CONTRIBUTION TO THE SYMPTOMATOLOGY OF EXPERIMENTAL BOVINE CYSTICERCOSIS

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Abstract. Results of clinical and laboratory examinations of animals experimentally infected with Taenia saginata eggs are described. At the early stage of infection, increased temperature, cough, muscle shaking and unstable pace were observed. The locomotive disorders disappeared only on day 60 p.i. Leukocytosis and peripheric eosinophilia were found at the early stage of infection. On days 14–28 p.i. the activity of serum creatinokinase (CK) significantly increased. The activity of other enzymes (AST, ALT, LD, ALP and ALD) examined was increased only slightly and irregularly. The lipid content in blood serum markedly increased on days 9–16 p.i.

During the complex studies on experimental bovine cysticercosis caused by C. bovis, also clinical symptoms of the infection were observed and some haematological and biochemical examinations were performed in order to gain a deeper knowledge of the pathological process induced by this parasitic zoonosis. The results obtained are reported in this paper.

MATERIAL AND METHODS

A total of 19 male calves at the age of 4 months and weighing 100–120 kg were used in the experiment. They originated from the breed of Czech dappled race in which cysticercosis did not occur. The calves were serologically negative (haemagglutination, precipitation) and were kept in an isolated stable. Six calves were infected with 200 000 eggs, 3 calves with 85 000 eggs of T. saginata, and one calf was used as a control. The infective material was fed to the animals in gelatine capsules by means of a pill feeder.

The calves were killed in the following order: two on day 21 p.i., one on day 23 p.i., one on day 42 p.i., one on day 81 p.i., two on day 112 p.i., one on day 168 p.i., and one on day 261 p.i. The intensity of infection was evaluated in all animals on the basis of the number of cysticeri found in the sections through muscles in different muscle groups and on the basis of the number of cysticeri found in a certain volume of muscles.

Blood was taken twice from vena jugularis at 7-day interval before infection. After infection, it was taken regularly, most often again at 7-day intervals, or at the intervals of 8–9 days. In case of longer observation, it was taken at the interval of 14 days.

Haematological examinations were performed by common methods. The following methods were used for biochemical examinations of blood serum: the activity of aspartate-aminotransferase (AST) and alanine-aminotransferase (ALT) was detected using the method after Rollman and Frankel (1957) with Szőve tests and serum dilutions in high activities (Mádrzová and Neumann 1960), lactate-dehydrogenase (LD) activity was detected after Šovels and Továrek (Homolka 1960), aldolase (ALD) activity after Sibley and Lehninger (Foírer et al. 1963), alkaline phosphatase (ALP) activity after King (Homolka 1960), and creatin-kinase activity by Bio-Loehne test.

The lipids were demonstrated after Homolka (1969). The levels of mineral substances in blood serum were demonstrated as follows: P by the colorimetric method of Schefer and Paierkamp (Anke 1964), Ca by a complexometric titration with trichrom black after Holásek and Flashka (Büchner and Gabš 1961), and Mg was demonstrated colorimetrically with titan yellow using polyvinylalcohol as a stabilizer (Homolka 1969).
RESULTS

Clinical symptoms

The clinical symptoms were observed in the infected animals only after a certain time. On day 15, and exceptionally till day 20 p.i., the body temperature fluctuated between 39.8°C and 41°C for 7—9 days, reaching the maximum on days 20—23 p.i. At the same time, apathy, tachycardia (P = 92), polynea (D = 34), inappetence, and even cough (on day 14 p.i.) occurred. The locomotive disorders (unstable pace), shaking and muscle pain were observed on days 15—21 p.i. Maximum symptoms in the muscle system occurred on days 30—35 p.i., and gradually disappeared only slowly, till days 50—60 p.i. Then the appetite and general health condition improved. These facts indicate that marked clinical symptoms appear at the early phase of C. bovis infection.

Fig. 1. Number of leukocytes during C. bovis infection. o massive infection, • control, v infection of medium intensity.

Fig. 2. Relative representation of eosinophiles in the blood during C. bovis infection. o massive infection, • control, v infection of medium intensity.

Fig. 3. CK values during experimental infection with C. bovis. — mean CK values in experimental animals, —— CK value in control animals.

Fig. 4. CK values in experimental infection with C. bovis. o massive infection, • control, v infection of medium intensity.

particularly if a massive infection is involved. These symptoms are induced by the development of the pathological process in the host tissue. At the time when young T. saginata larvae have just located in the muscles and parenchymatous organs, they cause there necrosis of the surrounding tissue and excessive cellular reaction. In massive infections, these foci are numerous and in the complex they represent a serious source of autointoxication. The intensity of clinical symptoms is related with the intensity of infection. The clinical symptoms appear particularly in massive infection, whereas a light infection is mostly inapparent.

Haematology

Some haematological and biochemical parameters were detected in 9 experimental calves (6 infected with 200,000 eggs and 3 infected with 85,000 eggs).
The number of erythrocytes and hemoglobin content remained unchanged in the course of the experiment. An increased number of leukocytes was observed in 4 of the 9 experimental calves (Fig. 1). In one of them, the number of leukocytes significantly increased already on day 9 p.i. reaching the maximum on days 16—30 p.i. In the calves observed for a long time, the number of leukocytes was later different; in the calf infected with 85,000 eggs, it was normal for several months p.i. (2-1/2—4 months), whereas in that infected with 200,000 eggs, the number of leukocytes was constantly increased at that time and another increase occurred 6 and 8 months after infection. A marked peripheral eosinophilia (Fig. 2) was found for the first time on day 14 p.i. and it was high still on day 35 p.i. On days 63—112 the number of eosinophils was still normal, but in case of massive infection (with 200,000 eggs), the proportion of eosinophils in the leukogram again increased 6 and 8 months after infection. After the reinfection made at the time of the second peak of blood leukocytosis and eosinophilia, the number of leukocytes suddenly slightly dropped and even the number of eosinophilic granulocytes decreased. These findings are in good agreement with the character of cellular reaction in the infected organ (Plate I, Figs. 1—4). The tissue eosinophilia is very conspicuous at the early phase of infection. It is not only the accumulation of eosinophils around the larvae, but also their high proportion in the inflammatory infiltrate in the wider vicinity. The peripheral eosinophilia is related with the intensity of infection.

Biochemistry of blood

The activity of serum creatine kinase (CK) was significantly elevated on days 14—28 p.i. with average maximum values (group average) on day 21. On day 35 p.i., the activity of this enzyme decreased below normal level and remained at this level during the further course of the experiment (Figs. 3, 4). In four of the nine calves infected at another period, the CK activity increased already on days 9—14 p.i. (average group maximum on day 9) and after a temporary decrease to almost normal level (on days 16—23 p.i.) it again increased on day 36 p.i. In the calves in which the first increase in CK activity was low, a much more marked elevation of CK values occurred on day 36 p.i. After this time the CK activity remained constant (Fig. 5).

The activity of aspartate-amino transferase (AST) increased only in three of the nine calves on days 19, 27 and 36 p.i., respectively.

The activity of alanine-amino transferase (ALT) increased in a group of experimental animals on days 8—14 p.i. and a new slight increase occurred on day 27 p.i. (Fig. 6). The activity of lactate-dehydrogenase (LD) in a group of animals rapidly increased on day 9 p.i. and maximum level was reached on day 10 p.i. Then it slowly decreased.

**Fig. 7.** Activity of serum LD during C. bovis infection. ○ massive infection, ▼ control, ▲ infection of medium intensity.

**Fig. 8.** Activity of serum ALP during C. bovis infection. Group average values.
of infection and are caused by the inflammation of lungs due to the localization of cysts cerei. These symptoms are described by Chroustová et al. (1981) and McIntosh and Miller (1960). Of importance and pathogenicity which is the inflammation of lungs due to the effect of the proper pathological process so that the state of nutrition becomes worse and sometimes even at the resting stage of the disease the weight increase may be lower than in healthy animals (Buchwalder et al. 1978). On the other hand, the weight increase is sometimes normal even in massive infections (McIntosh and Miller 1960). Also the clinical symptoms are apparent mostly in massive infection, but not always (Kozakiewicz 1977).

Our finding that the number of erythrocytes and haemoglobin content remain unchanged during the infection is in agreement with the results obtained by Kozakiewicz et al. (1977), who used the infection dose of 100,000 eggs, and Lloyd et al. (1980), who infected the animals with 32,000 eggs of T. saginata. The decrease in haemoglobin content at an unchallenged number of erythrocytes was recorded occasionally by Dewhirst et al. (1960). Alferova (1968 ex Gallie and Sewell 1976), on the contrary, observed an increased number of erythrocytes and haemoglobin content at simultaneous leukocytosis in infected one-month-old calves. However, the same infective dose did not cause any changes in the number of erythrocytes or in haemoglobin concentration in one-year-old calves. As it is evident from these results, the data on the number of erythrocytes are inconsistent, whereas most of the authors are in accord in that the number of leukocytes increases during C. bovis infection (Lloyd 1980, Gallie and Sewell 1976). It is probable that the changes (their early onset, dynamics etc.) in the white blood picture depend on many factors (individual reactivity of the organism, intensity of infection, and ratio of dead cysticerci), as it is indicated by the persistence of leukocytosis even at the late stage of infection in animals harboring a large number of dead cysticerci at the slaughter.

Also the age of the animal, its weight or health condition may play an important role. It would be interesting to ascertain which of the leukocytes participate in the detected leukocytosis. It was reported that lymphocytes and also eosinophils may be involved (Lloyd 1980), or the increased number of eosinophilic granulocytes in the peripheral blood is stressed (Gallie and Sewell 1976). In some cases, however, the eosinophils are not detected at all. In our experiments, lymphocytosis could not be confirmed, but a marked peripheral eosinophilia was detected. The peripheral eosinophilia appeared rather early, as it was reported by Gallie and Sewell (1976), even one week after peroral infection. Its origin is a sign of the active contact of the live migrating parasite with the host organism, since it does not occur in case that the oncospheres are injected directly into the muscle (Gallie and Sewell 1976). The origin of eosinophilia in our calves is related to the intensity of infection. It was found at the infection dose of 50,000 eggs, but no eosinophilia occurred after the infection with 5,000 eggs (Souilé et al. 1971). A light infection, however, is sufficient for the sensitization of the animal and for secondary eosinophilia after the reinfection.

Secondary eosinophilia is stronger and more prolonged than the eosinophilia in reinfection of heavily infected animals. The increase in eosinophils from low values kept for a long time, which is observed at the late phase of infection, indicates the disintegration of the dead cysticerci. The disintegration of larval bodies releases a larger amount of antigenically effective substances and represents a stimulus for the origin of eosinophils and tissue lymphocytosis (Blážek et al. 1981).

The results of our experiments indicate that the lymphocytes, or eosinophils, play an important role in the defence reaction of the host organism in C. bovis infection. Our studies on the ultrastructure of the tissue reaction (Blážek and Schramolová 1980) and detection of migratory inhibition factor (Blážek et al. 1980) confirm the findings by Lloyd (1980), who studied by another method the blood picture and immune
response of calves infected with C. bovis. He demonstrated that not only eosinophils, but primarily lymphocytes participated in the leukocytosis and that a blastic transformation of lymphocytes occurred.

Literary data on the results of biochemical tests in bovine cysticercosis are only sporadic (Bozcoń et al. 1974, Kozakiewicz 1977). Therefore in our set of experiments, also biochemical examinations of blood were performed during the infection. The interpretation of the increased activity of serum creatine-kinase (CK) is easy. It is an indication of the necrosis of skeletal muscle which takes place just at the early phase of infection (Blážek et al. 1981). The rapid decrease in the activity shows the relatively short duration of the necrosis and rapid restoration. The double-peak curve of CK level in the blood serum of some calves may suggest that the muscle fibres disintegrate even at the period of about 30 days, either due to the cellular reaction or due to the metabolites of larvae with retarded development in case of massive infection.

Kozakiewicz (1977) described the increased activity of aspartate-aminotransferase (AST) on days 19, 27 and 36 p.i. It seems to be associated with the dystrophic changes in muscles during the infection, but it may also indicate the necrosis of cells of other organs, e.g. liver cells, which occurs in massive infection with C. bovis.

The increased activity of alanine-aminotransferase (ALT) during experimental cysticercosis was described already by Kozakiewicz (1977). According to this author, the activity increased 3 times. This increase indicates changes in the skeletal and heart muscles, particularly in cattle, in which ALT is no specific liver enzyme (Kaneko and Cornelius 1971).

The highest activity of lactate-dehydrogenase (LD) was recorded on day 16 p.i. and a further increase occurred on days 62–75 p.i. Bozcoń et al. (1974) observed a light increase on day 30 p.i. The same authors also noted an increase in the level of LDH in the blood serum of larvae (Zdárský et al. 1981) at that time. Later, on day 42 p.i., the ALT activity is limited only to walls of capillaries and single fibroblasts at the pithy of organ reaction (Zdárský et al. 1981). It was demonstrated that also the immunologically active macrophages secrete, in addition to other enzymes, also alkaline phosphatase (Lorentz 1978). It is known that the macrophages are involved in immune response in bovine cysticercosis. This may, at least partly, explain the increased ALT activity in the blood serum on day 21 p.i. (Bozcoń et al. 1974) or on day 36 p.i. (own observation).

The increase in the lipide content in the blood serum on days 9–16 p.i. is associated with the fact that many muscle fibres of both skeletal and heart muscles disintegrate at that time. Kaneko and Cornelius (1971) found a high content of lipides by large muscular microscopic inclusions in the red muscle (of type 1). Lipoasma in the result of their disintegration.

Several conclusions can be drawn from the above data on bovine cysticercosis. The results supplement the pathological morphology of infected organs (Blážek et al. 1981, Blážek and Schramlová 1981) so that an idea can be made of the pathological process as a whole. The comparison of morphological and biochemical findings indicates the correlation between, e.g., the increased activity of some enzymes and the pathological changes, and extends the present knowledge of the possible use of some tests in the diagnostics of parasitic lesions. The knowledge of the significant increase in the activity of serum creatine-kinase at the early stage of cysticercosis can be used in the clinical practice. The test of CK may confirm or exclude the myogenic origin in case of the described clinical symptoms which may cause some hesitations in the diagnosis. Muscle strain during the transport and nutritional muscle dystrophy might be involved in case of a positive finding in cattle, but the clinical symptoms are different from those at the early stage of cysticercosis.

Other examinations, both haematological and biochemical, are of rather theoretical importance. Due to their non-specificity and sometimes even individual variability of the values demonstrated these methods are unsuitable for the diagnostics. Chronic cysticercosis with infective cysticeri (Plate II, Figs. 1 and 2) runs without any symptoms and cannot be detected by either haematological or biochemical examinations.

REFERENCES


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СИМПТОМАТОЛОГИЯ ЭКСПЕРИМЕНТАЛЬНОГО ЦИСТИКЕРОЗА КРУГЛОГО ГОРОДА СКУТА К. Блажек, К. Курса, Н. Шрамова и Я. Прокинч

Резюме. Описаны результаты клинико-лабораторного обследования животных, экспериментально инфицированных трематодами. В качестве примера была приведена серия клинических проявлений, обнаруженных в крови телят. Было обнаружено повышенное температура тела, кашель, дрожь и неуверенная ходьба. Расстройство движения исчезло только на 50-й день после заражения. Лейкоцитоз и периферическая гиперемия, а также гипергемия, развившаяся на 2-й неделе после заражения активность гликогеназы значительно увеличилась. Также заметны другие параметры (аспартат-аминотрансфераза, аланин-аминотрансфераза, лизоцим, фосфатаза) в крови и лимфоциты в кровотоке увеличивались на 9–16-й день после заражения.

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1981, Блажек и Схрамована (1981) так, что можно представить результаты патологического процесса как в целом. Сопоставление морфологических и биохимических данных указывает на корреляцию между, например, увеличением активности некоторых ферментов и патологическими изменениями, и расширяет текущее понимание возможного использования некоторых тестов для диагностики паразитарных инфекций. Знание значительного увеличения активности фермента цитратной киназы на ранних стадиях цистицеркоза может быть использовано в клинической практике. Тест на CK может подтвердить или исключить миогенный источник в связи с описанными сомнениями в диагностике. Мышечное напряжение при транспортировке и гиперемия мускулатуры могут быть вовлечены в случае положительного теста на цистицеркоз у коров, но клинические симптомы отличаются от тех, что связаны с ранним цистицеркозом. Другие исследования, как гематологические и биохимические, имеют теоретическое значение. Благодаря их неспецифичности и иногда индивидуальной вариабельности значений, выявленных при этих методах, они не подходят для диагностики. Хронический цистицеркоз с инфекционными гельминтами (Плате II, Фиги 1 и 2) проявляется без симптомов и не может быть выявлен с помощью других гематологических или биохимических исследований.
The first volume written by 19 specialists and covering Nematozoa and Brachiura Orthorynchus brings data on 1881 species belonging to 35 families. The introduction is followed by a brief history of research of Diptera in the territory of Slovakia and division of the latter into 26 geographic units. Over 200 text pages are devoted to the survey of species. In each species names of known and/or new localities of its occurrence and biologic characteristics in abbreviations are given. The next part brings in tabular arrangement data on distribution of individual species (except Chromonomidae) in 20 territorial units. The references contain only those not included in the bibliography of Czechoslovak Diptera published earlier. For medical entomologists of interest are data on 45 species of Culicidae, 109 species of Ceratopogonidae, 45 species of Simuliidae and 59 species of Tabanidae. The publication is an evidence of the great development of dipterology in Slovakia, especially in the last two decades.

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Fig. 1. Massive infection with C. baizai in heart, 3 weeks p.i. Foci with cysticerci are solid nodules with pus-like matter in the centre. Fig. 2. Cysticercus (C) in the node surrounded by eosinophils (EO). Macrophages and granulation tissue appear towards the periphery (P). (HE, x100). Fig. 3. Some of the nodules with necrotic centre (N) do not contain the cysticercus. The nodules are demarcated by connective tissue (ET), (HE, x100). Fig. 4. Character of the exudate in the centre of the nodule with cysticercus (C) (HE, x250).

Fig. 1. Skeletal muscles with cysticerci, 9 weeks p.i. No inflammatory reaction is visible around the cysts. Fig. 2. Resting cysts with morphologically differentiated cysticercus (C) have a fine, only little cellular wall (CW), which is thickened by histiocytic proliferation (PR) only at the site