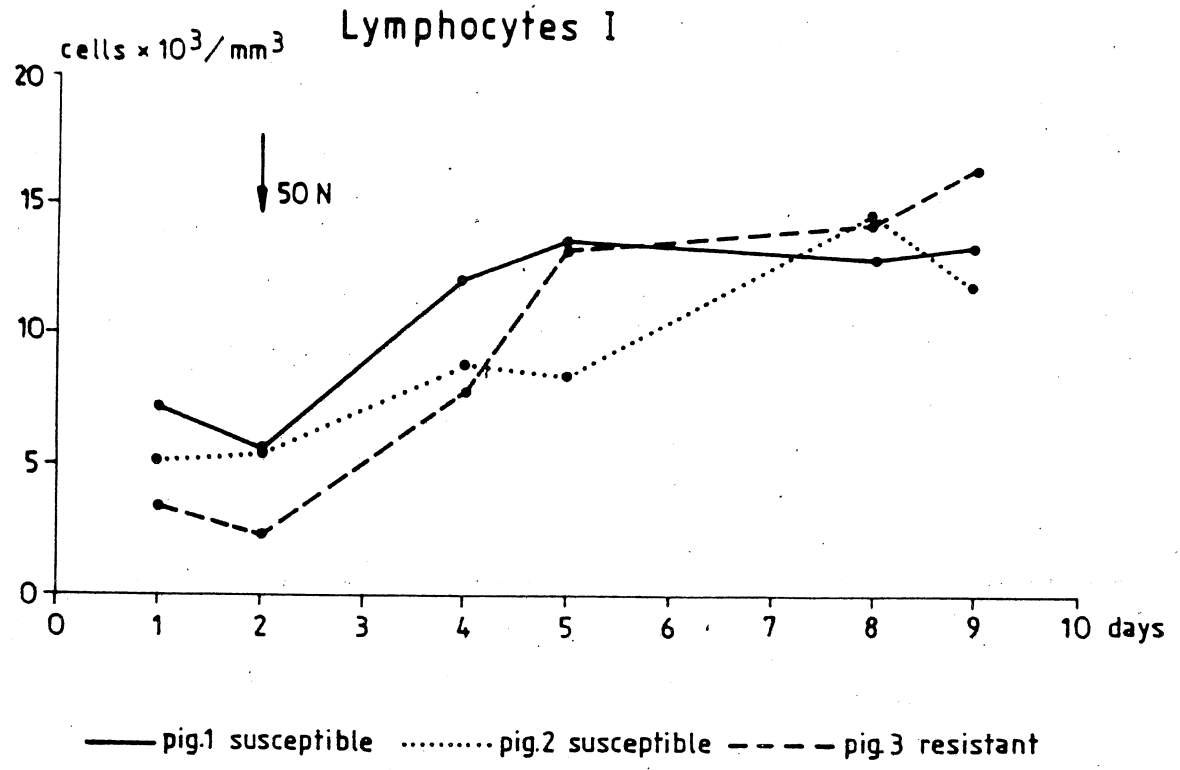

Lymphocytes I
cells × 10³/mm³
20
15
10
5
0
50 N
0 1 2 3 4 5 6 7 8 9 10 days
pig.1 susceptible
pig.2 susceptible
pig.3 resistant

Fig. 7.

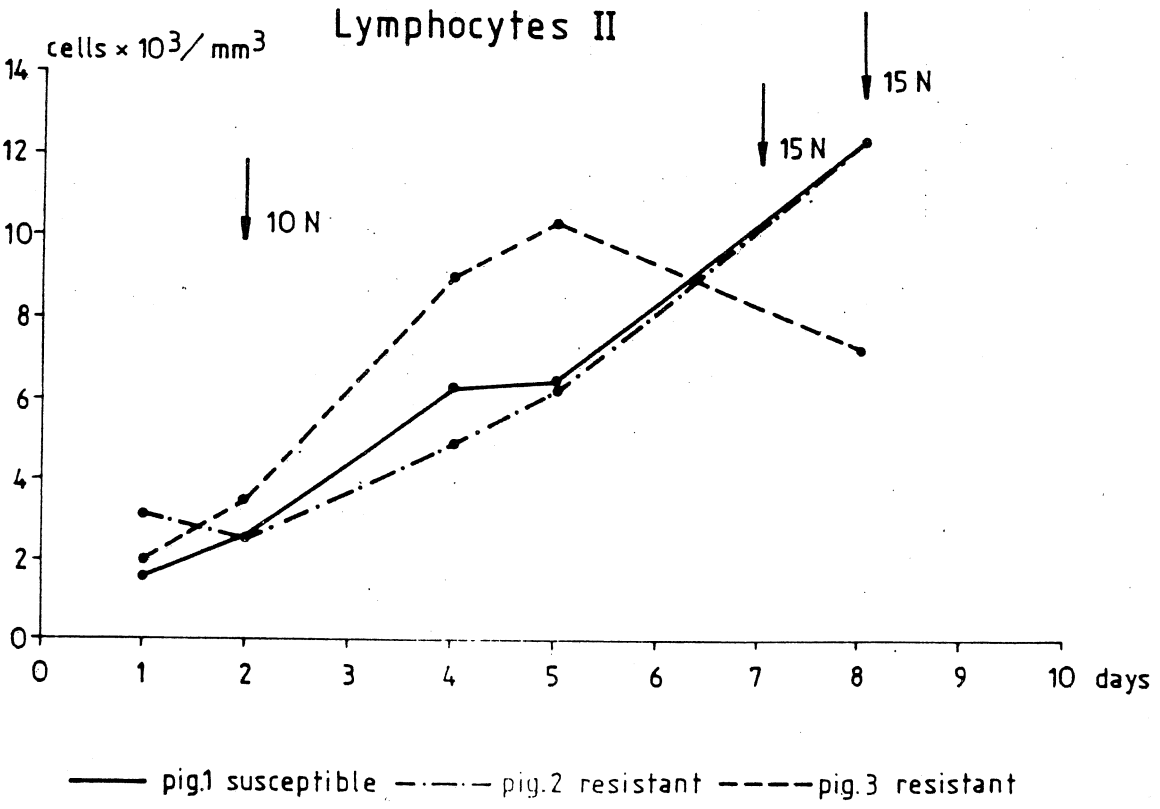

Lymphocytes II
cells × 10³/mm³
14
12
10
8
6
4
2
0
10 N
15 N
15 N
0 1 2 3 4 5 6 7 8 9 10 days
pig.1 susceptible
pig.2 resistant
pig.3 resistant

Fig. 8.

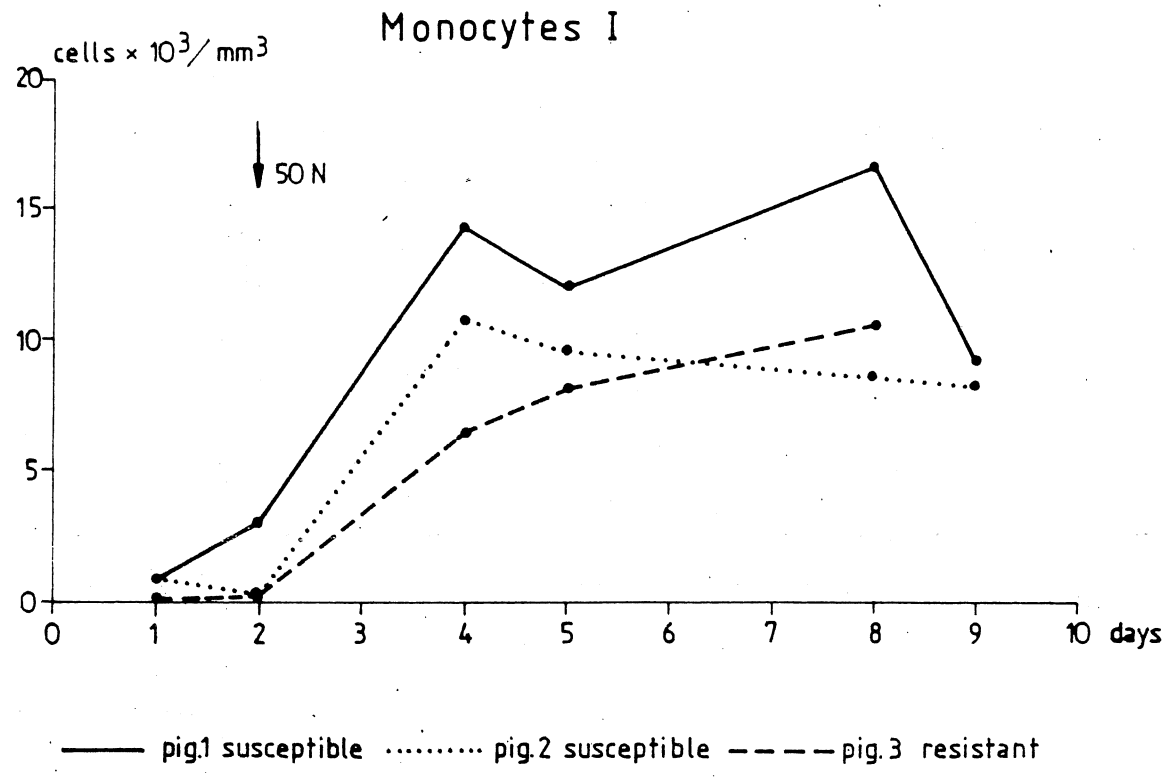

Monocytes I
cells × 10³/ mm³
50 N
20
15
10
5
0
0 1 2 3 4 5 6 7 8 9 10 days
pig.1 susceptible
pig.2 susceptible
pig.3 resistant

Fig. 9.

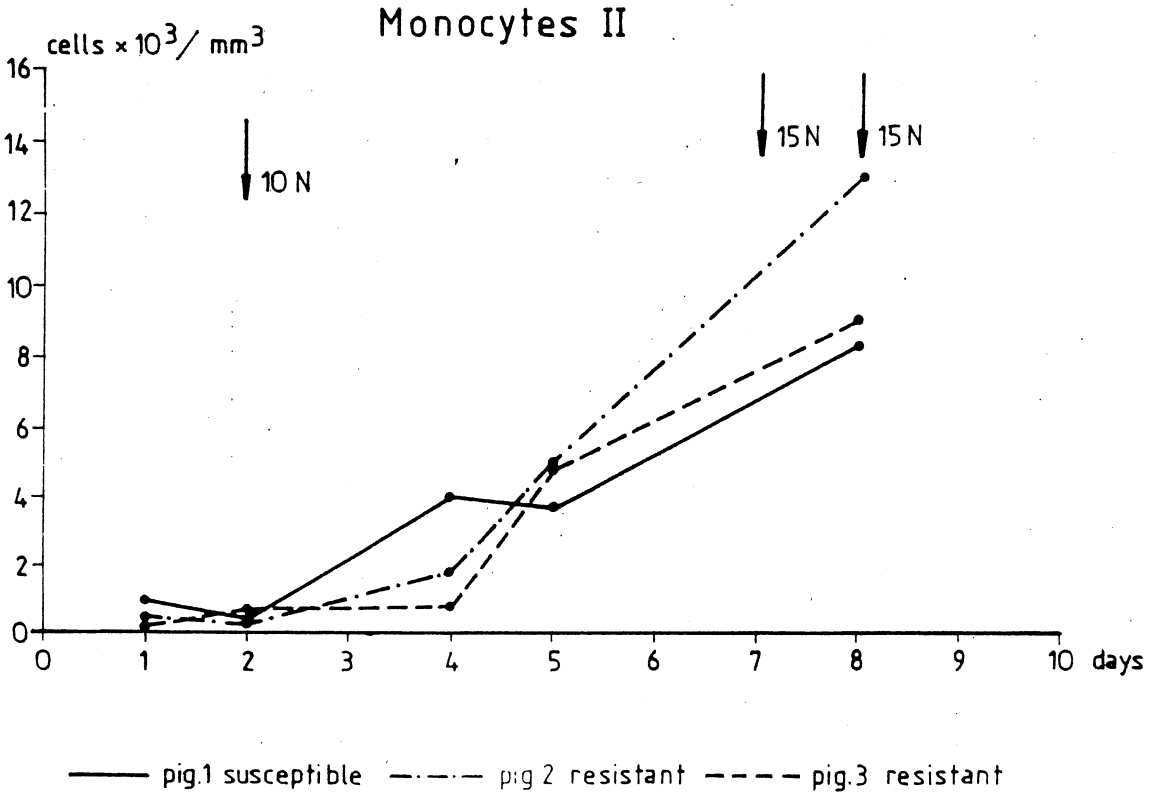

Monocytes II
cells × 10³/ mm³
10 N
15 N
15 N
16
14
12
10
8
6
4
2
0
0 1 2 3 4 5 6 7 8 9 10 days
pig.1 susceptible
pig 2 resistant
pig.3 resistant

Fig. 10

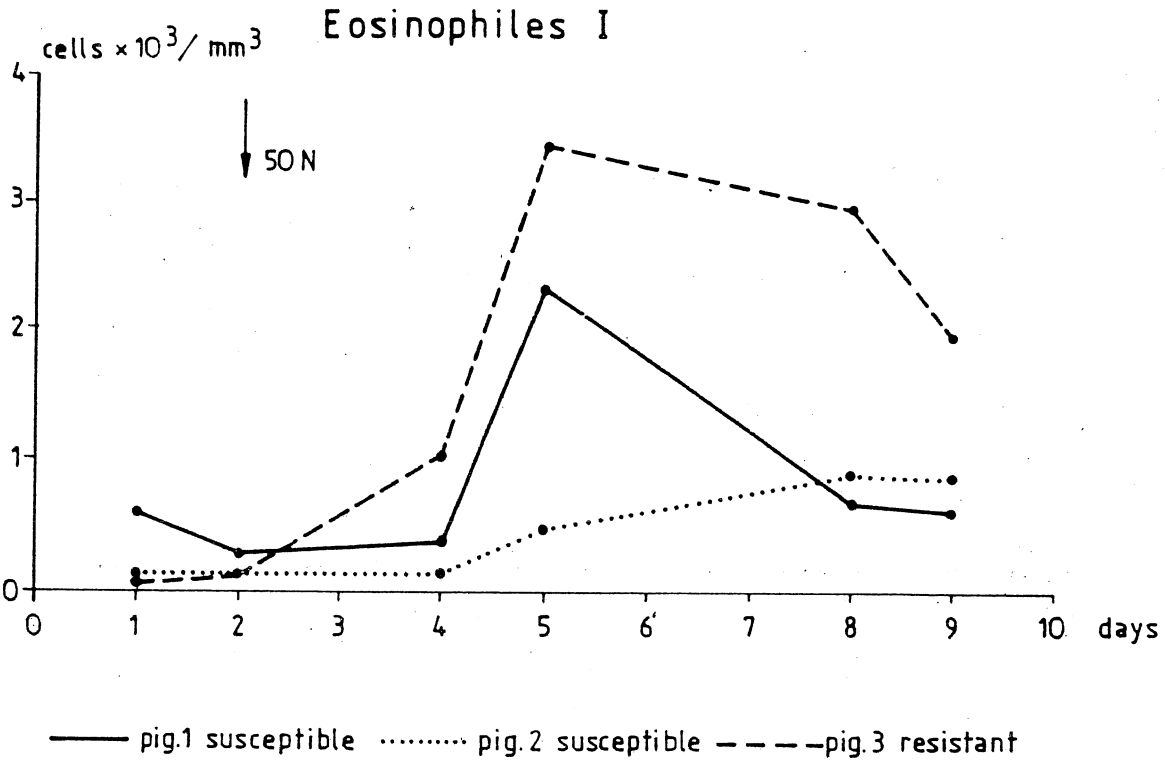

Eosinophiles I
cells × 10³/ mm³
50 N
0
1
2
3
4
0 1 2 3 4 5 6 7 8 9 10 days
pig.1 susceptible
pig. 2 susceptible
pig. 3 resistant

Fig. 11.

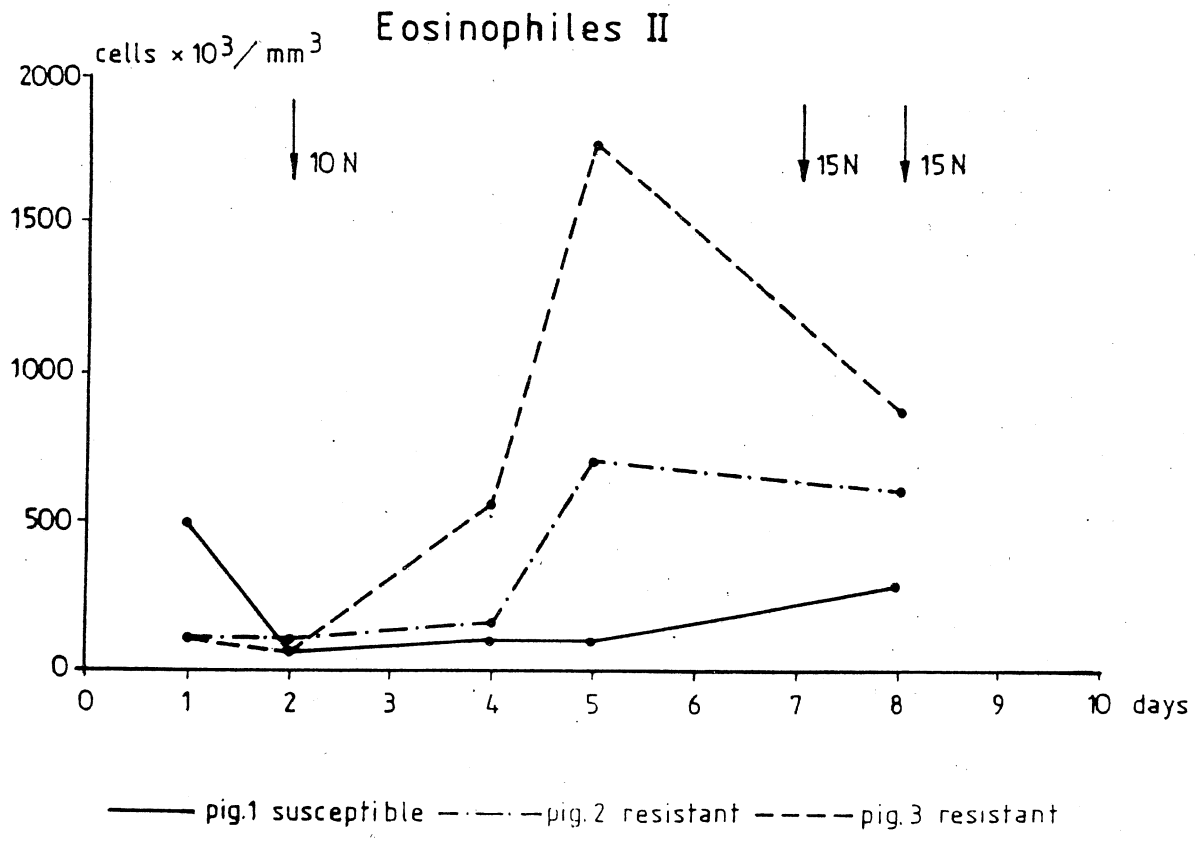

Eosinophiles II
cells × 10³/ mm³
10 N
15 N
15 N
0
500
1000
1500
2000
0 1 2 3 4 5 6 7 8 9 10 days
pig.1 susceptible
pig. 2 resistant
pig. 3 resistant

Fig. 12.

Fig. 13. The wall of stresses pig's stomach with larvae of *Anisakis simplex* B

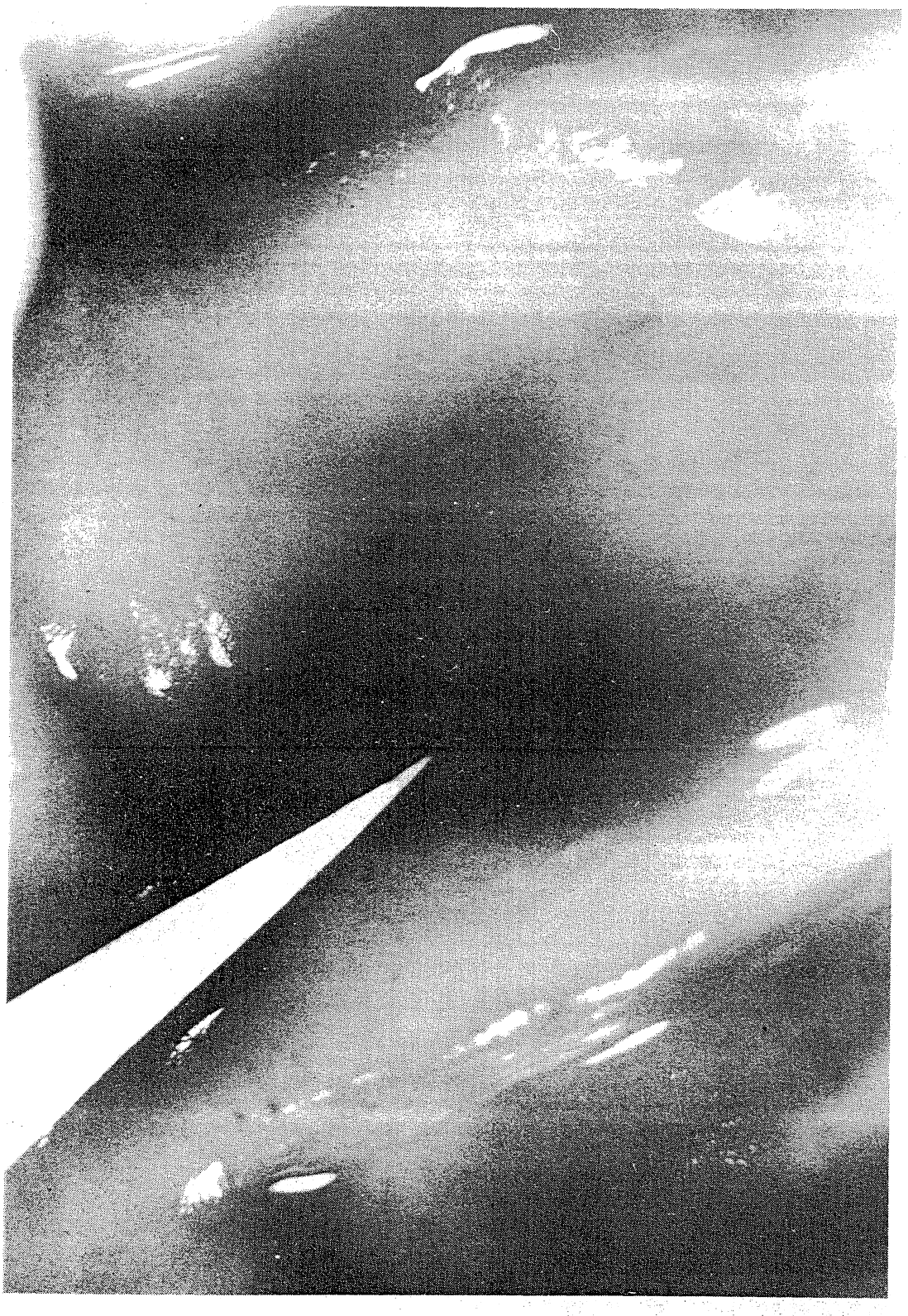


Fig. 14. The wall of stresses pig's stomach infected by larvae of *Anisakis simplex* B with haemorrhages

of the gullet part of the stomach. The rest of the worm was in the lumen of the stomach.

In sensitive pigs slight effusions appeared in the fundic, cardiae and pyloric areas of the stomach, especially around the nematodes present (Figs 13, 14). 21 parasites were found in one of the pigs in each of the parts mentioned. The colour of the cardial part was cream, that of pyloric area was light-pink and the stomach fundus was red.

## Discussion

Different species of mammals react in different ways to the *Anisakis* larvae infection. This results from the different anatomical construction and physiology of the random hosts, and what follows, the various behaviour of larvae. In small laboratory animals (guinea pigs, rats, rabbits) the nematode enters the body through the stomach wall. In large animals, including man, nematode penetration is restricted to the stomach mucosa and submucosa. ASHIZAWA et al. (1973a, 1973b) and USUI et al. (1973) observed three types of substantial changes in the stomach walls of pigs infected by *Anisakis* larvae (I). JACKSON et al. (1976) recorded their observations of the fundic area of the stomach in miniature pigs after the administering of *Anisakis* larvae.

Our experiments prove that after infection by *Anisakis* larvae the changes in stomach walls in stress-sensitive pigs were much greater than in stress-resistant ones. The changes were in the form of effusions in the submucosa and embraced the cardial and pyloric area as well as the fundus of the stomach. In resistant pigs the range of effusions was restricted to the fundus. In the dissection material of sensitive pigs 21 nematodes were found, while only one *Anisakis* larvae was present in one resistant pig. ASHIZAWA et al. (1973a, 1973b) and USUI et al. (1973) link the rate of mechanical changes in the stomach wall with the rate of nematode doses administered. On the other hand, BIER et al. (1976) maintain that the changes in the stomach walls in miniature pigs do not depend on the number of nematodes. They are usually much more numerous as compared with the number of parasites administered. This is connected with the fact that the nematode penetration is not restricted to only one part of the body. Our experiments make it clear that morphological changes occurring after nematode infection, apart from the dose rate, depend on the animals sensitivity to stress.

In the literature available to us little is said about the leucocyte image in animals infected by *Anisakis* larvae. It is mainly the changes in the number of eosinophilic granulocytes (eosinophenia), which is due to the non-specific reaction of the host to parasite invasion (RUITENBERG 1970). Our results support this popular thesis.

It is difficult, on the other hand, to answer the question as to why a substantial leucocytosis occurred in both stress-resistant and stress-sensitive

pigs, which was maintained until the end of the experiment, for six days, along with lymphocytosis and monocytosis. The authors presume that there might be other causes and mechanism for the occurrence of this phenomenon, despite the similar character of changes in the number of leucocytes. Thus in stress-sensitive pigs the anatomical-physiological features enable the larvae to penetration into the stomach wall. This leads to local inflammation reactions which in turn trigger a response in the immunological system, in the form of leucocytosis, mainly lymphocytosis and monocytosis. In stress-resistant pigs the changes observed in blood cell number may be of a „primary" character, i.e., the increase in blood leucocytes could form an obstacle preventing the nematodes form penetrating the stomach walls. The above findings are purely speculative and call for further research.

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## STRESZCZENIE

Badania hematologiczne i obserwacje morfologiczne przeprowadzono na 6 świniach. Trzy z nich należały do populacji wrażliwej na stres, a trzy inne do odpornej. Do zarażenia użyto larw trzeciego stadium *Anisakis simplex* forma B ze śledzi *Clupea harengus* z Południowego Bałtyku.

U badanych świń, a szczególnie odpornych na stres, stwierdzono przyrost liczby leukocytów we krwi. U wszystkich zwierząt wystąpiła limfocytoza jak i znaczny przyrost monocytów. Nie stwierdzono istotnych różnic w ilościach granulocytów obojętnochłonnych u obu badanych grup.

Niniejsze wyniki wskazują, że świnie zarażają się larwami *Anisakis simplex* B, co nie było dotychczas stwierdzone. Reakcje hematologiczne i morfologiczne mają nieco inny przebieg u wrażliwych i niewrażliwych na stres zwierząt.

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