STUDIES ON ECHINOCOCCOSIS XI.
OBSERVATIONS ON SECONDARY ECHINOCOCCOSIS

Jiro YAMASHITA, Masashi OHBAYASHI
and Tsukasa SAKAMOTO

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

(Received for publication, October 13, 1960)

The present authors have already published the results of experiments pertaining to experimental secondary echinococcosis in mice of dd strain as one of their series of studies of echinococcosis (YAMASHITA et al., 1957). As described in that paper, the scolices of larval *Echinococcus multilocularis* were used as inocula; it was concluded that the scolices injected into the peritoneal cavity and subcutis of dd mouse had a tendency to show a negative result in formation of typical multilocular echinococcosis. Thereafter, experiments have been carried out about both the multilocular and unilocular echinococcoses, using various experimental animals. In the present paper, the results of experiments on secondary unilocular and multilocular echinococcoses in Mongolian gerbil (*Meriones unguiculatus*) and mice (strains dba & C57BL/6) are dealt with.

MATERIALS AND METHODS

Unilocular echinococcus (abbreviated as E. g.) The inocula were obtained on 2/VII '59 from a unilocular echinococcus cyst in the lung of a sheep which had been affected orally with ova of E. g. Scolices (so-called hydatid sands) were collected from the cyst and a suspension of scolices in sterilized physiological saline was prepared. Eight Mongolian gerbils, 10 dba mice and 15 C57BL/6 mice were inoculated intraperitoneally with the suspension.

Multilocular echinococcus (E. m.) The inocula originated in hepatic echinococcus foci of orally inoculated cases of cotton rat, Mongolian gerbil and AKR mouse. Four Mongolian gerbils were given inoculation with material from cotton rat (15/XII '58); 10 Mongolian gerbils (14/V '59), 7 dba mice (6/VI '58) and 6 C57BL/6 mice (6/VI '58) with that from Mongolian gerbil; and 2 dba mice with material from AKR mouse (29/IV '58). Preparation of inoculum of E. m. case was different from that of E. g. Echinococcus tissue was lightly ground in a mortar with added physiological solution; this material was filtrated through a gauze and the filtrate was used as the inoculum.

Experimental animals were each given injection by syringe with 0.05 ml of inoculum. Number of scolices contained in the inoculum was various according to the animals from which the inocula originated. The numbers of scolices injected fluctuated from about 130 to 1,000 per animal. Position of injection was limited to the left hypogastric area of animals.

The animals were sacrificed at a proper time, dissected for macroscopical observation; histological examinations were also conducted.

RESULTS

E. g. Cases

1. Mongolian gerbils Three cases 35 days after the inoculation manifested no echinococcus foci, but echinococcus cyst formation was observed in 3 cases (Nos. 1~3) out of 5 animals 154 days after the inoculation.

No. 1: A unilocular cyst, 7 mm in diameter, with a pouch existed on the greater omentum; cyst wall was tense and fluid content was transparent.

No. 2: One spherical cyst, 4 mm in diameter, existed on the visceral surface of each the right lateral and caudate lobes of the liver respectively; its content was transparent fluid.

No. 3: A large cyst 15 mm in diameter was found on the greater omentum; cyst wall was tense and thin, surface showed slight elevations of 3~5 mm size and ramiform blood vessels.

These cysts showed a structure of typical unilocular echinococcus. The cuticular layer manifested laminated composition, 90 μ in thickness. The germinal layer was thin with dark-stained nuclei scattered sporadically and reddish granules. Brood capsules and calcareous corpuscles were absent.

2. dba mice A 341-day case (No. 1) and a 372-day case (No. 2) gave positive results. However, 3 animals of 35-days, one 250-day, one 348-day case and 3 of 372-days were diagnosed as negative.

No. 1: A unilocular cyst of 1 mm size was found on the diaphragmatic surface of the left lateral lobe of the liver and also a unilocular cyst 3 mm in diameter existed free in the abdominal cavity. The latter cyst comprised a laminated cuticular layer, 250 μ in thickness, and a considerably thick germinal layer with many nuclei.

No. 2: The left median lobe of the liver was occupied by a large echinococcus cyst, diameter 19 mm, with a slight pouching of 5 mm size. Histologically, the laminated cuticular layer of 250 μ thickness was remarkable and the germinal layer was thin. The adventitious layer showed the same thickness as that of the cuticular, became fibrous; cellular accumulation and vascularization existed.

3. C 57 BL/6 mice Positive results were obtained from a 35-day case (No. 1) and 2 cases of 372-days (Nos. 2 & 3), however 4 other animals of 35-day and 8 of 372-day cases manifested no echinococcus foci.

No. 1: A nodular focus, 1 mm in size, superficially at the portal area of the liver. The focus consisted of granulation tissue and was clearly demarcated from the hepatic parenchyma. Two holes, 400 and 300 μ in diameter respectively, could be found within the focus; they were surrounded by a necrotic mass. The former hole was filled with a scolex which was under way for cystic metamorphosis, its shape was ellipsoid, some hooklets remained and the parenchyma had become cystic for the most part except for the rostellar portion. The latter hole, however, was filled up by a unilocular cyst having cyst wall very thin with sporadically scattered nuclei of the germinal cells.

No. 2: A polymorphous cyst was found in the right median lobe of the liver. The

cyst was 12.5×13 mm and several pouches of 3~7 mm in size were seen. The cyst wall was thin and tense. The fluid content was light yellowish in color and transparent. Histologically, the cuticular layer was laminated, 30~40 μ in thickness, while the germinal layer was as very thin as 3~5 μ accompanied by sporadically existing dark-stained nuclei. The adventitious layer was fibrous and thin, there existed a histiocytic cell accumulation and, in contact with cyst wall, giant cells were found forming a simple layer.

No. 3: A unilocular cyst with a pouch was observed free in the left abdominal cavity; its size was 9×5 mm. The histological character was the same as that of No. 2.

E. m. Cases

1. Mongolian gerbils Out of 14 cases examined, 2 of 95-day cases, 1 of 150-day and 2 of 420-day were negative cases, however, echinococcus foci could be seen in one 33-day case (No. 1), 3 of 95-day (Nos. 2~4), 2 of 99-day (Nos. 5 & 6) and 3 of 420-day cases (Nos. 7~9).

No. 1: Three foci 1.5~3 mm in size were found in the portal area of the liver. One of these showed signs of being under way for cystic metamorphosis. This focus, 2 mm in diameter, consisted of granulation tissue with histiocytic cell accumulation and covered the hepatic surface. At the central portion of the focus, there could be noted a round hole. The hole was filled by a scolex of 180 μ size of which the parenchyma had become remarkably loose, however, sucker structure yet remained.

No. 2: A focus, 4 mm in diameter, was noted on the visceral surface of the diaphragm and a nodular focus, size 1 mm, was also found in the pelvic cavity. The diaphragmatic focus, microscopically, was seen to be granulation tissue in which many regressively degenerated scolices were found; some of these scolices were already organized. Two echinococcus cysts with very thin cuticular layer, 2 and 0.7 mm in size respectively, were also found in the focus. The cysts showed scolex formation; scolices of one cyst were immature and the thickness of the germinal layer was proportioned to maturity of scolices.

No. 3: A focus of 1 mm size in the diaphragm. In the focus necrotic echinococcus tissue was enveloped with connective tissue.

No. 4: A spherical mass, 3 mm in diameter, was seen on the serosa of right uterus horn and, in contact with this mass, a 2-mm-sized mass was also found. The former was necrotic multilocular echinococcus tissue surrounded by poor connective tissue layer. The latter, however, was completed multilocular echinococcus tissue with numerous matured scolices and extremely thin cuticular layer.

No. 5: Two minute foci were found in the portal area of the liver. In one of them, histologically, many necrobiotic scolices were observed; among these scolices granulation tissue had proliferated showing organization process. Undulated fragments of the cuticular layer were seen accompanied by many giant cells. In a corner there can be found an echinococcus cyst composed of germinal layer with many calcareous corpusles and thin cuticular layer. The germinal layer manifested some thickness, but no scolex formation was proven.

No. 6: Multilocular echinococcus foci were found superficially in the liver, stomach, greater omentum, mesenterium and testis. Histologically, these foci were multilocular echinococcus tissue composed of cystic structures of which the diameter was 250~700 μ. Numerous scolices contained in brood capsules were conspicuously observed. The germinal

layer was rich in calcareous corpuscles and the cuticular layer was as thin as about 3 μ in thickness.

Nos. 7~9: Each one focus of about 3 mm size was found free in the pelvic cavity (No. 7), on the diaphragmatic surface of the left lateral lobe of the liver (No. 8) and on the visceral surface of the right median lobe of the liver (No. 9). These foci, histologically, were multilocular echinococcus tissue. The germinal layer with numerous calcareous corpuscles was well-developed and reticular; abundant brood capsules with matured scolices were noted. Thickness of the cuticular layer was 3~5 μ and the adventitious layer was poorly developed.

2. dba mice Two animals of 61-day cases (Nos. 1 & 2), 3 of 135-day (Nos. 3~5) and 2 of 153-day (Nos. 6 & 7) manifested echinococcus focus formation, although 2 animals of the 38-day cases showed no such formations.

No. 1: Two and one foci in the left median and right lateral lobes of the liver respectively, 2 in the lesser omentum and 1 on the serosa of urinary bladder were found; all the foci were minute. These foci were in the form of an organized nodular focus of which the central portion was necrotic tissue; sometimes shady scolices figure could be differentiated.

No. 2: One, 2 and 1 minute foci respectively were observed in the greater omentum, and the portal area and visceral surface of the left lateral lobe of the liver. All the foci were organized granulation tissue nodules with central necrotic echinococcus tissue.

No. 3: A superficially-existent focus, 1×3 mm in size, was noted on the diaphragmatic surface of the left lateral lobe of the liver. Histologically, a multilocular echinococcus tissue was surrounded by thick layer of granulation tissue with histiocytic cell accumulation. The cuticular layer was less than 10 μ in thickness, the germinal layer had scattered calcareous corpuscles sporadically and a very few examples of immature scolices were formed. Tissue necrosis, in the adventitious layer, was observed in contact with cyst wall.

No. 4: A yellowish focus was seen on the diaphragmatic surface of the right median lobe of the liver. The focus was a granulation tissue nodule which showed advanced organization.

No. 5: One, 2 and 1 foci, 1~5 mm in size, were found in the right lateral, right median and left median lobes of the liver respectively; large foci were composed of an aggregation of minute cystic structures. As to microscopical findings, the focus contained aggregated minute cystic structures, the multilocular echinococcus; the cuticular layer was thin and, irrespective of existence of calcareous corpuscles, the germinal layer was also not very thick. Initial scolex formation was observed. The diaphragm showed a focus nearby the left thoracic wall; this focus was an organized one with marked cell accumulation and the central portion was necrosed tissue with intermingled some liberated hooklets. The central portion had fallen into calcification.

No. 6: In the liver, a focus of 6×2 mm size and one of 1.5 mm were found on the diaphragmatic surface of the left lateral and left median lobes respectively; a botryoid-shaped mass of 5 mm size attached to the papillary process by a peduncle of 5 mm length; a minute focus was also seen at the marginal portion of the right median lobe. The foci, histologically, were composed of multilocular echinococcus tissue and well-proliferated adventitious granulation tissue; the cuticular layer was thin. The pedunculated mass, however, had a thin fibrous adventitious layer and initial scolex formation was discernible.

No. 7: In the liver there existed 3 minute foci and a cystic focus of 2 mm size in the left lateral lobe, a minute focus on the diaphragmatic surface of the left median lobe and a focus, 3.5 mm in diameter, composed of minute cystic structures in the papillary process. In other portions, a mass of 4×2 mm size was found on the uterine serosa while the greater omentum had also 3 foci of 3~4 mm size and a minute focus. Microscopical examinations of the foci revealed well-developed adventitious granulation tissue and a germinal layer which was not very thick, although calcareous corpuscles were conspicuously distributed. Scolex formation in the beginning stage was observed. The cuticular layer was about 3 μ in thickness. The adventitious tissue occasionally showed remarkable histiocytic cell accumulation and some giant cells were also detected.

3. C 57 BL/6 mice Three animals of 61-day cases were negative for echinococcosis, but 3 of 135-day (Nos. 1~3) manifested focus formation.

No. 1: The left median lobe of the liver was occupied by a large mass of echinococcus tissue. It was made up of 10 large cystic structures, 3~13 mm in size, and areas, 10×11 and 6×6 mm, composed of minute cystic structures. The focus was swollen especially on the diaphragmatic surface. A focus, 5 mm in size, was also found on the diaphragmatic surface of the left lateral lobe. Five foci of 5~10 mm were found on the greater omentum and mesenterium, a mass of 7×5×5.5 mm was free in the abdominal cavity and the uterus showed on its serosa 2 foci, respectively 4.5 and 8 mm in diameter. Multilocular echinococcus tissue composed of numerous various-sized cystic structures, histologically, could be observed embedded within well-proliferated granulation tissue or adventitious tissue. Minute pouches were only 100~200 μ in size. Regressive changes of adventitious tissue were seen in areas which were in contact with small-cystic echinococcus tissue. Giant cells appeared sporadically being attached to large-cyst walls. Cyst wall was very thin and the cuticular layer was 2~3 μ in thickness. The germinal layer was thin, although it showed reticular structure and sporadical appearance of incompleted calcareous corpuscles. No scolex formation could be detected. Echinococcus tissue other than in the liver was similar to the above, but regressive changes were generally noticeable.

No. 2: A yellowish-white nodular focus of 2 mm size was found on the greater omentum and, histologically, was identified as an organized focus.

No. 3: On the greater omentum a nodular focus, 2.5 mm in size, was detected. Histologically it was identified as an organized focus.

Discussion

As to experimental secondary echinococcosis of E. m. in dd strain mice, it was clarified that the scolex inoculated into the abdominal cavity of a host rarely terminated to secondary echinococcus formation (Yamashita et al., 1957). In animals dealt with in this paper, however, positive results were obtained with respect to secondary echinococcosis.

The time when intraperitoneally injected scolex becomes cystic can be explained by reference to the description of the foci found in C 57 BL/6 No. 1 of E. g. case and Mongolian gerbil No. 1 of E. m. case. That is to say, the foci

manifested the very stage of cystic metamorphosis of scolex. It can be concluded that at least one month is needed for cystic metamorphosis. Cyst formation in experimental cases of oral administration of E. m. ova occurs in as early stage as about 3 days after inoculation (OHBAYASHI, 1960). The authors, therefore, can say that an unexpectedly long period is necessary for cyst formation in secondary echinococcosis. This relationship is also applicable for scolex formation; this phenomenon takes place in secondary multilocular echinococcosis by scolex injection about a month later than in the cases of oral infection by E. m. ova (YAMASHITA et al., 1958; OHBAYASHI, 1960). Initial scolex formation was found in 3- and 4-month cases of secondary echinococcosis in Mongolian gerbils and dba mice respectively; 4-month cases of C57BL/6 mice did not exhibit the formation.

Between secondary echinococcoses of E. g. and E. m. cases, there exists remarkable differences similar to those in oral infection by ova of the two species. Differences can be found in shape of cyst, characteristics of the germinal layer, structure of laminated cuticular layer etc. as described above. Also among E. m. cases, indeed, histological findings of Mongolian gerbils and C57BL/6 mice manifest different types as YAMASHITA et al. (1958) pointed out in cases of oral infection. Namely, in Mongolian gerbil (type 1), individual cysts are large in size, development of echinococcus tissue is rapid, scolex formation takes place in an earlier stage and host tissue reaction is not very severe. In C57BL/6 mouse (type 2), contrary to the above, minute cysts play the leading role, development is slow in tempo, scolex formation is delayed and conspicuous host tissue reaction can be found.

Susceptibility of experimental animals to secondary echinococcosis sets an interesting problem. When the results of intraperitoneal scolex injection and oral administration of ova are compared, the interest becomes great. Although the animals dealt with in experiments described in the present paper show negative results to oral infection by ova (unpublished data), positive results could be obtained by intraperitoneal scolex injection in E. g. cases as above described. Contrary to the E. g. cases, susceptibility of intraperitoneal E. m. cases was lower than that of oral cases as shown in table 1. In this table, however, the intraperitoneal E. m. cases in which only organized nodular foci were found, are omitted (Mongolian gerbil No. 3, dba Nos. 1, 2 & 4, C57BL/6 Nos. 1 & 2).

As to the cause of decline of infection rate in intraperitoneal cases (secondary echinococcosis) of multilocular echinococcosis, various factors should be considered. The present authors, however, would like to attach importance to particular histological characteristics of multilocular echinococcus. That is to say, as pointed out in the preceding paper (OHBAYASHI, 1960), in animals such as those from

TABLE 1. *Susceptibilites of Experimental Animals (No. of positive cases / No. of cases examined)*

ANIMALS	E. G.		E. M.	
	Oral (Ova)*	Intraperitoneal (Scolices)	Oral (Ova)	Intraperitoneal (Scolices)
Mongolian gerbil	0/ 6	3/ 8	5/ 5	8/14
dba mouse	0/12	2/10	6/ 6	4/ 9
C 57 BL/6 mouse	0/23	3/15	15/19	1/ 6

*: Authors' data unpublished

which the inocula in the present experiment were obtained, the germinal layer with numerous scolices develops extremely. Scolices are always contained in brood capsules and the brood capsules are embedded in thickened germinal tissue. The germinal tissue is not very fragile; it is accompanied by numerous calcareous corpuscles. In unilocular echinococcus, however, the germinal layer is generally a simple thin layer and scolices liberate easily in fluid content suspended as so-called hydatid sands. Multilocular echinococcus does not take such a form as hydatid sands; to make scolices liberate mechanically is not so easy when inoculum is prepared. The authors, therefore, ground the echinococcus tissue in a mortar using saline solution. The inoculum thus prepared contains a mixture of liberated scolices and pieces of germinal tissue with scolices. Consequently, it is easy to suppose that, in multilocular cases, scolices injected intraperitoneally manifest a decline of percentage to settle on visceral surface as compared with E. g. cases. Severe host tissue reaction also strengthens the inhibition of development of a scolex and, in fact, organized foci are frequently found in secondary echinococcosis. As a matter of fact, scolices which can develop to the stage of an echinococcus cyst are a part of injected scolices.

Age and sex must be taken into consideration in the host parasite relationship. SCHWABE et al. (1959) investigated the age resistance in E. g. cases of secondary echinococcosis and they obtained interesting results. The present authors have disregarded the problem of age resistance for the present. They, however, wish to add that no differences by age can be observed so far as the present cases are concerned. The authors would like to attach importance to different susceptibilities due to differences of animal species or strains in E. m. cases of secondary echinococcosis similar to oral cases by ova.

SUMMARY

Secondary echinococcosis in Mongolian gerbils, dba and C 57 BL/6 mice caused by *Echinococcus granulosus* and *E. multilocularis* was investigated through

intraperitoneal injection of scolices prepared from larval echinococcus tissue. The following conclusions were arrived at:

1) A part of scolices injected develop to echinococcus cyst about a month being needful for cyst formation.

2) In E.g. cases, some animals become affected by intraperitoneal inoculation of scolices irrespective of insusceptibility to oral administration of ova. In E.m. cases, however, a decline of susceptibility takes place in intraperitoneal cases.

3) As to a factor influencing the decline of susceptibility in intraperitoneal E.m. cases, the authors attached importance to a particularity of the histological structure of multilocular echinococcus.

References

1) OHBAYASHI, M. (1960): *Jap. J. vet. Res.*, **8**, 134.
2) SCHWABE, C. W., L. A. SCHINAZI & A. KILEJIAN (1959): *Amer. J. trop. Med. Hyg.*, **8**, 29.
3) YAMASHITA, J., M. OHBAYASHI, Y. KITAMURA, K. SUZUKI & M. OKUGI (1958): *Jap. J. vet. Res.*, **6**, 135.
4) YAMASHITA, J., M. OHBAYASHI & S. KONNO (1957): *Ibid.*, **5**, 197.

Explanation of Plate

Figs. 2~8 were photographed from hematoxylin-eosin preparations; Figs. 3~8 are the same magnification, × 45.

Fig. 1. Mongolian gerbil E.g. No. 3, 154 days after inoculation, × 1.2. A unilocular cyst.
Fig. 2. C 57 BL/6 E.g. No. 1, 35 days, × 130. At right is a scolex showing cystic metamorphosis and at the left is an initial cyst.
Fig. 3. dba E.m. No. 5, 135 days. A focus showing organization.
Fig. 4. Mongolian gerbil E.g. No. 3, 154 days. A part of a unilocular cyst showing thick laminated cuticular layer.
Fig. 5. Mongolian gerbil E.m. No. 6, 99 days. Fully developed tissue of multilocular echinococcus.
Fig. 6. dba E.m. No. 7, 153 days. Multilocular echinococcus without scolex formation.
Fig. 7. C 57 BL/6 E.g. No. 2, 372 days. Showing wall of a typical unilocular cyst.
Fig. 8. C 57 BL/6 E.m. No. 1, 135 days. Multilocular echinococcus composed of minute cystic structures.