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| Author(s) | YAMASHITA, Jiro; OHBAYASHI, Masashi; SAKAMOTO, Tsukasa |
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STUDIES ON ECHINOCOCCOSIS
XII. OVINE EXPERIMENTAL CASES OF
UNILOCULAR ECHINOCOCCOSIS

JIRO YAMASHITA, Masashi OHBAYASHI
and Tsukasa SAKAMOTO

*Department of Parasitology,
Faculty of Veterinary Medicine,
Hokkaido University, Sapporo, Japan*

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Natural and experimental cases of sheep echinococcosis were investigated and described in the first⁶⁾ and fifth⁷⁾ reports respectively of the present series of studies. In these reports, it was made clear that the hydatid cysts manifest a type *Echinococcus polymorphus* in the ovine viscera; some discussion of the histogenesis was presented. In ovine cases, the development of hydatid cysts is not very rapid, although the sheep is well-known as a suitable intermediate host for *Echinococcus granulosus*. Specifically, hydatid cysts in the authors' cases required more than 1 year to reach a size of approximately 1 cm, in which the scolex could not be found.

The authors, in the present paper, deal with 3 ovine experimental cases of unilocular echinococcosis, on which longterm observation was conducted. These cases manifested the findings of each the prodromal, the active and the declining stages of scolex formation.

MATERIALS AND METHODS

Hydatid sand of Australian strain of *Echinococcus granulosus* of ovine case No. 2 described in the first report were given orally to dogs; adult cestodes and eggs were obtained. The eggs were given to six 2-year-old Corriedale sheep on January 26 and February 2, 1955, which were also infected on March 11, 1955 with the proglottids of the cestode. Three of these sheep were already described in the fifth report. The present cases No. 1, No. 2 and No. 3 were sacrificed and autopsied on September 17, 1957, October 23, 1958 and July 2, 1959 respectively, each 32, 45 and 54 months after the first ingestion of *Echinococcus* eggs.

After formalin fixation of tissue materials, paraffin sections were stained with hematoxylin-eosin, v. GIESON, Azan and MACMANUS's PAS.

RESULTS

Case No. 1

Macroscopical findings Liver: Thirty-two polymorphous-shape hydatid cysts, 5~12 mm in size, were scattered in the liver. Small cysts were nodular and hard as a result of severe

calcification. It comprised thick wall and some minute cavities. Large cysts usually consisted of a few or several loculi of which the cavities communicated with each other by a narrow portion or were fused to each other. Lung: Eight and 15 hydatid cysts, 3~22 mm in size, were recognized in the right and left lungs respectively. Calcification was slight except for a few nodular foci. The cysts, excepting some simple globular ones, manifested the shape of *Echinococcus polymorphus*. The majority of the cysts contained serous fluid, however some contained a caseous mass or crumpled cuticular layer. Mediastinal lymph node: It was enlarged remarkably as to 60×25×17 mm. The surface showed unevenness and partial calcifications. On cutting, the lymph node was found to have a polymorphous cystic structure with transparent fluid; on the inner surface reticular trabecular elevations were observed.

Microscopical findings Liver: Cysts were well-developed, but had no brood capsule and scolex. The germinal layer was simple-layered with small nuclei. The cuticular layer was laminated, 20~130 μ in thickness. Adventitious tissue around the cyst was about 450 μ in thickness; it was divided into two parts. The inner layer was composed of dense collagenous fibers with scarce nuclei while the outer one was composed of connective tissue which became fresh toward the periphery. Accumulation of lymphocytes and histiocytes, sometimes also lymph-nodular foci, was found at the boundary portion between the liver parenchyma and adventitious tissue. The outer margin of inner adventitious tissue layer was irregularly demarcated and there was observed calcification in contact with outer layer. Accumulation of lymphocytes, plasma cells and histiocytes was recognized in GLISSON's sheaths nearby the foci. The cuticular layer of small cysts was increased in thickness as compared with that of the large cyst; 100~160 μ in thickness and crumpled. Lung: No formation of brood capsule or scolex was recognizable, however accumulation of the germinal cells, which could be regarded as a prodromal stage of brood capsule, was found occasionally. The cuticular layer was thick, 18~210 μ , and the germinal layer was thin. Adventitious connective tissue resembled very closely that observed in the liver. In some large cysts, regressive changes were seen. Colloidal mass with intermingled degenerated or detrited cell elements, neutrophil leucocytes and round cells, was recognized in the cystic cavity. The thickness of cuticular layer was increased and crumpled. Mediastinal lymph node: The germinal layer had eosinophil granules. No formation of the brood capsule or scolex could be found. The thickness of cuticular layer and adventitious connective tissue was 35~70 μ and 175~1300 μ respectively.

Case No. 2

Macroscopical findings Liver: The size was 42.0×23.6×7.8 cm. About 50 polymorphous-shaped hydatid cysts were distributed through the liver. The cysts were large in general; the smallest cyst was 15×9×9 mm and the largest 70×47×40 mm. Cyst wall was thick being 0.7~3.8 mm in thickness. Numerous brood capsules could be detected on the inner surface of cyst wall. Spleen: The spleen was 13.7×11.5×3.1 cm in size. A cocoon-shape hydatid cyst was found, 30×20×54 mm in size; the thickness of cyst wall was 1~1.5 mm and there existed numerous brood capsules. Lung: The right and left lungs were 12.0×22.6×6.2 cm and 13.4×24.7×6.1 cm in size respectively. Hydatid cysts, 12~35 mm in diameter, were large and manifested polymorphous-shape. Thirteen cysts were recognized in each the right and left lung. The thickness of cyst wall was 0.5~2.5 mm. Brood capsules could be seen on the inner surface of cyst wall.

Microscopical findings Liver: The hydatid cysts were fully developed. Formation of brood capsule and scolex was remarkable. The brood capsule, 240~300 μ in diameter, contained 1~7 scolices (average 4) on section preparations. The scolex was usually invaginated type of a pear-shape; its size was 130~192 μ (average 160 μ) \times 96~113 μ (100 μ). A few scolices exhibited regressive findings such as vacuolation and rupture. The germinal cell layer and the wall of brood capsule were considerably thick with eosinophil granules. The thickness of cuticular layer was 35~100 μ (average 44 μ). The adventitious connective tissue bore resemblance to that of case No. 1; accumulation of eosinophil cells and plasma cells, and proliferation of bile ducts were observed in the outer portion. The liver tissue showed proliferation of interstitium, some small hemorrhagic foci and accumulation of plasma cells and histiocytes, in contact with the cyst. Lung: Hydatid cysts were similar to those of the liver and showed formation of brood capsule and scolex. These cysts were polymorphous echinococci with considerably complicated form. Regressive changes were observed similar to those of case No. 1. The pulmonary parenchyma manifested atrophy resultant from pressure in contact with the hydatid focus, and accumulation of plasma cells, lymphocytes and histiocytes was found. Spleen: Microscopical characteristics of the hydatid cyst were similar to those of the liver or lung.

Case No. 3

Macroscopical findings Liver: A polymorphous hydatid cyst was found in the caudate lobe; it was 16 \times 12 \times 9 mm in size. The cyst wall was about 1.5 mm in thickness, and its inner surface was smooth. Lung: A hydatid cyst, 29 \times 41 \times 29 mm, consisting of three loculi existed on the diaphragmatic surface of the left lung. A round-shaped hydatid cyst, 24 \times 25 \times 15 mm, was found in the cardiac lobe. Each one large and one small hydatid cyst was also observed on the costal surface of the left lung; they measured 43 \times 35 \times 22 mm and 16 \times 17 \times 17 mm respectively; the former manifested fibrous adhesion with the costal pleura, consisted of some loculi, and the inner surface of cyst wall showed a reticulate figure as a result of trabecular elevation. A hydatid cyst, 28 \times 55 \times 24 mm in size, was also found in the diaphragmatic surface of the right lung. The thickness of wall of these cysts was increased in places to 7 mm, but was about 2 mm in general. Brood capsules were detected on the inner surface of large type cysts.

Microscopical findings Liver: Although brood capsule and scolex formation was non-recognizable, the finding of hydatid cysts resembled that of case No. 2. Lung: Remarkable regressive changes were observed. The cuticular layer was thickened, being 34~270 μ in thickness. The number of brood capsules was decreased. In some cysts, the cuticular layer was crumpled within the cavity; the germinal layer fell into degeneration and desquamation. Conspicuous degenerative changes of brood capsule and scolex were observed. Among degenerated scolices some had progressed to vacuolation of the parenchyma; this change could be understood as metamorphosis for daughter cyst formation. In the inner layer of adventitious connective tissue, calcification became conspicuous. Remarkable accumulation of plasma cells, lymphocytes and eosinophil cells were found in the outer layer of adventitious tissue.

DISCUSSION

In their 5th report⁷⁾, the present authors reported morphological findings of unilocular hydatid cysts at 112, 115 and 399 days after the infection. Those hydatid cysts, however, were considered to be still in course of development; they were small in size, even the largest one of the 399-day case was only $15 \times 10 \times 10$ mm. It was not possible to find any formation of brood capsule and scolex and even an accumulation of germinal cells which corresponds to a prodromal stage of brood capsule. In case No. 1 in the present report, however, accumulation of germinal cells was rarely observed; the meaning is that development of the hydatid cyst was on the prodromal stage of brood capsule formation. Active formation of scolex and brood capsule was recognized in case No. 2; it can be said that the hydatid cyst of this case was at the maximum active stage of scolex formation. In case No. 3, on the contrary, the number of brood capsules and scolices was remarkably decreased; the latter manifested regressive changes such as rupture and vacuolation, and the cuticular membrane crumpled within the cyst cavity showing degeneration and desquamation; this case can be explained as one in the declining stage. As far as the present cases are concerned, it can be considered that brood capsule and scolex formation begins from about 35 months after the infection, it reaches to the maximum at about 45 months, and that the decline of scolex formation begins at about 54 months. DENAPOLITANO et al. (1953) obtained results different from the present authors': the scolex was observed for the first time 9 months after the infection. In their report with regard to experimental multilocular echinococcosis, YAMASHITA et al. (1958) stated that there was significant difference in development of hydatid cysts among uniform mouse strains. As to the breed used in both experiments by the present authors and DENAPOLITANO et al., it was described as Corriedale breed. It is well-known that the Corriedale is not a sufficiently fixed breed. The authors, therefore, can easily suppose the existence of different data resultant from these experiments. Pertaining to the time of scolex and brood capsule formation in sheep, the authors can refer to the data of text-books which make the supposition that variation among individual animals is not unusual.

DEW (1926)¹⁾ and FAIRLEY and WRIGHT-SMITH (1929) classified the hydatid cysts in sheep into three types; simple univesicular (unilocular), multilocular and multicystic. They similarly concluded that the majority of hydatid cysts in sheep belonged to the uni- and multilocular types. They pointed out that those cysts manifested a type of so-called polymorphous hydatid cysts and that this type was formed by external herniation of cyst wall through relatively weak portions of the adventitia as a result of rising intracystic pressure. All these cysts, however,

belong to the unilocular cyst according to the classification by the present authors; the above multilocular cyst is not a multilocular (alveolar) cyst in the true sense, but a variation of the unilocular type. In the fifth report of this series, the present authors expressed agreement with the herniation theory except for its causal genesis.

The authors would like to consider the relation between the hydatid cyst and adventitious connective tissue. In the prodromal and active stage of scolex formation, the cuticular layer which is lined with the germinal layer was tense and considerably thin as a result of rising intracystic pressure, which also spread out the adventitious tissue. The adventitious tissue was not very thick and cellular accumulation other than lymphocytes was slight. On the contrary, in regressive hydatid cysts, the cuticular membrane was crumpled within cyst cavity by weakened intracystic pressure. The cavities, therefore, were narrow, and even disappeared in the severe regressive focus. Degeneration and desquamation of germinal membrane and abnormal thickening of adventitia were recognized. And there were also observable an expanded necrotic area with calcification in the inner adventitious layer and accumulation of plasma cells, eosinophil cells and lymphocytes in the outer layer. It is supposed that necrosis of the inner adventitious tissue provokes an unfavorable result upon the nutritional currency for a hydatid, and the development of hydatid cysts is also obstructed because of the limited narrow space surrounded by thick adventitious tissue. On the other hand, the hydatid with regressive changes naturally provokes conspicuous proliferation of granulation tissue. Anyway, it is a fact that the remarkable reaction of adventitious connective tissue is found in sheep, and the development of hydatid cyst is likely to be suppressed. As to cellular reaction in the adventitious tissue, DEW¹⁾ reported as follows: "No doubt in the earliest stage there is an intense cellular reaction with characteristic eosinophil and endothelial cells, but even in small cysts the inner layer rapidly becomes acellular and avascular." The authors, however, have no data of their own concerning the hydatid cyst at its initial developmental stage. In the present case No. 1, lymphocytes were the main element, but accumulation of eosinophil cells and plasma cells had a tendency to increase from case No. 2 to case No. 3 parallel to the regressive changes. DEW¹⁾ also stated: "Endogenous daughter cyst formation which is so typical of human cysts, is extremely rare and it was noticed only in three cases in all of which some abnormal condition was present". On the other hand, with regard to hydatid cysts in sheep, oxen and pigs, FAIRLEY and WRIGHT-SMITH reported that degeneration was never observed and that degeneration of mother cyst with daughter cysts is a secondary phenomenon. In the present case No. 3, a few scolices manifested the beginning change of endogenous daughter cyst formation.

within the mother cysts which had fallen into regressive changes. This phenomenon seems to follow the same course as daughter cyst formation in echinococcosis multilocularis of mice which has been reported by one of the authors (OHBAYASHI; 1960). The present authors consider that daughter cyst formation does not take place while the scolex is immature and that the phenomenon occurs later than the active stage when the regressive changes appear. Although no degeneration of mother cyst was described morphologically in FAIRLEY and WRIGHT-SMITH's report, the mother cyst itself of their cases, the present authors consider, was past its active stage.

DEW²⁾ stated that the daughter cysts usually arise as a result of progressive evolution of the cells of the original germinal membrane or from brood capsules and more rarely that they may develop from scolices. On the contrary, FAIRLEY and WRIGHT-SMITH concluded on the basis of their observation in domestic animals that daughter cyst formation is essentially an alternative method of reproduction manifested by vesiculating and probably aged scolices. At any rate, the present authors would like to register agreement with the opinion that the endogenous daughter cyst arises only from the scolex by vesiculation when the mother cyst falls into a regressive condition. However, it can be said that daughter cyst formation is a rare phenomenon in ovine cases.

SUMMARY

The authors investigated 3 ovine cases; 32, 45 and 54 months after experimental infection by *Echinococcus granulosus*. These cases corresponded to the prodromal, active and declining stages of scolex formation respectively. The following facts were emphasized.

The hydatid cyst in sheep usually shows polymorphous shape, and is surrounded by thick and fibrous adventitious tissue. Although the sheep is a suitable intermediate host, development of cyst is slow and regressive changes are not uncommonly found. With occurrence of regressive changes, some scolices manifest metamorphosis in the direction of an endogenous daughter cyst by vesiculation, but this phenomenon seems to be rare in sheep.

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EXPLANATION OF PLATE

- Fig. 1. Case No. 2. The liver showing numerous polymorphous-shape hydatid cysts. $\times 1/4$.
- Fig. 2. Case No. 2. Cut surface of the liver; fully-developed hydatid cysts. $\times 1/3.6$.
- Fig. 3. Case No. 3. Cut surface of the lung; a hydatid cyst with regressive change. $\times 1/2$.
- Fig. 4. Case No. 2. Active formation of brood capsule and scolex. Hematoxylin-eosin stain. (H.-E.), $\times 35$.
- Fig. 5. Case No. 3. Crumpled cuticular membrane in a regressive hydatid cyst. H.-E., $\times 35$.
- Fig. 6. Case No. 3. Degeneration and fragmentation of hydatid cyst wall. H.-E., $\times 35$.
- Fig. 7. Case No. 3. A scolex showing metamorphosis toward a daughter cyst (vesiculation). H.-E., $\times 170$.
- Fig. 8. Case No. 3. A daughter cyst. H.-E., $\times 50$.

