# Spontaneous *Echinococcus multilocularis* Infection in Swine in North-Eastern Hokkaido, Japan

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## Introduction

Echinococcus multilocularis is an important zoonotic parasite in eastern Hokkaido, Japan and the voles serve as its natural intermediate host. Unlike *E. granulosus*, which has domestic ungulates as its intermediate host, reports of *E. multilocularis* infection in these ungulates have been almost non-existent with the exception of that of Lukashenko (1968, 1971). We present in this paper spontaneous cases of *E. multilocularis* infection in swine raised and slaughtered in north-eastern Hokkaido. This report led to that area being designated as an enzootic area of *E. multilocularis*.

## Materials and Methods

Of the 58,567 swine slaughtered between December 1982 to July 1983, at the Higashimokoto Meat Inspection Center, Hokkaido, 93 were observed to contain nodules which were found only in the liver and not in any other organs. No clinical symptoms were observed in the affected swine before slaughtering.

The hepatic nodules were fixed in 10 % formalin solution, dehydrated in an ethanol series and paraffin sections of  $4 \mu$  thickness were made. These sections were then stained with either haematoxylin-eosin or periodic acid Schiff (PAS) stain for histological examination.

#### Results

The affected swine were raised in the areas consisting of 2 cities, 7 towns and 1 village in north-eastern Hokkaido. Namely, they are Abashiri, Monbetsu, Bihoro, Tokoro, Tsubetsu, Koshimizu, Memanbetsu, Shari, Kiyosato and Higashimokoto, respectively (Map 1).

Histopathological examination of the hepatic lesions of the 93 cases revealed the presence of *E. multilocularis* cyst in 34 cases and lesions resembling those of that cestode in 40 cases. The rest were diagnosed as nodules due to other causes, which include other parasitic infection, atypical mycobacterial infection, lymphosarcomatosis, hepatic abscess and lymph follicle formation (Table 1). The swine infected with *Echinococcus* consist of 1 boar, 3 brood sow and the rest fattened swine.

At gross examination, the nodules were observed only in the liver and not in any other visceral organs. The size, consistency and the colour of the affected liver appeared normal. The hepatic nodules were oval in

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Map 1 Distribution of *E. multilocularis* in swine in eastern Hokkaido.
Area designated enzootic for *E. multilocularis* until the present report
Area positive for *E. mutilocularis* infection in swine

Higashimokoto
Tsubetsu
Tokoro
Bihoro
Koshimizu

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2:	Bihoro	5:	Koshimizu	8:	Shari
3:	Abashiri	6:	Memanbetsu	9:	Kiyosato

shape, clearly defined and range from 1 to 20 mm in diameter with most of them at around 5 mm. Most of the nodules were found superficially in the parenchyma of the liver. The nodules were yellowish white in colour and either bear a resemblance to an abscess or a caseous lesion. Adventitious layer was made up of a grayish but clear mem-

branous structure (Figs. 1, 2).

Histological examination revealed that the central portion of the nodule consisted mostly of degenerated necrotic eosinophiles, together with a small number of neutrophiles. Aggregation of many small cysts, lined internally by a layer of germinal cells, was also observed at the center of the nodule and the cuticular

Fig. 1 Macroscopical appearance of a hepatic echinococcal nodule

Fig. 5 A nodule showing regressive cuticular layer. PAS stained.  $\times 15$ 

Fig. 2 Cut surface of a nodule

Fig. 3 Histological view of a nodule showing aggregation of echinococcal cysts in the center. H-E stained.  $\times 40$ 

Fig. 4 Magnification of the central portion of a nodule showing multilocular echinococcal cysts. PAS stained.  $\times 100$ 



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Locality	No.	Positive for echinococcal foci		Nodules due to non-	Total
	examined	With larva	Without larva	echinococcal causes	
Higashimokoto	11,873	13	8	5	26
Bihoro	13,301	2	6	1	9
Abashiri	4,789	8	3	4	15
Tsubetsu	1,581	4	1	0	5
Koshimizu	9,752	3	13	5	21
Memanbetsu	5,593	2	1	1	4
Tokoro	7,480	2	5	2	9
Shari	2,715	0	1	1	2
Kiyosato	977	0	1	0	1
Monbetsu	225	0	1	0	1
Kunnepu	129	0	0	0	0
Engaru	51	0	0	0	0
Ikutawara	28	0	0	0	0
Saroma	27	0	0	0	0
Kitami	16	0	0	0	0
Rubeshibe	10	0	0	0	0
Tanno	10	0	0	0	0
Total	58,567	34	40	19	93

Table 1 Incidence of E. multilocularis infection in swine (Number of cases)

layer of the cyst was strongly eosinophilic as well as PAS positive. No brood capsule nor protoscolex was seen. Strong cellular response of the host, in the form of a granuloma which consisted of a large number of epithelioid cells and occasionally the foreign body giant cells, lymphocytes, histocytes and dense fibrous proliferation, was observed around the central portion. Proliferation of the interstitial connective tissue, eosinophile infiltration, lymphocyte accumulation, deposition of hemosiderin and centrilobular congestion were observed in the tissue around the nodule (Figs. 3, 4 and 5).

From the above observation, the lesion was diagnosed as the initial developmental stage of larval *E. multilocularis*.

#### Discussion

Many reports on *E. granulosus* but not *E. multilocularis* infection in domestic ungulates, namely, swine, sheep, cattle and horses in Hokkaido have been published (Ida *et al.*, 1955; Kanemaru *et al.*, 1976; Ohbayashi, 1975, 1978; Ono et al., 1963; Ueda et al., 1958; Yamashita, 1973). More than half of these were imported animals. E. multilocularis infection in foxes, dogs and voles has been reported in eastern Hokkaido and Rebun Island, which is north of Hokkaido (Kamiya et al., 1977; Ohbayashi, 1975; Yamashita, 1973) and these areas have been designated as enzootic area of that cestode. However, E. multilocularis infection in ungulate has never been reported in Japan. Ohbayashi et al. (1971), in their study of the development of E. multilocularis in experimentally infected horse and goats, produced negative results and found only regressive foci in the livers of these animals.

The only reports of *E. multilocularis* infection in domestic ungulates were that of Lukashenko (1968, 1971). He showed that although sheep, cattle and swine can be experimentally infected with *E. multilocularis*, they cannot serve as intermediate host because of the sterility of the larvocyst. Our observation of the strong host cellular response and the regressive change of the cyst in the swine

liver support his findings. Our observation led to the finding of a high incidence of *E. multilocularis* in the voles caught in the surrounding areas of the infected swine farm (Yagi *et al.*, 1983). This study also resulted in the affected areas being designated as enzootic area of *E. multilocularis*.

### Summary

Of the 58,567 swine slaughtered between December 1982 to July 1983, at the Higashimokoto Meat Inspection Center, Hokkaido, 93 were observed to contain nodules in the liver. These hepatic lesions revealed the presence of *Echinococcus multilocularis* cyst in 34 cases and lesions resembling those of that cestode in 40 cases. The rest were diagnosed as nodules due to other causes.

The hepatic nodules were oval in shape, clearly defined and range from 1 to 20 mm in diameter. Histological examination revealed that the central portion of the nodule consisted mostly of degenerated necrotic eosinophiles, together with a small number of neutrophiles. Since aggregation of many small cysts was observed at the center of the nodule and the cuticular layer of the cyst was strongly eosinophilic as well as PAS positive, the lesion was diagnosed as the initial developmental stage of larval *E. multilocularis*.

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1982年12月から1983年7月までの間に,北海道網走保 健所東藻琴食肉検査事務所で検査した豚58,567例のう ち,93例の肝臓に結節病巣が認められた. 組織学的検索 の結果,34例に多包虫が認められ,他の40例に虫体は検 出されなかつたが,類似病変が認められた.残りの19例 は,他の原因によるものであつた. 病巣は、円形の比較的境界明瞭な結節で、直径が1か ら20mmであつた. 組織学的には、結節の中心部に多数 の変性壊死好酸球と少数の好中球の集塊があり、周囲に 肉芽組織層を伴つていた.病巣中心部に多数の小形シス トの集りからなる多包虫が存在し、シストの壁に好酸性 で PAS 陽性のクチクラ層が証明された.