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Ewa SOBECKA

Anisakidosis

EFFECTS OF *ANISAKIS SIMPLEX* LARVAE ON BLOOD PARAMETERS OF EXPERIMENTALLY INFECTED SYRIAN HAMSTERS, *MESOCRICETUS AURATUS* (WATERHOUSE)*

WPŁYW LARW *ANISAKIS* SIMPLEX NA OBRAZ KRWI ZAKAŻANYCH DOŚWIADCZALNIE CHOMIKÓW SYRYJSKICH *MESOCRICETUS AURATUS* (WATERHOUSE)

Institute of Ichthyology,
Academy of Agriculture,
Szczecin

The paper summarises a part of studies on *Anisakis simplex* (Rudolphi, 1809) larvae carried out in the Fish Diseases Department under the supervision of the late Dr Jadwiga Grabda.

The experiment involved twenty eight Syrian hamsters assigned to three groups and fed the larvae. Anatomo-pathological changes in the experimental animals were assessed and some blood parameters analysed.

INTRODUCTION

In the late sixties, possibilities of anisakiasis diagnosing other than by an operation were beginning to be considered. As the *Anisakis simplex* larvae do not mature in human

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bodies, faeces testing for the presence of the parasites' eggs is out of the question. Therefore the laboratory mammals' blood serum was tested (Ruitenberg, 1970; Gibson, 1970). An accurate and fast diagnosis procedure was urgently needed.

The main objective of the work presented was to evaluate a possibility of using hematological tests as an auxiliary diagnosing method for anisakiasis. The available literature contains only a few relevant data on changes in mammalian blood, the data, however, concerning the blood serum only.

MATERIALS AND METHODS

When assessing changes in the experimental animals blood, an attempt was made to eliminate all irrelevant effects so that changes in hematological parameters could be objectively ascribed to the factor under study (Barańska et al., 1862; Krzanowska et al., 1974).

The following parameters were assessed in blood samples taken:

1) erythrocyte count; the blood was mixed with the Türk solution and assayed in the Bürker chamber;
2) leukocyte count; the blood was mixed with the Türk solution and examined in the Thom-Zeiss chamber;
3) hematocrit (microhematocrit technique);
4) hemoglobin content (colorimetric cyanmethemoglobin technique);
5) quantitative and qualitative changes in erythrocyte and leukocyte patterns (May-Grünwald Giemsa-stained blood smears).

The tests were made on 3-months-old male Syrian hamsters. Two experimental variants were run between January 6 and May 26, 1981. The first variant involved two groups of animals, one (Group I) fed stage 3 *Anisakis* larvae collected from the Baltic herring and kept at +5°C in the physiological solution, the other (Group II) fed with the stage 4 larvae obtained in the same way and cultured in a pH 2 medium kept in a thermostat at 38°C. When fed, members of Group I obtained 5 larvae each, while the Group II hamsters got 2 larvae each. The second variant involved hamsters (Group III) fed the *Anisakis simplex* larvae obtained from spice-salted herring. The larvae were collected 1, 2, and 3 weeks after salting. Each animal was fed 3 larvae. A total of 28 hamsters were used: 16 formed the experimental and 12 control groups. A pair of tweezers was used to feed the hamsters. On the termination of the experiment, both the experimental and control animals were first ether narcotized and then the blood was collected from the heart chamber and the dissection of the animal was made. The hamsters' responses to the larvae fed was observed as well.

RESULTS

In each group the tests were made at different times following feeding the larvae. The Group I animals infected with the stage 3 larvae kept in the physiological solution were first assayed. The hamsters were dissected 1h, 11h, 16 h, 1 wk, 2 wk, and 3 wk after feeding. In the first hamster (1) a larva was found that had pierced the stomach wall and moved out of it. The remaining larvae were not found. The blood showed an increased number of leukocytes and the presence of younger developmental stages of these blood cells. The second animal (2), showing clear symptoms of poor health, contained 2 whole live larvae and two parts of another larva in its stomach. This animal also showed a slight increase in the leukocyte count and slightly decreased hematocrit. Additionally, an increase in the eosinophil count was recorded. The next hamster (3) was found to contain a larva in its small intestine and 2 larvae in its large intestine, one larva of the latter two being broken in two parts. The blood showed changes similar to those in the preceding animal. The fourth hamster (4) dissected a week after feeding with the larvae possessed a live larva in the lower part of the body cavity, close to the testes. The larva was placed in the thermostat at 37°C and cultured in a bloodenriched medium. The larva survived for 67 days, metamorphosing regularly. The animal's blood showed no marked qualitative changes, the erythrocyte count and hematocrit being decreased somewhat. Some monocytes showed vacuolised cytoplasm. The next hamster (5), examined 2 wk after feeding, did not suffer any change in its viscera. No quantitative changes in its blood cell pattern were discernible either, except for a slight increase in the leukocyte count. Immature neutrophils and monocytes with vacuolised cytoplasm were observed. No larvae were found.

The second group contained animals fed the stage 4 *Anisakis* larvae. The first hamster of the group (7) was dissected 1 wk after feeding. No inflammational lesions in the intestine were found, only the corner of the stomach was strongly swollen and tight. No larvae were found. The blood cell patterns showed a slight increase in the eosinophils and immature neutrophils. The hamsters dissected 2 wk (8) and 3 wk (9) after feeding did not contain any larvae either. No changes in the internal organs and blood were observed, the third hamster only showing slightly increased leukocyte and eosinophil counts (Table 1).

The next stage of the experiment involved feeding the hamsters (Group III) with live *Anisakis* larvae collected from spice-salted Baltic herring. As in the previously described variant, the animals were fed 3 larvae each following a 24-h starving period. The larvae were collected 1 wk after salting the fish. The first animal (10) was dissected 24 h after feeding, the animal being drowsy, its fur bristled up, clear symptoms of pain being evident. The autopsy revealed strongly swollen stomach and duodenum, no larvae being found, however. The blood showed a marked increase in the leukocyte count and eosinophilia (Table 1). The blood smear showed the leukocyte pattern to be shifted to the left, i.e., younger forms in the granulopoiesis system were present. Myeloblasts and myelocytes appeared; cells with large, circular or oval nuclei, slightly granulated chromatin and poorly, if at all, visible cytoplasm were seen. It is difficult to identify these

Table 1

Blood hematologic indices for *Anisakis simplex* – infected and control syrian hamsters

Animal No.	Erythrocyte count ($x10^6/mm^3$)	Hemato-crit (%)	Hemoglobin (g%)	Leukocyte count ($x10^3/mm^3$)	Granulocytes (%)					Agranulocytes (%)	
					Myelo-blastes	Myelo-cytes	Rod Neutro-philes	Filamented Neutro-philes	Eosino-philes	Monocytes	Lymphocy-tes
Group I											
1	6.42	46	17.04	17.80			2	24	1	21	52
2	5.80	43	15.72	11.80				31	2	16	51
3	6.38	46	16.84	13.42			1	19	2	13	65
4	5.28	41	16.53	6.40				27		23	50
5	6.81	49	16.78	14.00			3	29	1	17	50
6	6.78	49	16.36	16.20				46	1	10	43
Group II											
7	5.69	42	16.22	6.35			3	52	2	9	29
8	6.42	47	15.92	5.10				23	1	13	63
9	6.52	52	16.72	11.30				39	3	16	42
Group III											
10	6.72	54	17.04	21.40	1	3	6	20	8	21	41
11	6.84	54	16.36	8.60		2		31	5	13	49
12	6.14	45	15.24	4.20				42	2	18	38
13	5.92	43	13.94	7.40				40	1	18	41
14	5.84	43	14.68	12.80			1	29	3	21	46
15	5.95	42	13.94	8.20	2	2	2	27	7	17	43
16	6.34	48	15.46	10.20			3	33	4	16	44
Control											
1	5.98	42	13.94	9.20			1	36	1	21	41
2	6.56	49	15.72	6.56				32		26	42
3	5.90	42	14.48	5.80				39		19	42
4	6.72	50	15.84	7.20				27	2	16	55
5	6.24	49	16.28	6.40				27		21	52
6	6.28	49	15.92	6.00			1	32		21	46
7	6.40	48	16.28	6.42				24	1	15	60
8	6.15	47	15.24	9.80				41		14	45
9	6.42	50	15.84	5.56				29	1	21	49
10	6.67	49	15.98	6.20				31	1	16	52
11	5.92	45	15.76	7.62				29		24	47
12	6.15	49	16.36	5.40				29	1	26	44

cells as they cannot be with certainty assigned to any system. Monocytes with many vacuoles in the cytoplasm were observed as well. Anisocytosis characteristic of the erythrocyte pattern.

The next hamster (11) was examined 48 h after feeding. No behavioural changes were recorded and no larvae found. No larvae were found in the animal (12) dissected 72 h following the feeding with larvae. Both hamsters showed no quantitative changes in their erythrocyte and leukocyte patterns; however, the blood smear of the latter animal revealed an increased eosinophil count and the presence of juvenile neutrophils. The cytoplasm-less cells with large nuclei were seen as well, similarly to the animal No. 10 blood smear. Anisocytosis was again characteristic of the erythrocyte pattern.

At the last stage of the experiment, the hamsters fed 3 larvae each were examined, the larvae being obtained from spice-salted herring 2 wk after salting. The first (13) and second (14) animals were autopsied 3 d and 5 d after feeding, respectively. Both hamsters suffered no changes in their internal organs, the second one only showing a slight increase in the leukocyte count and eosinophilia. No larvae were found. A live larva was found in the hamster (15) fed the *Anisakis simplex* larvae obtained from spice-salted herring 3 wk after salting, the animal being dissected 5 h after feeding. Another (damaged) larva was found in the small intestine near the duodenum. The live larva survived 4 days in the medium. No changes in the dissected hamster's internal organs were found. The only characteristics recorded were eosinophilia, the presence of juvenile neutrophils, and anisocytosis in the erythrocyte pattern. Another hamster (16) was dissected 3 d after feeding and no changes except eosinophilia were observed.

DISCUSSION

The tests showed the *Anisakis simplex* larvae introduced into the hamster digestive tract to produce clinical symptoms and anatomopathological changes. The symptoms were observed in some of th he hamsters fed the stage 3 larvae regardless of the origin of the larvae, be it the physiological solution cultures (Group I) or sspice-salted herring (Group III). Some animals showed clear symptoms of ill-being (drowsiness, fur bristled up, signals of pain) and – in one case – a pathologically changed digestive tract. The Group I autopsies revealed a high degredd of larval survival; 4 of the 6 examined animals contained 1 to 3 larvae located in the stomach, intestine, and body cavity. One of those larvae found in the body cavity was very viable as evidenced by its 67-d survival in the medium during which time it molted twice and reached sexual maturity. The larval survival in Group III was different: out of the 6 hamsters autopsied, only 1 was found to contain 2 larvae; one of them survived 4 d in the medium.

Somewhat different results were obtained in the experiment involving the stage 4 larvae offered to 3 hamsters. No larvae were found in the animals; the stomach of one hamster dissected 1 wk after the infection was strongly swollen, which may have been the result either of larval toxins or of a mechanical damage inflicted by the larvae. The

absence of the larval nematodes both in the remaining 2 animals of the group and in other hamsters examined a long time after feeding can be accounted for by digestive processes. A possibility of the larvae being destroyed by the hamster mouth radula cannot be excluded.

The larval *Anisakis simplex* survival and effects of the nematodes on laboratory mammals were studied by numerous authors. Among them Myers (1963) found the larvae in the internal organs of Guinea pigs infected, the larvae being encysted under the skin. Actively migrating larvae produced a mechanical damage in tissues and a local reaction. Kikuchi et al. (1970) studied the behaviour of stage 3 larvae offered to dogs and rabbits. When the nematodes reached the stomach they pierced its walls and migrated into the intestine, damaging its walls or remaining within its lumen. Gibson (1970) studied the larval survival in rats and recorded a high viability: as early as after 4 h the larvae penetrated into the body cavity having pierced the stomach wall. Many of them (10–20%) were being found there 21 d later. Margolis and Beverley-Burton (1977) observed a similar response of mink to the offered larvae.

When comparing the present results with the literature data a similar survival, mainly in Group I (the larvae kept in the physiological solution) is evident. Also the hamsters responded in a way similar to that displayed by other experimental animals.

The hematological data showed some parameters to deviate from the control. In the Group I hamsters fed the stage 3 larvae kept in the physiological solution the changes involved an increase in the leukocyte count. This condition points out to the defense mechanisms of the body being mobilised. Additionally, the presence of rod neutrophils and a slight increase in the eosinophil count were recorded. Eosinophilia is a reaction typical of invermination.

The Group III hamsters fed the stage 3 larvae obtained from spicesalted herring showed similar deviations of their blood parameters. The leukocyte counts increased in 2 animals, in one of them the increase being two-fold relative to the control. Eosinophilia was much more marked than in Group I; also a rejuvenation of the leukocyte system was evident and myeloblasts and myelocytes appeared.

The blood changed to the lowest degree in the Group II hamsters fed the stage 4 larvae. A slight leukocyte count increase was recorded in one case only, and a slight increase in the number of eosinophils occurred in two hamsters.

The qualitative changes in the Group I and III blood parameters were usually accompanied by pathologically transformed cells. Monocytes with vacuolar cytoplasm degeneration and cells with large nuclei and almost invisible cytoplasm occurred. These forms were difficult to identify. In some cases anisocytosis was typical of the erythrocyte system.

The available literature contains no data on hematological parameters of *Anisakis simplex* larvae-infected laboratory animals. The qualitative changes observed are similar to those described as accompanying other types of invermination. Dorosz (1981) reported increased leukocyte and eosinophil counts in Guinea pigs, white mice, and rabbits affected by natural helminthiases. Also Blaski and Szliman (1981) reported similar effects

in the blood of experimental rats infected with larval nematodes Nippostrongylus brasiliensis.

CONCLUSIONS

1. The laboratory animals differ in their responses to larvae ofered. The stage 3 larvae are most active, the reaction being most rapid, while the stage 4 larvae are less active and are likely to become digested much faster. The stage 3 larvae obtained from spice-salted herring are less viable than those kept in the physiological solution, their effects on animals being, however, evident.
2. Increased leukocyte and eosinophil counts are observed in the blood of those hamsters infected with the stage 3 larvae.
3. Most hamsters infected with the stage 3 larvae yielded blood smears showing a rejuvenated leukocyte system and pathologically changed cells. Additionally, some smears show anisocytosis.
4. Owing to the non-specific reaction to the presence of the *Anisakis simplex* larvae, basic hematological assays are of little value in diagnosing anisakiasis. On the other hand, the nature of pathological changes in the leukocyte system elements deserves attention.
5. Syrian hamsters are difficult to feed orally with the larvae due to a likelihood of the larvae being mashed in the mouth and narrow esophagus.

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Translated: Dr. Teresa Radziejewska

Ewa Sobecka

WPŁYW LARW *ANISAKIS SIMPLEX* NA OBRAZ KRWI ZAKAŻANYCH DOŚWIADCZALNIE CHOMIKÓW SYRYJSKICH

STRESZCZENIE

Przebadano 28 chomików syryjskich, którym podano larwy III i IV stadium *Anisakis simplex*. Zwierzęta podzielono na trzy grupy, z których jedna stanowiła chomiki skarmiane larwami III stadium uzyskanymi ze śledzi bałtyckich i przechowywanymi w płynie fizjologicznym. Drugą grupą były zwierzęta skarmiane larwami III stadium ich zasolenia. Trzecią grupę zwierząt skarmiano larwami IV stadium hodowanymi w pożywce z dodatkiem krwi. Badania chomików dotyczyły ich reakcji na podane larwy, zmian patologicznych w ich narządach wewnętrznych oraz zmian niektórych parametrów krwi i jej obrazu. Stwierdzono, że w organizmie zwierząt doświadczalnych larwy III stadium przechowywano w płynie fizjologicznym są najaktywniejsze, a we krwi chomików zauważono zwiększoną liczbę leukocytów i eozynofili. Obraz krwi wykazywał odmłodzenie układu białokrwinkowego oraz występowanie form patologicznie zmienionych.

Ewa SOBECKA

ВЛИЯНИЕ ЛИЧИНОК ANISAKIS SIMPLEX НА КАРТИНУ КРОВИ ИСКУССТВЕННО ИНФИЦИРОВАННЫХ СИРИЙСКИХ ХОМЯКОВ

Р Е З Ю М Е

Исследовали 28 сирийских хомяков, кормленных личинками III и IV стадий Anisakis simplex. Животных разделено на 3 группы. Первую группу кормили личинками III стадии, полученными из балтийской сельди. Личинки хранились в физиологическом растворе. Вторую группу животных кормили личинками III стадии, полученными из сельди пряного соления, различавшимися периодом времени с момента их соления. Животных третьей группы кормили личинками IV стадии, разведение которых производили в питательной среде с добавкой крови. Исследовали отзывчивость хомяков на кормление личинками, патологические изменения внутренних органов, а также изменения некоторых параметров крови и её картины. Установлено, что самыми активными в организме опытных животных являлись личинки III стадии, хранившиеся в физио-

логическом растворе. В крови хомяков наблюдалось увеличенное количество лейкоцитов и эозинофили. Картина крови отличалась омоложением системы белых кровяных телец, а также присутствием патологически изменённых форм.

Author's address:
Mgr Ewa Sobecka
Instytut Ichtiologii
ul. Kazimierza Królewicza 4
71–550 Szczecin, Polska (Poland)

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