5. CHAPTER Ⅳ

Etiologically Study of Mesothelioma in Cattle 64 Autopsy Cases during 23 Years (1974-1996)

5.1 *INTORODUCTION*

Mesothelioma is a tumor originating from the mesoderm of celomic serous membranes in the thoracic and peritoneal cavities. Although the tumors occur in various animals, it is rare, except for cattle [5,12,22,30,31,36,52,54,60,62]. However, the incidence in cattle is not high, accounting for 0.22-1.4% of entire tumors [5,34]. Although mesothelioma has been found in the form of a congenital tumor in fetuses in the terminal gestational period and in young cattle, it is more likely to be found in mature animals other than cattle [12,22,36,57]. The mechanism of the occurrence of mesothelioma in animals including cattle is unknown [11,17].

In humans, the annual incidence is 0.34-3.5 per one million persons [62]. Since Wagner's report in 1962 [60], there have been many reports in which asbestos has been involved [5,13,16,33,52,61,58]. In the central area of Turkey, there have been some reports on mesothelioma in which volcanic ash is involved [6,7,8,9,32,51].

Within the jurisdiction of the Miyakonojo Meat Inspection Office, there have been many cattle mesothelioma cases reported, including "15 intraperitoneal tumors" by Kawabata et al in 1982 and "cattle malignant mesothelioma" by Goto et al in 1985. In 1997, we made a presentation concerning mesothelioma at the 123rd meeting of Japan Veterinary Science Association. We conducted a pathological study in 64 mesothelioma cases detected at Miyakonojo Meat Inspection Office for the 23-year period from April, 1974 to March, 1997, including our past reports [27,28]. The incidence of cattle

mesothelioma was compared to national figures, Miyazaki prefecture, and the jurisdiction of Miyakonojo Meat Inspection Office. In addition, we attempted to clarify the epidemiology and etiology using surveys of livestock farmers and breeding sites in the Miyakonojo area.

5.2 *MATERIALS AND METHODS*

Intrathoracic and peritoneal tumors were detected in 138 of 162, 328 cattle for the 23-year period from April, 1974, when Miyakonojo Meat Inspection Office was established, to March, 1997. Among those cases, 64 mesothelioma cases were used for comparative data. In the recent cases and for cases for which usable data/specimens has survived and can be used for investigation, fixation was conducted with 10% neutral buffered formalin, masked formalin, and Carnoy's fluid and paraffin slices were made. The slices were stained according to conventional methods using various staining materials, including HE, Azan, PAS, Alcian Blue, toluidine blue (pH2.5, pH 7.0). The immunohistochemistry staining was conducted by avidin-biotin-peroxidase complex (ABC) method using cytokeratin (Pre-diluted, Dako), vimentin (1:200, Dako), and a carcinoembryonic antigen (CEA, pre-diluted, Dako) as primary antibodies (Vector stain ABC kit, Vector Lab, USA).

For comparison of individual regional incidences of mesothelioma, we used the statistic documents from surveys about mesothelioma at five meat inspection offices in Miyazaki prefecture, not including Miyakonojo, and from those about actual situation of tumors in meat animals (cattle, pigs, horses, and others) conducted by the Pathology Division of the National Meat Sanitary Inspection Council (1978-1993) for 16 years.

The survey concerning the actual situation of livestock farmers investigated the use of "Shirasu" (the volcanic ash) at 254 livestock farmer sites in the Miyakonojo area

where mesothelioma has been found frequently.

5.3 *RESULTS*

Among 162, 328 cattle examined for 23 years from April, 1974 to March, 1997, intrathoracic and peritoneral distributed tumors were detected in 138 cases, including mesothelioma as tumor-like lesions in 64, adnocarcinoma as liver or lung primary lesions or others in 20 (Fig. 90, 19, 92, 93, 94, 95), squamous cell tumors in seven (Fig. 100), malignant ovarian granule cell tumors in seven (Fig. 96,97, 98, 99), lymphomas in five (Fig. 100) and unknown cases in 16. Non-neoplastic lesions detected included peritonitis in 12 (Fig. 102, 103), actinomycosis in five (Fig. 105), and granulomatous inflammation in two (Fig. 104) (Table 8).

The detection rate of the 64 mesothelioma cases (Fig. 66, 67, 68, 69. and Fig. 70, 71, 72, 73.) was 39.4 / 100,000 in average for 23 years. The breakdown of breed was 61 Japanese black cattle and three Holstein cattle and all were females aged 1-20, including one cow under one year-old. The majority of the cattle was the Japanese black breed with an average age of 10.9 years (Table 9).

Clinical findings included traumatic gastritis, expanded abdominal circumference, leanness, anorexia, astasia, respiratory distress, pregnancy, pyrexia and others. A total of 38 sick cattle (59.4%) were transferred in. Ascites and pleural effusion were confirmed in 28 cases (43.8%).

Macroscopic findings were a large number of distributed nodal lesions with various sizes similar to millet seed ~ rice grain ~ soybean ~ small finger's tip ~ hen's egg sizes and with white ~ milky white ~ yellow ~ dark red colors in the peritoneum, greater omentum, liver, spleen, diaphragm and pleura. The majority of those were found in the peritoneal cavity, including the peritoneum in 62 (96.9%), greater omentum in 60 (93.8%),

spleen in 60 (93.8%), liver in 55 (85.9%), and diaphragm in 53 (82.8%). In the thoracic cavity, 20 cases (31.3%) had the lesions at the pleura and only two cases showed nodal lesions in the thoracic cavity only. There were 38 lesion cases at the mediastinal lymph nodes (59.4%) (Table 9).

To compare the sites of the occurrences, the primary site data for mesothelioma were investigated at the national level. During 16-year period from 1978 to 1993, 194 mesothelioma cases had been detected. The primary sites were the peritoneum in 115 (about 90%) of the 129 cases excluding an undocumented 65 cases, pleura in five, pericardium in two, diaphragm in two, and other sites in five (Table 10).

As histological findings, time series pictures from the serous membranes indicated that a large number of nodal lesions were formed in the membrane faces (Fig. 80,87, 88, 89.). There were 43 epithelial type cases (Fig. 74, 75, 76, 77, 78, 79.) (67.2%) presenting papillary proliferation and luminal formation of the tumor cells, 13 sarcomatous fibrous type cases (Fig. 80, 81, 82, 83.) (20.3%) having marked stoma hyperplasia and eight biphasic type cases (Fig.84, 85.) (12.5%) presented in both the findings. In 30 cases which could be re-examined, 24 cases were positive for PAS staining (80.0%) and 17 cases (56.7%) were positive for toluidine blue staining of pH 7.0. At pH 2.5, metachromasia was found only rarely (Table 11).

In immunohistochemical staining, the ABC method was attempted in a total of 12 cases, including four epithelial types, four fibrous types and four biphasic types. All the 12 cases were positive for cytokeratin and keratin and negative for CEA. Nine cases (75%) were negative for vimentin (Table 11).

As for the national situation concerning incidence of mesothelioma for the 16-year period from 1978 to 1993, there were 57 cases (53.13 / 100,000 cattle) at Miyakonojo Meat Inspection Office and 17 cases (4.73 / 100,000) in Miyazaki prefecture,

excluding Miyakonojo, and 120 cases (0.71 / 100,000) for Japan (Table 12). The situation of the incidence at individual production sites in Miyazaki prefecture was 27 cases in Miyakonojo city, 11 cases in Kobayashi city, indicating higher incidence in the western region of Miyazaki prefecture. Moreover, mesothelioma cases detected at individual meat sanitary inspection centers all over Japan were investigated involving individual production sites (For various different Prefectures). The highest incidence was 62 cases in Miyazaki prefecture, followed by 22 in Kagoshima prefecture, 11 in Saitama prefecture, 10 in Miyagi prefecture, nine in Kanagawa prefecture, six each in Hokkaido, Hyogo prefecture, and Shimane prefecture. Besides those, there were four cases in nine other prefectures, three in three prefectures, two in five prefectures and one in seven prefectures (Fig.58).

Among the livestock farmers in Miyakonojo area where mesothelioma had occurred frequently, the use of "Shirasu" was confirmed in 149 (58.7%) of 254 farm sites investigated. Especially, small-scale breeding of Japanese black cattle sites, cases were found in 127 farm sites, a large number (Table 13). The breeding sites were cattle barns or grounds, and "Shirasu" was used as bedding in place of sawdust. Reasons for use were said to be because it was cheaper than sawdust, economic, had better water permeability, and the stools could be handled easily.

5.4 *DISCUSSION*

The mesothelial cells which form the serous membranes covering the body cavities originate from the mesoderm. The mesothelium is slightly squamous and arranges with one layer like epithelium and forms distributed tumors at the pleura, peritoneum, and pericardium. The mesothelioma presents a form similar to an epithelial tumor and also a fibromatoid form. Thus, its pathological diagnosis is not often easy to

differentiate from various adenocarcinomas such as lung and liver cancers that present distributed metastases in the peritoneal cavity, and ovarian granule cell carcinoma [5,12,22, 30,31,36,42,52,54,60,62].

We investigated 64 cattle mesothelioma cases detected during the 23 year-period from 1974 to 1997 concerning macroscopic, histological, and epidemiological findings and were able to summarize them as follows. We obtained similar results to the past reports for cattle [11,17,30,34,36,57] and for humans [10,13,18,32,33,35,42,51,52,54,62] as follows: Macroscopically, a tumor lesion appearing to be primary was not found in the other organs and the primary lesion was sufficiently indicated to be at the serous membrane (peritoneum and pleura). Histologically, time-series pictures for the serous membrane were found to indicate that the lesion nodes were formed at the membranes in so far as can be judged from appearances. The immunohistochemical staining showed a positive result for cytokeratin, and a partial positive result for vimentin. An epithelial tendency was clearly shown with more of the epithelial types than the fibrous types similarly to the classification as based on Ackerman [1].

Mesothelioma in humans occurs rarely and Bruckman et al. [14] reported a morbidity of 0.4 ~ 3.7 / one million persons. For total Japan, the morbidity was 0.034 ~ 3.5 [62]. The site of the occurrence was the pleura in about 89%, the highest, followed by the peritoneum and pericardium [18]. As for differences by sex, more males had pleural / peritoneal mesothelioma. The ages were reported to range from 50 to 70 years old [18]. On the other hand, in animals, mesothelioma occurs rarely, except for cattle [7], and has been found in many animals, including horses, pigs, dogs, cats [22] and rats. The incidence in cattle is reported to be 0.22% to 1.4% of all tumors [5,34]. However, our surveys [27,28] showed 64 mesothelioma cattle (17.0%) from among 377 cattle tumor cases within the jurisdiction of the Miyakonojo Meat Inspection Office and a higher

incidence of 39.4 / 100,000 cattle than the other areas. A report by Miyazaki University surveying [44] about tumors in various animals in the Southern Kyushu area showed a high incidence of 14 mesothelioma cases (14.7%) among 95 cattle tumor cases. Using the survey concerning the actual situation for tumors in meat animals (cattle, pigs, and horses) which the National Meat Sanitary Inspection Center Council executed in 1995, we attempted to conduct a comparison with the nationwide data. The incidence of cattle mesothelioma in Japan for the 16-year period was 0.71 (120 / 16, 819, 090). The incidence at the Miyakonojo Meat Inspection Office during the same period was 53.1 / 100,000 cattle, about 75 times higher than the national incidence.

In human mesothelioma, many cases involved the thoracic cavity and there were more males than females [18]. In cattle, peritoneal mesothelioma was found in many cases and most of the cases were females. Our surveys [27,28] showed similar results. A reason for the low incidence in the males was inferred to be because male cattle for fattening are not bred for a long period such as three years or longer.

Wagner [60] and Sleggs et al.[51] pointed out first that a factor likely related to mesothelioma is asbestos. It is stated that a common factor related to mesothelioma in humans is exposure to asbestos at work sites in many cases, and there have been many reports concerning mesothelioma [9, 13, 14, 16, 18, 33, 35, 38, 58, 60, 62].

On the other hand, in epidemiological surveys in central Turkish area, Baris et al. [6,7,8,9]reported about human pleural mesothelioma in 1978, 1979, 1981 and 1987, and Metintas et al. [32] reported it in 1999. There was no hiding of asbestos underground and asbestos product factories in Ankara and Cappadocia in the central area of Turkey volcanic ash tuff is used as a wall material for houses in this area and is called "Aktoprak" (white soil) so named because of its color. The material has been used as baby power and for pottery production. As such, "Aktoprak" is being used specifically and directly by

residents of this area. Because the volcanic ash (white soil) contains a large amount of fibrous etionite, the cause-effect relationship and the frequent incidence of use, the high incidence human mesothelioma can be observed.

Stanton and Pott et al. reported from an experimental study that inducers of mesothelioma include radiation, chronic inflammation, mineral oil, glass fiber, beryllium, and silica, besides natural minerals such as asbestos. Wagner et al. [60, 61] and Baris et al. [6,7,8,9] also reported about the necessity to pay attention to natural fibrous minerals.

In Miyakonojo area within the jurisdiction of Miyakonojo Meat Inspection Office, which locates at Kirishima piedmont, volcanic ash of welded tuff gushed at the great eruption of Mount Kirishima 20,000-30,000 year ago [50] exists all over the Shirasu terrace and is several ten's of meters deep in some areas (Fig. 59). This volcanic ash resembles asbestos-like substances such as crocidolite, chrysolite and amoacyte and is a volcanic glass substance with SiO_2 as a main component (Table 14). The grain sizes vary with 40 to 300μm and it is called "Shirasu" (white sand) due to the color [50] as "Aktoprak" (white soil) in central area of Turkey. Moreover, because it is cheap and has a high water permeability and white color, indicating purity, in Japan, it is used for a unique Japanese custom during the New Year's season that white soil be placed over the garden or hallway to eliminate dark-colored substances (Fig. 60). Livestock farmers in Miyakonojo area use the water permeability of Shirasu for cattle barn bedding to prevent the appearance of being dirty or on outside breeding grounds (Fig. 61, 62, 63, 64.). The survey of actual use of Shirasu revealed that about 60% of the livestock farmers were using Shirasu. Especially, side-work and small-scale livestock farmers with only a few Japanese cattle tended to use the cheap and highly water-permeable Shirasu, but not sawdust. In the 64 mesothelioma cases most of them were old Japanese cattle with an average age of 11 which had been bred by side-work farmers with only few cattle. Shirasu

had been in use in many cases. Those findings were interesting. Zeolite beds containing volcanic ash as well as Shirasu are distributed in Hokkaido, Tohoku, Kanto, and Sannin areas [50], where the incidences of mesothelioma showed a similar tendency to those of the prefectures with the high incidence. Thus, in the future, it will be necessary to conduct further studies on the cause-effect relationship between mesothelioma and natural fibrous minerals such as volcanic ash.

In an animal experiment using rats, there has been a report that asbestos absorbed into the lungs by aspiration exposure caused pleural mesothelioma [58]. In humans, there have been many cases of pleural mesothelioma caused through the air ducts [18]. Among 64 cases in this study, 62 cases were peritoneal mesothelioma and only two were pleural mesothelioma. The national situation concerning the incidence rate tended to indicate that most of the cases were peritoneal mesothelioma. Because of the unique preference of cattle to eat foreign matter, it is speculated that carcinogens of mesothelioma may be taken in orally and transferred to the digestive organs, particularly the gastric mucosa of the abdominal cavity, to cause peritoneal mesothelioma at a high rate. In the past 13 years, the gastric morbidity of cattle detected in ordinary meat inspections at Miyakonojo Meat Inspection Offices was only 4.2%, including traumatic gastritis. However, when we conducted thorough examinations of the gastric mucosa of the I ~ IV stomachs in about 350 cattles after gastric contents were removed and the stomach was washed, gastritis was confirmed in about 23% of the I stomach, including ulcers, and about 46% of the III and IV stomachs. In the future, studies about the relationship of cattle mesothelioma with gastric diseases including traumatic gastritis will be necessary.

The composition of Shirasu consists of volcanic glasses resembling asbestos very closely. In the Miyakonojo area, residents have the unique custom of using Shirasu in their everyday life. Because Shirasu is economic and easily available, side-work farmers who

breed Japanese cattle for production use Shirasu for their cattle barns. Such a situation has many features in common daily life environmental aspects of the white soil, a cause of mesothelioma in Turkey. Because the morbidity of cattle gastric diseases is higher than the other diseases detected at ordinary meat inspections, it will be also necessary to conduct comparative studies to elucidate why the incidence of peritoneal mesothelioma is higher than the pleural type. Although as far as the adverse effects of Shirasu on humans is concerned, there has been no report on frequent incidents of mesothelioma in the Miyakonojo area. Taking into consideration the fact that in humans, such oral intake of foreign materials as seen in cattle is absent and the morbidity of pleural mesothelioma by aspiration exposure in is low in cattle, the effect of Shirasu on humans is inferred to be very low. However, follow-up investigations of workers engaged in the collection of used Shirasu over a long period will be necessary in cooperation with local medical organizations.

Here, at the end of this paper, we wish to deeply thank the persons related to Yokohama City Meat Sanitary Inspection Center Pathology Department of the National Meat Sanitary Inspection Center Council and all of the meat sanitary inspection centers in the prefectures of Kyushu, including those in Miyazaki prefecture who provided precious tumor statistics, and those related to the Miyazaki prefecture Chemical Center who provided documents concerning the chemical composition of Shirasu and other related information.

5.5 *ABSTRACT*

At the Miyazaki Prefecture Miyakonojo Meat Inspection Office, mesothelioma was detected in 64 of 162,328 cattle for the 23-year period from April, 1974 to March, 1997. The incidence was 39.4 / 100,000 cattle. Macroscopic findings were for nodal lesions distributed over a range of different sizes similar to millet seed~rice grain~soybean~small finger tip~hen egg and with white~milky white~yellow~dark red colors in the peritoneum, greater omentum, liver, spleen, diaphragm and pleura.

Histopathologically, it was shown by time-series pictures of the serous membranes that the lesions had formed on the membrane face. The lesion types were epithelial type with papillary proliferation or luminal formations of tumor cells in 43 cases (67.2%), sarcomatous fibrous type with marked stoma hyperplasia in 13 (20.3%) and biphasic type with both found in eight animals (12.5%). The 64 cases included 61 Japanese black cattle and three Holstein cattle and all were females. The mean age was 10.9 and ranged from 1-20.

There have been only a few reports concerning the high incidence of cattle mesothelioma in specific areas in Japan. In Miyakonojo / some Northern prefectural areas within the jurisdiction of the Miyakonojo Meat Inspection Office, volcanic ash called "Shirasu", found specifically in Southern Kyushu has been used frequently for bedding in cattle barns and outside breeding grounds. This volcanic ash contains chemical components similar to asbestos. Thus, it was inferred that the ash might be involved in the occurrence of mesothelioma as "Aktoprak" (white soil), ash is in the central area of Turkey.

KEY WORDS : mesothelioma, Southern Kyushu, volcanic ash, Shirasu, asbestos.

Table 8. Disseminated peritoneal masses of 138 autopay cases

Classidiction＼Organ	cases	Normal cow	Disease	Pleura	Diaphragm	Peritoneum	Great omentum	Mesentery	Lung	Liver	Spleen	Uterus	Ovary	Urinary bladder	Other	Mediastinal lymph	Abdomenal lymph	Trunk lymph	Peritoneal fluid
Neoplastic lesions																			
Malignant mesothelioma	**58**	21	37	20	50	56	56	53	12	53	56	18	10	16	0	37	8	10	28
Mesothelioma	**6**	5	1	0	3	6	4	1	0	2	4	1	2	0	0	1	0	0	0
Adenocarcinoma	**20**	6	14	8	12	17	13	9	7	12	12	5	3	3	7	12	5	6	3
Guranulosa cell tumor	**7**	2	5	1	2	6	7	3	1	4	4	0	7	1	2	3	0	0	0
Squmous cell tumor	**7**	0	7	1	4	6	5	4	4	6	6	1	1	0	1	3	3	0	0
Leukemia	**5**	0	5	0	0	5	2	4	2	4	4	3	0	0	3	5	3	3	0
Unknown	**16**	9	7	5	8	15	13	6	2	12	8	3	5	0	4	5	4	1	1
Non-neoplastic lesions																			
Peritonitis	**12**	7	5	1	5	12	7	2	0	4	7	2	0	0	0	2	0	0	2
Actinomycosis	**5**	2	3	2	2	5	3	1	1	4	3	0	0	0	0	1	0	0	1
Glanulomatous inflammation	**2**	2	0	0	1	1	1	1	0	2	0	0	0	0	0	0	0	0	0
Total	**138**	**54**	**84**	**38**	**87**	**129**	**111**	**84**	**29**	**103**	**104**	**33**	**28**	**20**	**17**	**69**	**23**	**20**	**35**

Table 9. Summary of findings in 64 cases of mesothelioma

Case No	Fiscal Year	Breed	Gender	Age	Hair color	Weight(about)	Production site	Normal cow	Disease cow	Clinical sign	Pleura	Diaphragm	Peritoneum	Greater omentum	Mesentery	Other	Lung	Liver	Spleen	Uterus	Ovary	Urinary bladder	Mediaastinal lymph	Abdomen lymph	Trunk lymph	Other	Peritoneal fluid	Histological types
1	1977	JB	♀	10	B	310	Miyakonojo	○					◎	◎	◎			◎	◎									fibrous
2	1978	JB	♀	8	B	380	Takasaki		○	Pyrexia,Tachypnea		◎	◎	◎	◎			◎	◎	◎			○	○	○		◎	biphasic
3	1978	JB	♀	8	B	450	Mimata		○	unknown		◎	◎	◎	◎			○	○				○		○		◎	biphasic
4	1978	JB	♀	11	B	360	Kobayashi		○	After surgical operation		◎	◎	◎	◎			○	◎	◎				○			◎	biphasic
5	1978	JB	♀	12	B	450	Sueyoshi		○	Abdominal swelling,Tachypnea		◎	◎	◎	◎			○	○								◎	epithelial
6	1978	JB	♀	10	B	380	Kobayashi		○	Strenal recumbency		◎	◎	○	○			○	○									epithelial
7	1978	JB	♀	13	B	300	Kobayashi		○	Sternal recumbency,Emaciation		◎	◎	◎	◎		○		◎	◎							○	epithelial
8	1979	JB	♀	16	B	350	Nojiri		○	Emaciation,Sternal recumbency		◎	◎	◎	◎			○	○						○	○	◎	biphasic
9	1979	H	♀	11	BW	590	Miyakonojo	○				◎	◎	◎	◎		○	○	○	○		○		○	○			fibrous
10	1979	JB	♀	8	B	450	unknown		○	Abdominal swelling		◎	◎	◎	◎			○	○	○			○				◎	epithelial
11	1979	JB	♀	11	B	480	Miyakonojo	○			○	◎	◎	◎	○			○	○									epithelial
12	1980	JB	♀	5	B	530	Miyakonojo		○	Strenal recumbency	◎		◎	◎	◎		○	◎	◎				○	○	○		○	epithelial
13	1980	H	♀	12	BW	430	Miyakonojo		○	Abdominal swelling		◎	◎	◎	◎		○	◎	◎				○		○		○	fibrous
14	1980	JB	♀	13	B	480	Takarabe		○	Abdominal swelling		○	◎	○	○			○	○				○				◎	epithelial
15	1981	JB	♀	16	B	450	Sueyoshi		○	Pyrexia,Tachypnea	◎	◎	◎	◎	◎			◎	◎	◎		○	○				◎	fibrous
16	1982	JB	♀	17	B	400	Miyakonojo		○	Pyrexia,Tachypnea			◎	◎	◎			◎	◎				○					epithelial
17	1984	JB	♀	13	B	400	Takajo		○	Abdominal swelling	◎	◎	◎	◎	◎		○	◎	◎		○		○				◎	epithelial
18	1984	JB	♀	10	B	—	Miyakonojo	○			○	○	◎	◎	◎			◎	◎				○					epithelial
19	1985	JB	♀	10	B	490	Miyakonojo	○				◎	◎	◎	◎			◎	◎				○					epithelial
20	1985	JB	♀	10	B	500	unknown	○					○	○														fibrous
21	1985	JB	♀	10	B	520	Miyakonojo	○				◎	◎	◎	◎			◎	◎	○			○					biphasic
22	1985	JB	♀	12	B	450	Miyakonojo		○	non-appetite	◎	◎	◎	◎	◎			◎	◎	○	○	○	○					epithelial
23	1985	JB	♀	12	B	400	Sueyoshi		○	Fracture,Pregnancy		◎	◎	◎	◎			○	◎				○		○			fibrous
24	1985	JB	♀	13	B	500	Miyakonojo	○				○	○	○	○			○	○				○					epithelial
25	1985	JB	♀	14	B	350	Miyakonojo		○	Mesenteric fat necrosis		◎	◎	◎	◎			◎	◎				○				○	epithelial
26	1985	JB	♀	10	B	410	Miyakonojo	○			○	○	○	○	○			○	○				○					epithelial
27	1986	JB	♀	13	B	400	Aira		○	non-appetite			◎	◎	○				○				○	○				epithelial
28	1986	JB	♀	8	B	570	Miyakonojo	○					◎	◎	◎			◎	◎				○				○	biphasic
29	1986	JB	♀	12	B	—	unknown	○				◎	◎	○	○			○	○									epithelial
30	1986	JB	♀	1M	B	50	Miyakonojo		○	non-appetite			◎	○														epithelial
31	1986	JB	♀	20	B	495	unknown	○			○	◎	◎	◎	◎			◎	○				○		○			epithelial
32	1986	JB	♀	10	B	550	Miyakonojo	○				◎	○					○	◎									epithelial
33	1986	JB	♀	12	B	450	Miyakonojo		○	Fracture,Sternal recumbency	○	◎	◎	◎	◎			○	○	○	◎	○	○				○	biphasic
34	1987	JB	♀	3	B	400	Takarabe		○	Pregnancy,Impaction		◎	◎	◎	◎			◎	◎			○						epithelial
35	1987	JB	♀	8	B	530	Miyakonojo	○				◎	◎	○				○	◎	○	○		○					epithelial
36	1987	H	♀	5	BW	550	Miyakonojo		○	Impaction	○	◎	◎	◎	◎		○	◎	◎		○	◎					○	epithelial
37	1987	JB	♀	13	B	400	Takajo		○	Pyometra		◎	◎	◎	◎			◎	◎	○			○					fibrous
38	1987	JB	♀	15	B	450	Yamada		○	Traumatic reticulitis		◎	◎	◎	◎			◎	◎								◎	fibrous
39	1987	JB	♀	14	B	400	Yamada		○	Pregnancy			◎	◎	◎			◎	◎	○		○						fibrous
40	1987	JB	♀	—	B	—	unknown		○	Pregnancy,Diarrhea		○	◎	◎	◎			◎	◎			◎	○				◎	epithelial
41	1987	JB	♀	9	B	450	Takajo	○					◎	◎	◎			◎	◎				○					epithelial
42	1988	JB	♀	13	B	400	Miyakonojo	○				○	○	○	○			○	○								◎	epithelial
43	1988	JB	♀	11	B	300	Sueyoshi		○	Emaciation,Pregnancy	◎	◎	◎	◎	◎			◎	◎				○	○			◎	epithelial
44	1988	JB	♀	10	B	500	Miyakonojo	○			○	○	○	○	○			○	○				○					epithelial
45	1988	JB	♀	15	B	450	Shibushi		○	Mesenteric fat necrosis		○	○	○	○			○	○	○		○					◎	fibrous
46	1988	JB	♀	10	B	500	Miyakonojo	○				○	○	◎	○			○	○				○		○			epithelial
47	1988	JB	♀	15	B	500	Miyakonojo		○	Traumatic reticulitis		○	◎	○	○			○	○	○	○	○	○				◎	epithelial
48	1988	JB	♀	18	B	400	Takarabe		○	Abdominal swelling	◎	◎	◎	○				○	○	○		○	○	○				epithelial
49	1988	JB	♀	8	B	550	Miyakonojo	○				○	◎	◎	◎			○	○	○		○	○				◎	epithelial
50	1988	JB	♀	15	B	450	Sueyoshi		○	Pneumonia		◎	◎	◎	◎			○	○				○					epithelial
51	1989	JB	♀	11	B	450	Sueyoshi		○	Traumatic reticulitis	○	◎	◎	◎	◎		○	◎	◎	○	○						○	epithelial
52	1989	JB	♀	9	B	—	Takajo		○	Traumatic pericarditis		○	◎	◎	◎			◎	◎	○			○		○		◎	epithelial
53	1989	JB	♀	7	B	500	Miyakonojo	○			○	○	◎	○	○		○	○	○	○	○	○	○				◎	epithelial
54	1992	JB	♀	19	B	350	Miyakonojo		○	Emaciation,nonapitaite			○				○		○									epithelial
55	1992	JB	♀	11	B	500	Miyakonojo		○	non-appetite	○	○	○	○	○			○	○									fibrous
56	1992	JB	♀	17	B	400	Osumi		○	nonapitaite,Abdominal swelling		○	◎	○	○		○	○	○								○	epithelial
57	1993	JB	♀	10	B	400	Miyakonojo	○			○	○	○	○	○			○	○									fibrous
58	1993	JB	♀	5	B	500	Miyakonojo	○				○	◎	◎	○				○									biphasic
59	1994	JB	♀	14	B	400	Miyakonojo		○	non-appetite	◎	◎	◎	◎	◎			◎	◎									epithelial
60	1994	JB	♀	8	B	400	Miyakonojo	○					◎	◎					◎		○							fibrous
61	1995	JB	♀	10	B	400	Matuyama		○	non-appetite		◎	◎	◎				○	○		○	○	○					epithelial
62	1995	JB	♀	3	B	650	Yamanokuchi	○			◎	◎					○				○		○					epithelial
63	1996	JB	♀	8	B	600	Osumi	○				◎	◎	◎	◎			○	○		○	○	○				◎	epithelial
64	1996	JB	♀	3	B	545	Kawaminami	○			◎	◎					○						○					epithelial

◎: Multiple nodules ○: Scatter nodules NO62 and NO64: pleural mesothelioma

JB:Japanese black cattle H:Holstein

Table 10. Site of the occurrence mesothelioma

Primary site	Bovine(case)	(%)	Human(case) *	(%)
Pleura	5	2.6	4181	88.8
Peritoneum	115	59.3	454	9.6
Diaphragm	2	1.0	30	0.6
Pericardium	2	1.0	33	0.7
Vaginl tunics	—	—	9	0.2
Other	5	2.6	—	—
Unknown	65	33.5	3	0.1
Total	**194**		**4710**	

* Hillerdal-1983

Table 11. The results of special staining for the tumors

Staining for	Epithelial type	Sarcomatous type	Biphasic type	Total
Cases	**43**	**13**	**8**	**64**
PAS stain	14/18	4/6	6/6	24/30
Toluidine blue stain				
(pH2.5)	1/18	0/6	0/6	1/30
(pH7.0)	11/18	5/6	1/6	17/30
Alcian blue stain	10/18	6/6	3/6	19/30
Immuno-chemo stain				
(Cytokeratin)	4/4	4/4	4/4	12/12
(Vimentin)	2/4	4/4	3/4	9/12
(C E A)	0/4	0/4	0/4	0/12

Table 12. Incidence of mesothelioma for 1978-1993

Fisical year	Miyakonojo	Miyazaki pre.	Japan	Total
1978	6		6	12
1979	4		1	5
1980	3	1	3	7
1981	1		4	5
1982	1	2	6	9
1983		2	10	12
1984	2	2	4	8
1985	8	1	7	16
1986	7	2	4	13
1987	8	5	6	19
1988	9		13	22
1989	3	1	13	17
1990			9	9
1991			5	5
1992	3	1	21	25
1993	2		8	10
Total	**57**	**17**	**120**	**194**
Nomber of inspection cases	**107,283**	**359,416**	**16,819,090**	**17,285,789**
/100,000	**53.13**	**4.73**	**0.71**	**1.12**

Table 13. The use of "Shirasu" in 254 farm sites

Use of Shirasu	Breeding Farm	Dairy Farm	Fattening Farm	Total
Use	127	22	0	149
No use	84	13	8	105
Total	211	35	8	254

Table 14. Chemical composition of Shirasu and Asbestos

Component	Shirasu	Chysotile	Crocidolite	Amosite
SiO_2	72.58	40.75	52.00	49.70
Al_2O_3	12.29	3.37	-	0.40
Fe_2O_3	1.86	0.44	16.05	0.03
FeO	-	0.28	17.65	39.70
MnO	-	0.03	-	0.22
MgO	0.18	41.28	4.28	6.44
CaO	1.04	0.35	1.20	1.04
Na_2O	3.32	0.07	6.21	0.09
K_2O	3.31	0.04	0.06	0.63
Ig.loss	5.43	13.65	2.68	1.92
Total	99.81	100.69	100.14	100.17

Fig. 58 Incidence of mesothelioma for 16-year (1978-1993)

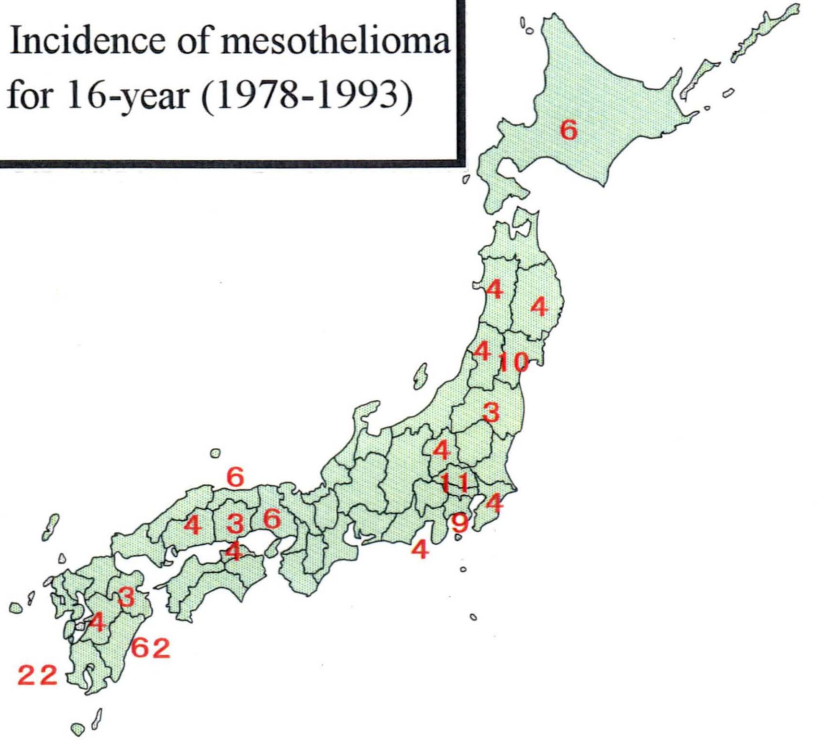


Fig.59

Fig.59 The Shirasu terrace is volcanic ash laying several ten's meters deep in some areas.

Fig.60

Fig.61

Fig.60 Shirasu productions that the volcanic ash is a volcanic glass substance with SiO_2 as a main component.

Fig.61 Unique Japanese custom during the New Year's season the white soil be placed over the garden.

Shirasu using of the livestock farmer

Fig.62

Fig.63

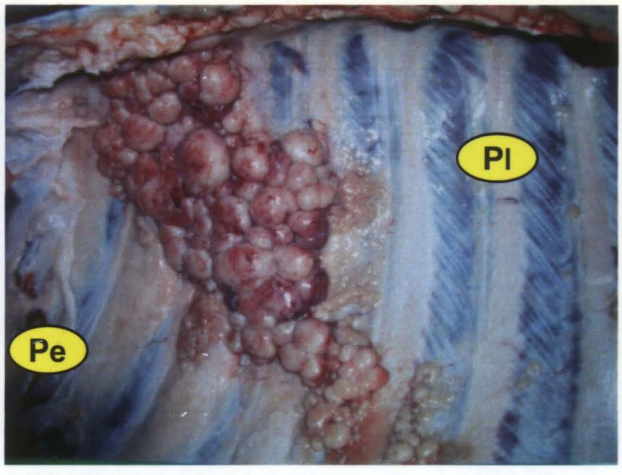
Fig.62 Shirasu at the garden of the livestock farmer in Miyakonojo area.
Fig.63 Many livestock farmers in Miyakonojo area use the water permeability of Shirasu for cattle barn bedding.

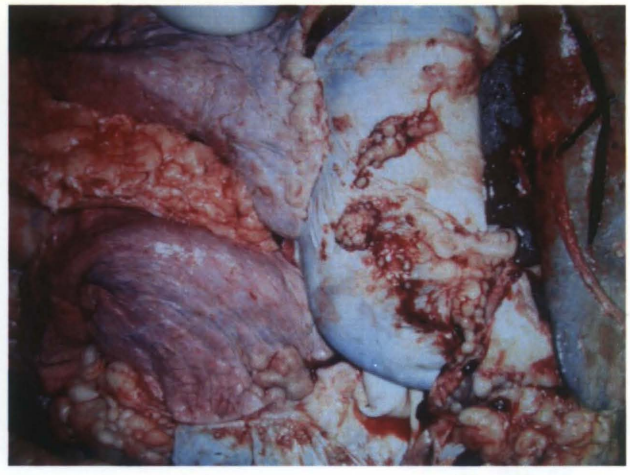
Fig.64

Fig.65

Fig.64 Shirasu at outside breeding grounds.
Fig.65 Shirasu adhere to the body of cattle.

Malignant mesothelioma (epithelial type)



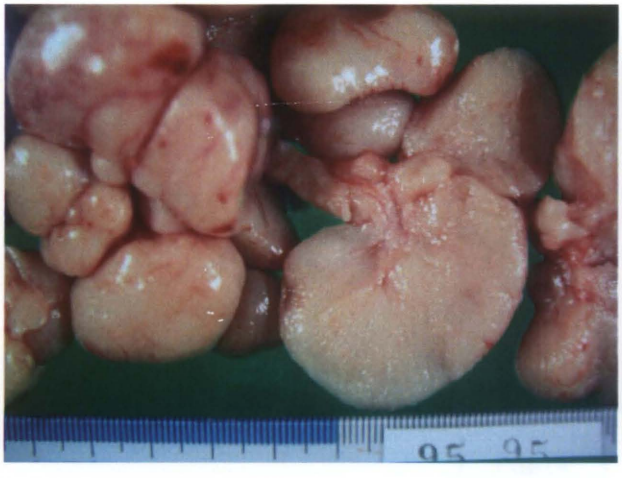


Pl :Pleura Pe :Peritoneum

Fig.66

Fig.67

Fig.66 and Fig.67 Malignant mesothelioma of No 62, a 3-year-old female Japanese black cattle, weighing about 650kg. The masses of pleura and the superficial parts of the lungs and diaphragm, were multiple about 1 to 5 cm in diameter, from gray-white to yellow colored nodules which were consisted of hard tissues like fibrous peduncles.



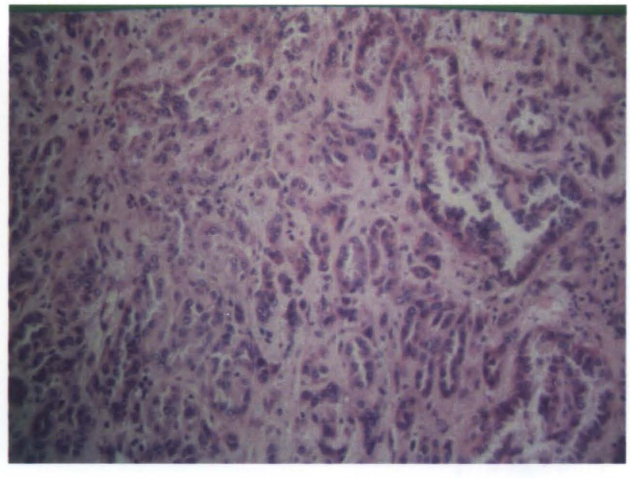
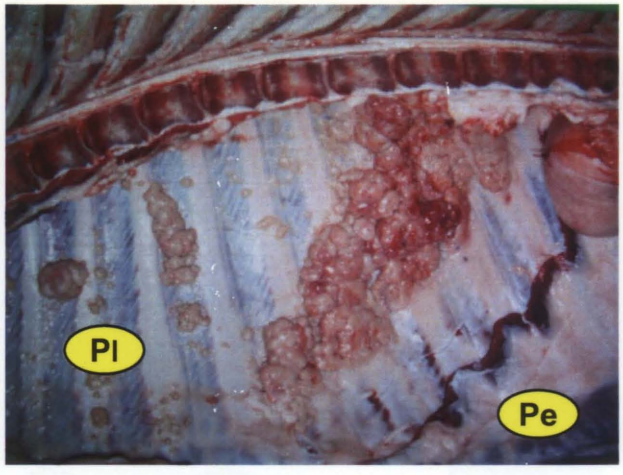


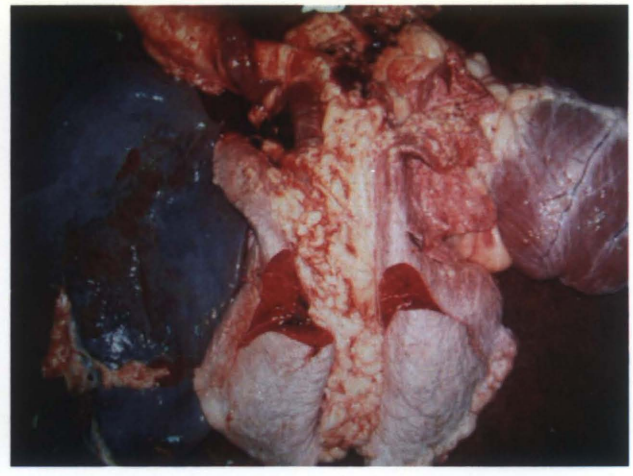
Fig.68

Fig.69

Fig.68 Cut surface of the pleural mass were gray-white.
Fig.69 The pleura tumor cells showing tubular pattern of the epithelial form. HE stain. ×200.

Malignant mesothelioma (epithelial type)





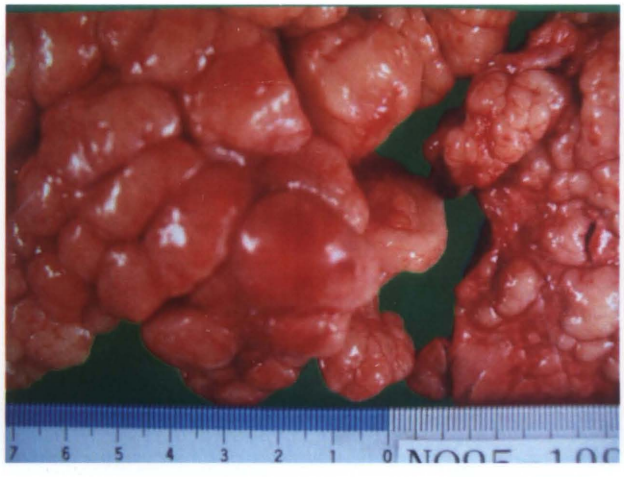
Pl :Pleura Pe :Peritoneum

Fig.70

Fig.71

Fig.70 Malignant mesothelioma of No 64, a 3-year-old female Japanese-black cattle, weighing about 545kg. The pleural surface was disseminated with numerous small neoplastic nodules.

Fig.71 Some superficial nodules founding in the parts of the lungs and diaphragm.



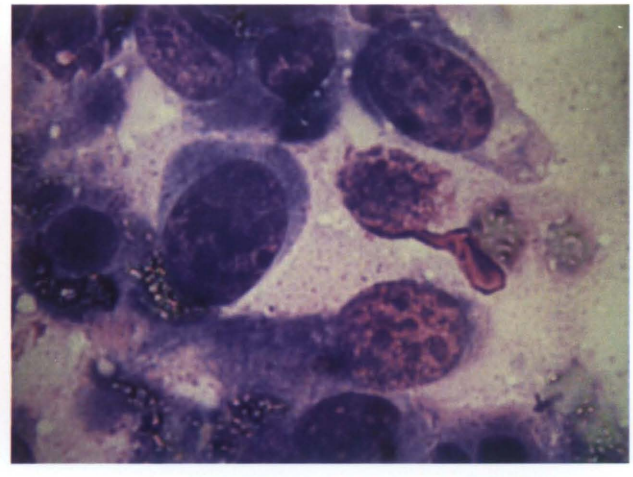


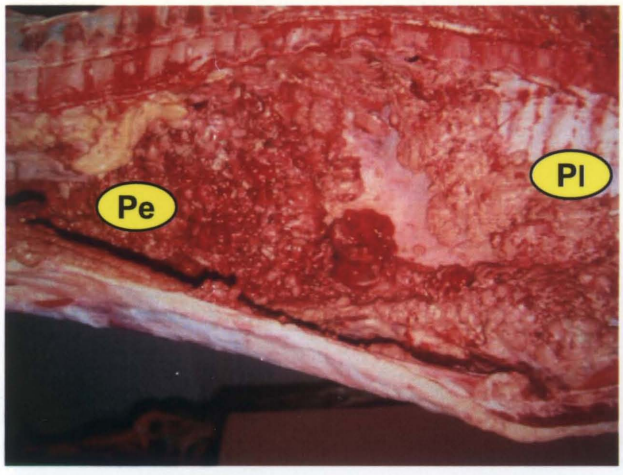
Fig.72

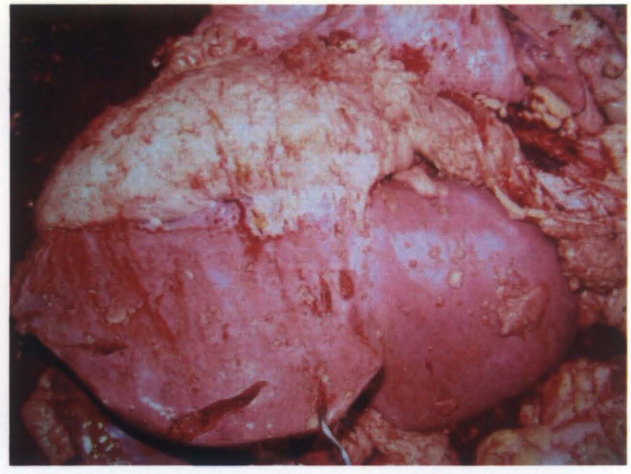
Fig.73

Fig.72 The masses of pleura were multiple about 1 to 3cm in diameter, from gray-white to reddish-yellow colored nodules like fibrous peduncles.

Fig.73 The neopastic cells had moderate amount of eosinophilic cytoplasm and round to ovoid nuclei with the defined chromatin and a distinct nucleolus.
Diff Quick stain. × 1000.

Malignant mesothelioma (epithelial type)



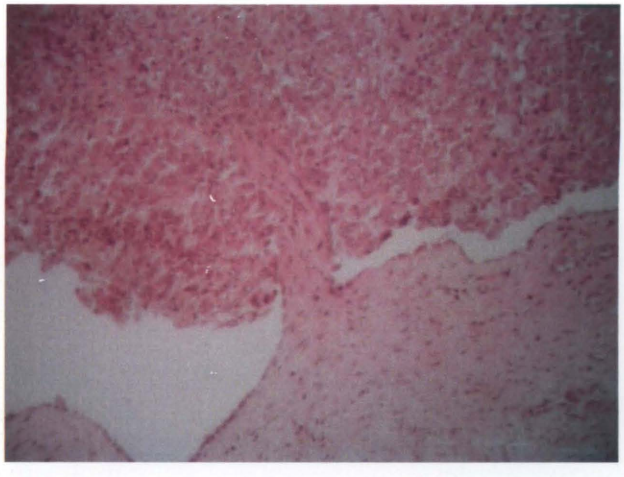


Pl :Pleura Pe :Peritoneum

Fig.74

Fig.75

Fig.74 and Fig.75 Diffusely disseminated malignant mesothelioma of No52, a-9-year-old female Japanese black cattle. Dissemination of numerous small neoplastic nodules showing extend on the peritoneal, abdominal surface. Clinical symptoms were noted traumatic pericarditis, traumatic reticulitis and peritoneal fluid.



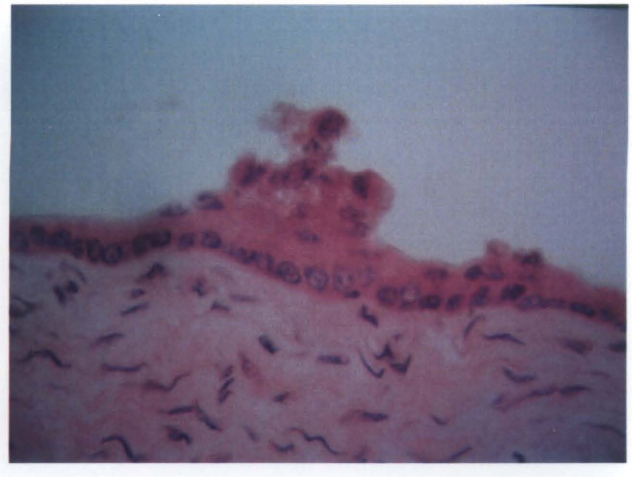
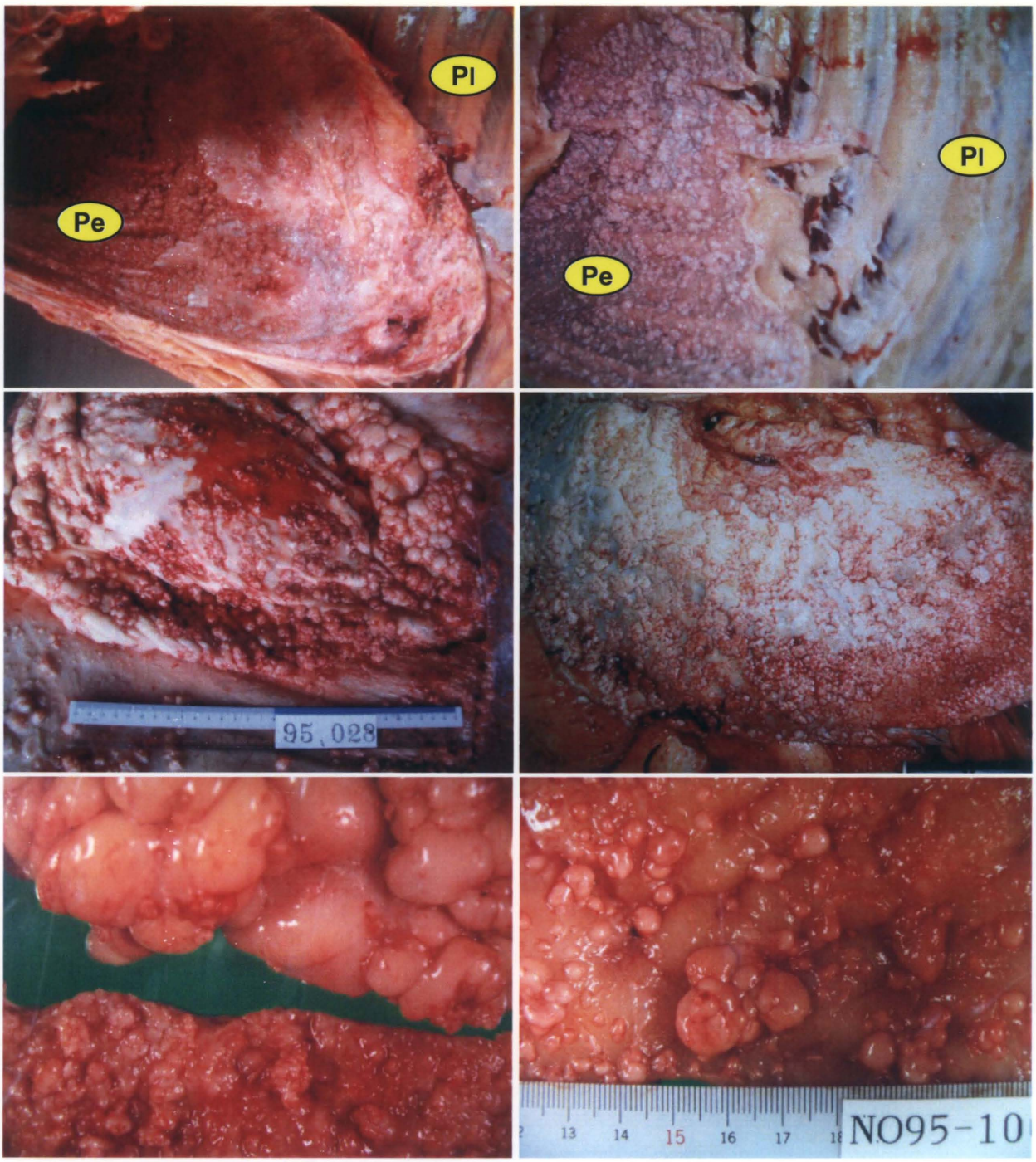


Fig.76

Fig.77

Fig.76 The pedunculated nodule with papilliform growth. HE stain. ×100.
Fig.77 The neopastic cells showing arise from normal mesothelial cells. HE Stain. ×200.

Malignant mesothelioma (epithelial type)



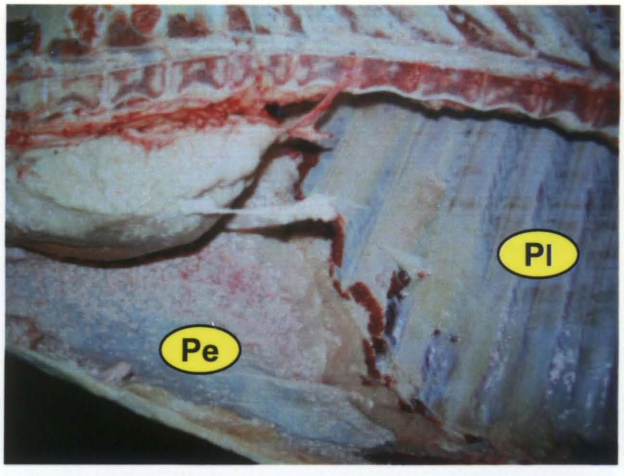
Pl :Pleura Pe :Peritoneum Fig.78

Pl :Pleura Pe :Peritoneum Fig.79

Fig.78 Diffusely disseminated malignant mesothelioma of No61, a 10-year-old female Japanese black cattle, weighing about 400kg. The masses showing extend to the peritoneum and abdominal viscera.

Fig.79 Diffuse malignant mesothelioma of No63, a 8-year-old female Japanese black cattle. The masses showing extend to the peritoneum and abdominal viscera.

Malignant mesothelioma (biphasic type)

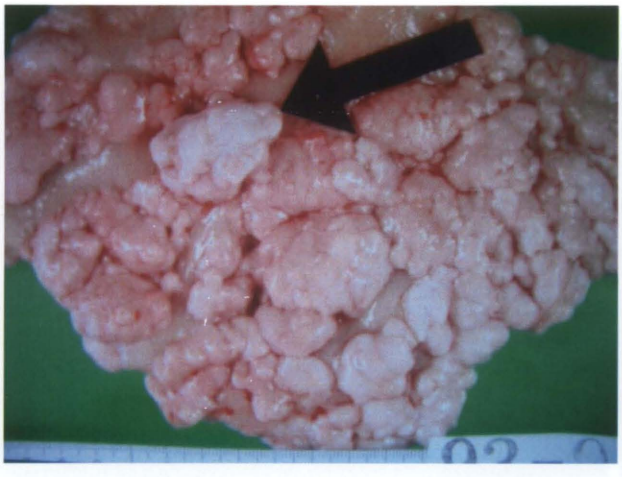


Pl :Pleura Pe :Peritoneum

Fig.80

Fig.81

Fig.80 and Fig.81 Malignant mesothelioma of No58, a-5-year-old female Japanese black Cattle, weighing about 500kg.
The masses showing extend to the peritoneum and abdominal viscera.



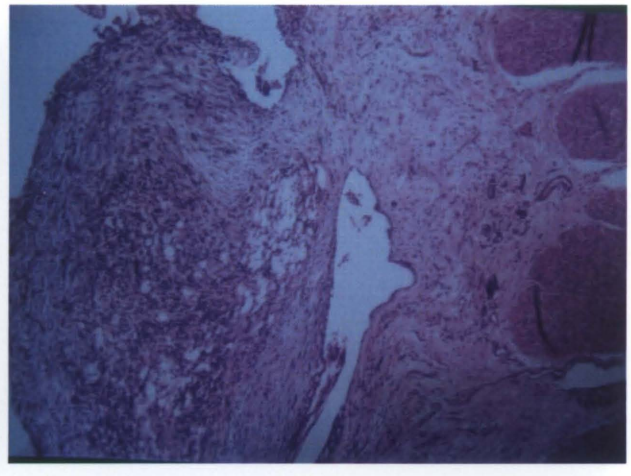


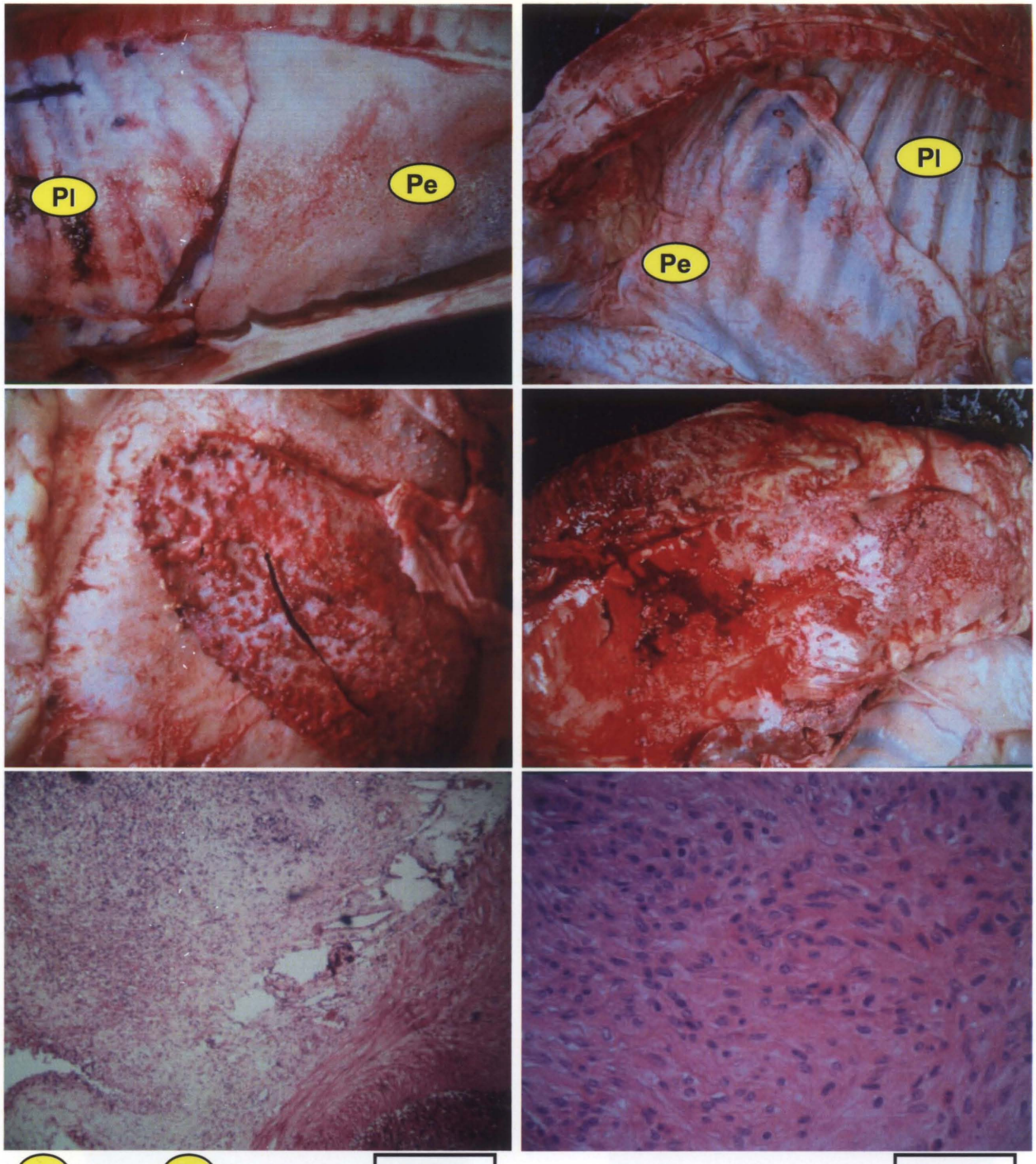
Fig.82

Fig.83

Fig.82 The masses of peritoneum and abdominal viscera were multiple about 1 to 5 cm in diameter, from gray-white to white colored nodules.

Fig.83 The scattered neoplastic nests formed by epithelial cells are surrounded by fibrousarcomatous cells, peritoneum and abdominal viscera tumor cells.
HE stain. ×40.

Malignant mesothelioma (fibrous type)



Pl :Pleura Pe :Peritoneum

Fig.84

Fig.85

Fig.84 Fibrous form of fuse malignant melanoma, No37, a-13-year-old female Japanese black cattle, weighing about 400kg. The neopastic cells are spindle-shaped. HE stain. × 40.

Fig.85 Fibrous form of fuse malignant melanoma, No39, a-14-year-old female Japanese black cattle, weighing about 400kg. The neopastic cells in this form showing spindle-shaped and have an ovoid or elongated nucleus with well developed nucleoli. HE stain. × 200.

Fig.86

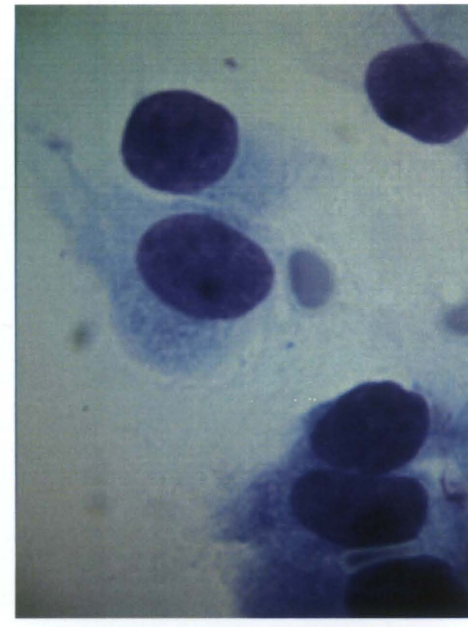


Fig.87

Fig.86 The normal mesothelial cells lining the peritoneum.
HE stain. ×1000.

Fig.87 The normal mesothelial cells. Diff Quick. stain. ×1000.

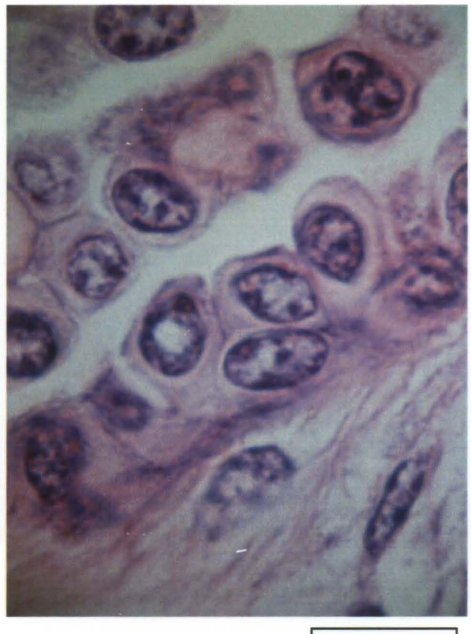


Fig.88

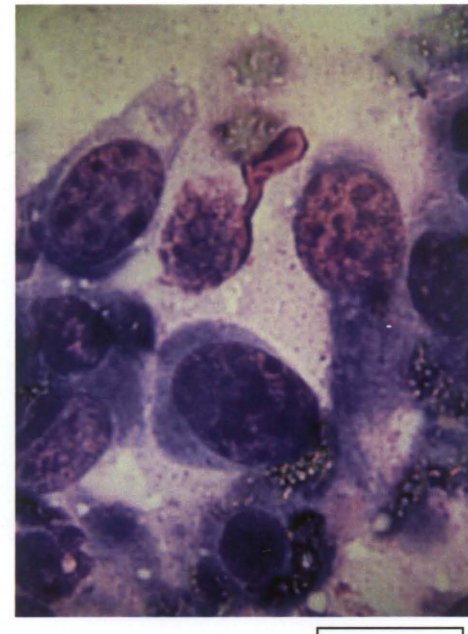


Fig.89

Fig.88 The Malignant mesothelioma, neopastic cells. HE stain. ×1000.

Fig.89 The neoplastic cells. Diff Quick. stain. ×1000.

Disseminated peritoneal masses : Adenocarcinoma

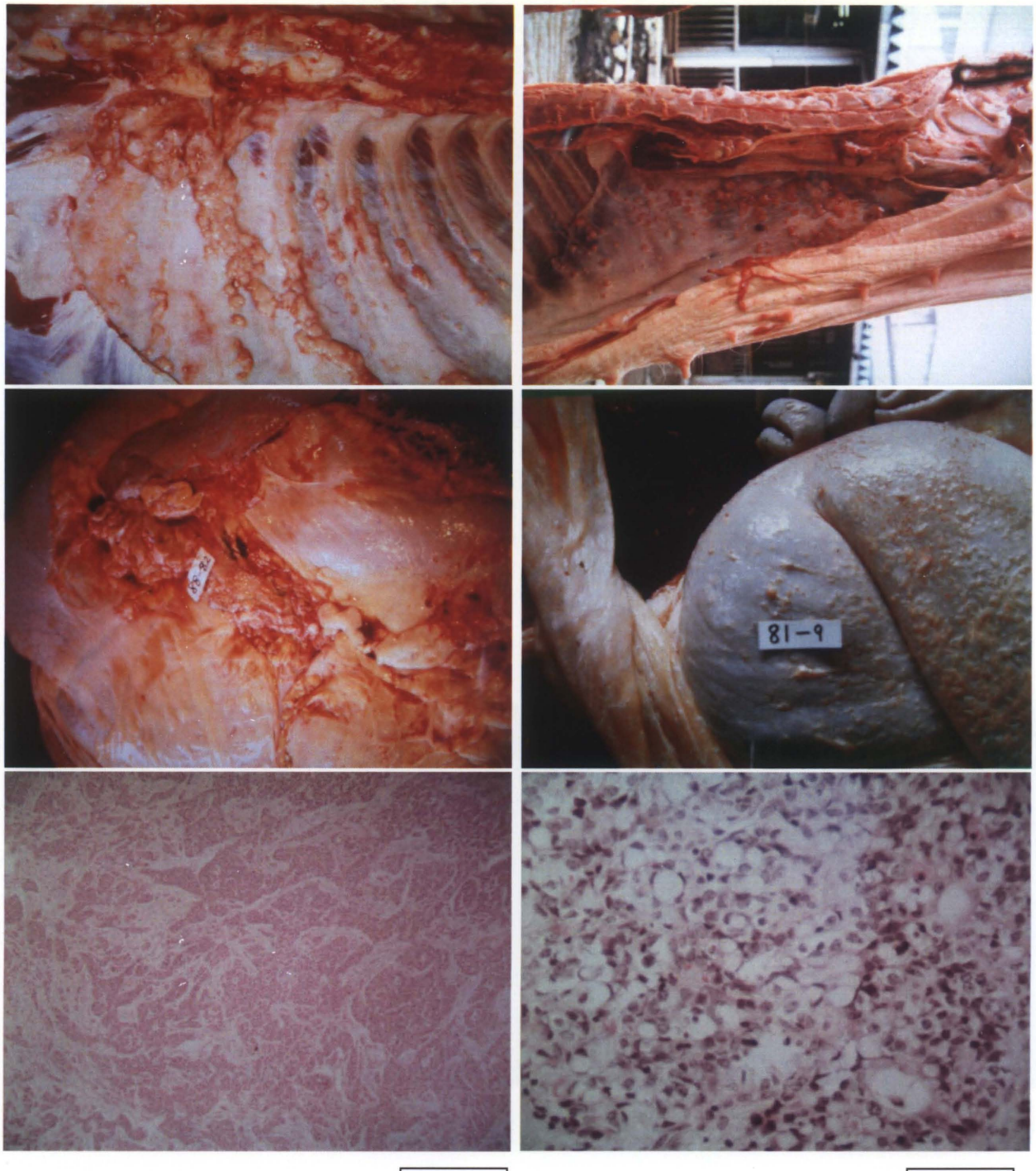


Fig.90

Fig.91

Fig.90 Adenocarcinoma of the colon, a 14-yeat-old female Japanese black cattle, weighing about 400kg. The metastases showing extend to the peritoneum and pleura. The tumors showing discrete nodules 20 to 40 mm in diameter, gray- white colored. The premediastinal lymph nodes were enlarged. HE stain. × 40.

Fig.91 Adenocarcinoma of the rectum、a 14-yeat-old female Japanese black cattle, weighing about 350kg. Dissemination of numerous small neoplastic nodules showing extend on the abdominal surface. Higher magnification of proliferating tumor cells. HE stain. × 200.

Disseminated peritoneal masses : Cholangiocarucinoma

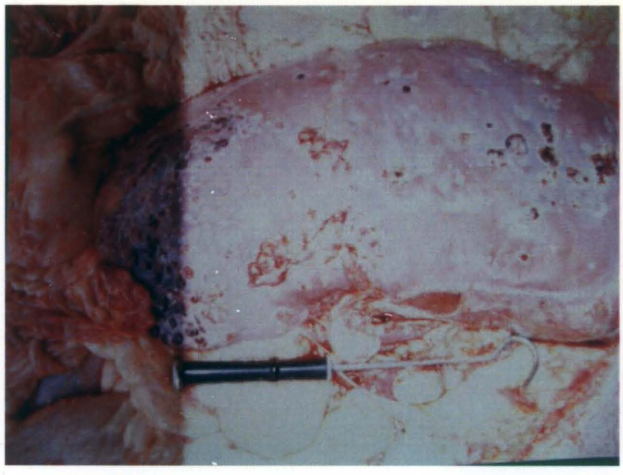


Fig.92

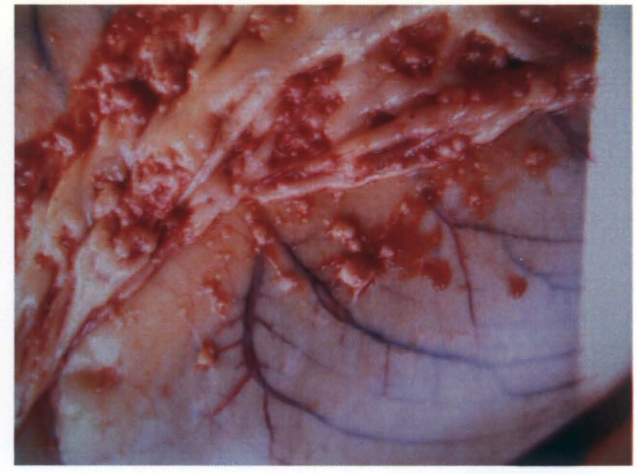


Fig.93

Fig.92 and Fig.93 Cholangiocarucimoma of a 10 year-old female Japanese black cattle. Dissemination of numerous small neoplastic nodules showing extend on the liver and abdominal surface.

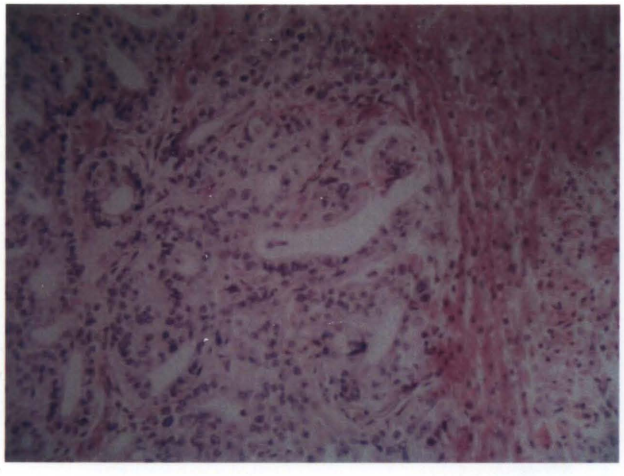


Fig. 94

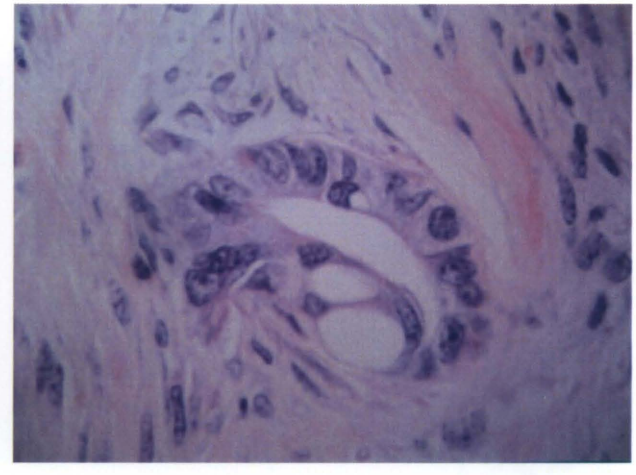


Fig. 95

Fig.94 The tumor of the peritoneum showing tubular form. HE stain. × 100.
Fig.95 The tumor cells are composed of small glandlike structure. HE stain. × 400.

Desseminated peritoneal masses : Malignant granulose cell tumor)

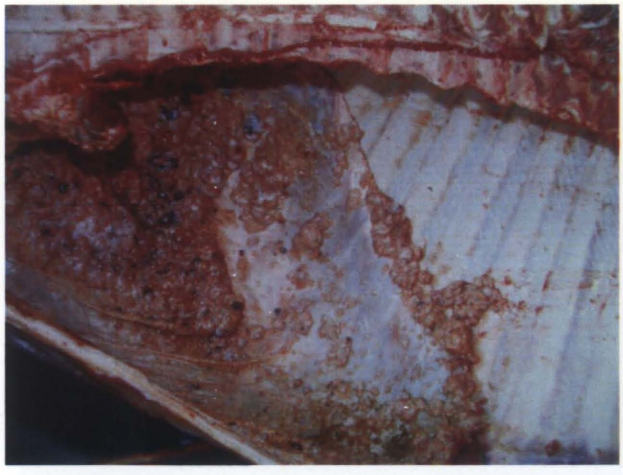


Fig.96

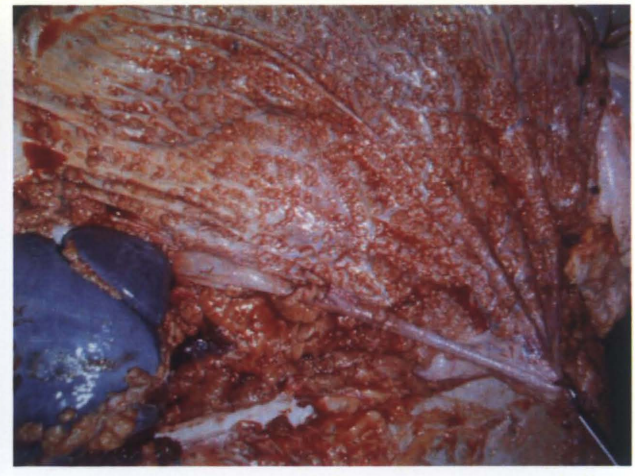


Fig.97

Fig.96 and Fig.97 Malignant granulosa cell tumor in the left ovary of a 15-year-old female Japanese black cattle, weighing about 460kg. Multiple nodules disseminating on the partietal and visceral peritoneal surface are seen.

Fig.98

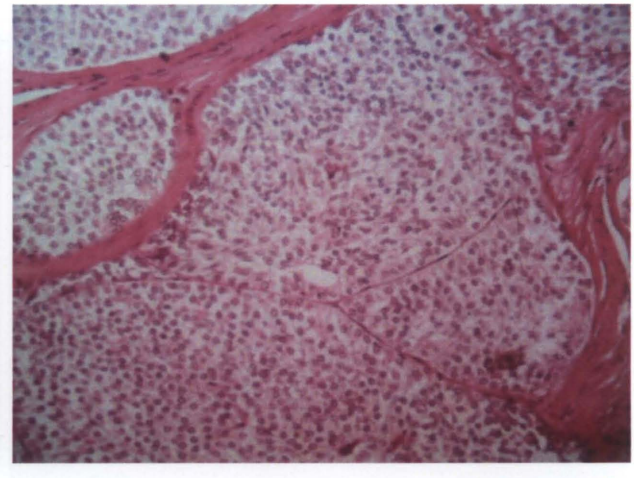


Fig.99

Fig.98 The left ovary was enlarged to approximately 10 × 10 × 10cm in size. The cut surface showing grayish-white and subdividing irregular.

Fig.99 Primary tumor showing the follicular pattern type of guranulosa cell tumor. HE stain. × 100.

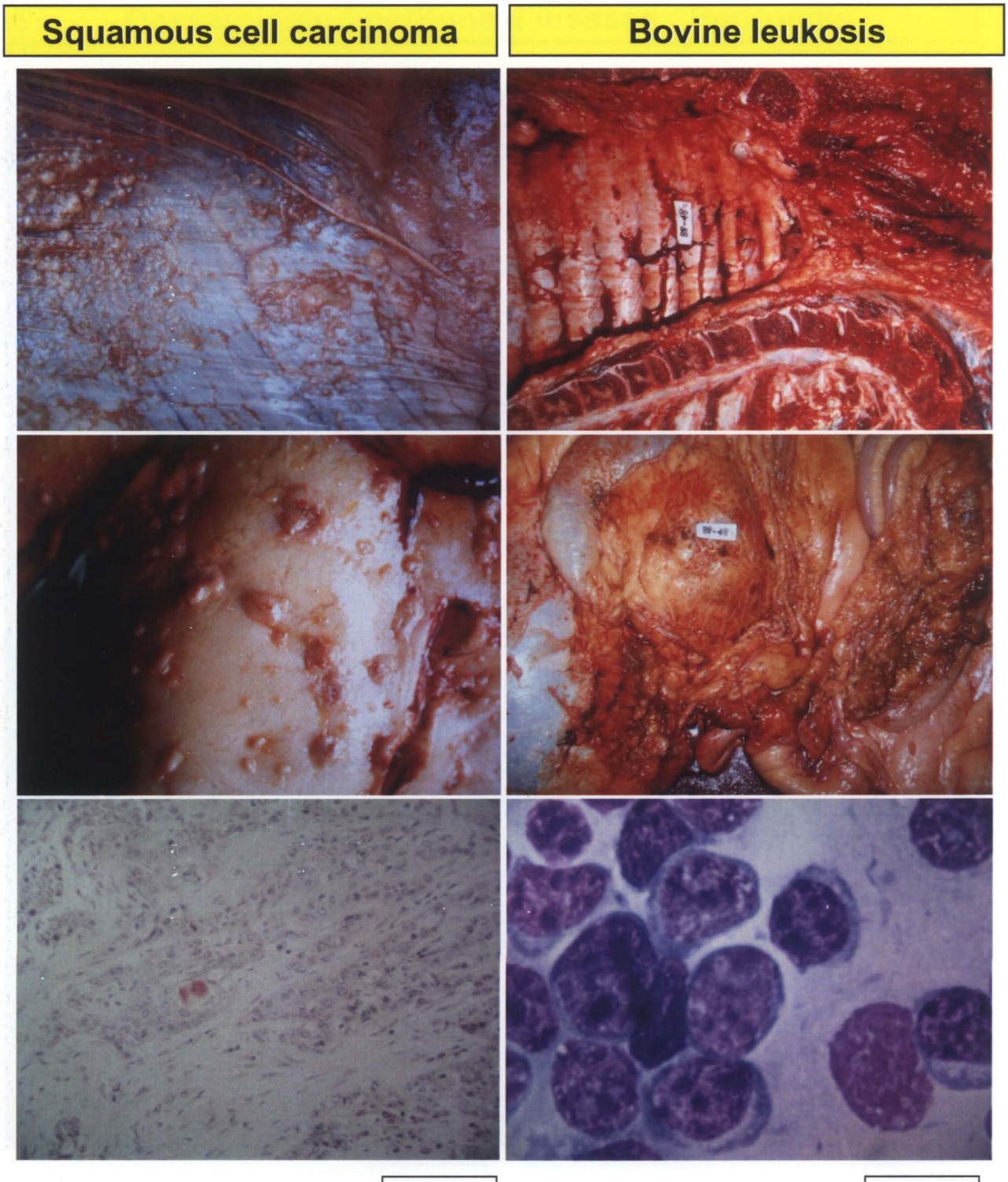


Fig.100

Fig.101

Fig.100 Squamous cell carcinoma of a 18-year-old female Japanese black cattle, weighing about 430kg. Multiple gray-white colored nodules distributed in the lungs, ,pleural, abdominal surface.In histological feature of the metastasis of the lungs, the horn pearls are composed of concentric layers of squamous cells showing increasing keratinization toward the center. HE stain. × 100.

Fig.101 Bovine leukosis of a 5-year-old male Japanese black cattle, weighing about 500kg. The masses showing extend to the pleural, abdominal surface. The liver swelling,massive lymph node enlargements in the mesentery. Observe the lymphoid cells are large with fine chromatin and obvious nuclei. Diff Quick stain. × 1000.

Desseminated peritoneal masses : Peritonitis

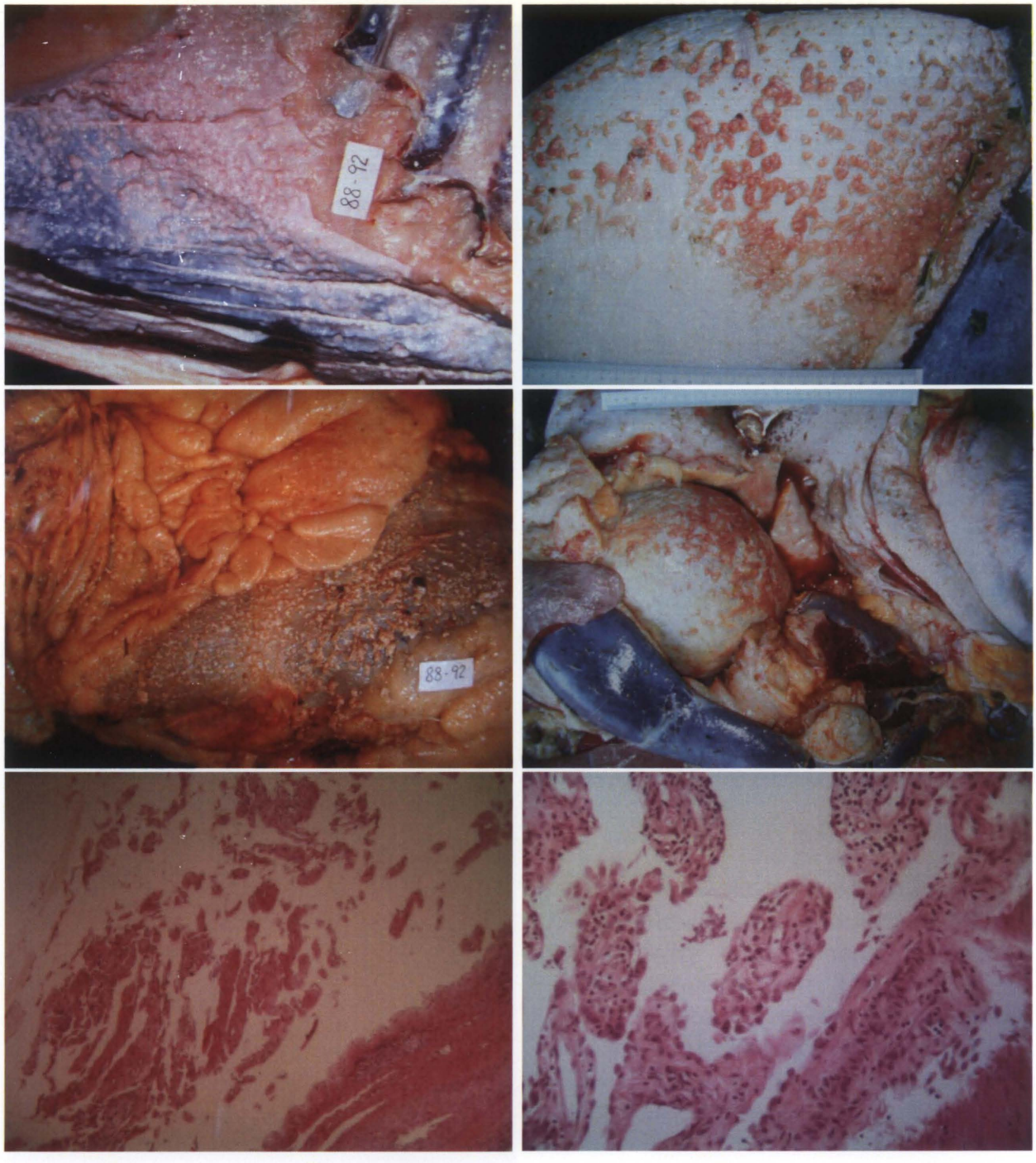


Fig.102

Fig.103

Fig.102 Diffuse fibronous peritonitis of a 10-year-old female Japanese black cattle, weighing about 520kg. Gross appearance of the pleura, peritoneum. The masses of fibrin are present between the omentum and the abdominal wall. HE stain. × 40.

Fig.103 Diffuse fibronous peritonitis of a 18-year-old female Japanese black cattle, weighing about 400kg. Gross appearance of the peritoneum. Fibrins covering the visceral and serosal surfaces of the abdominal cavity. The inflammation is initiated. HE stain. × 100.

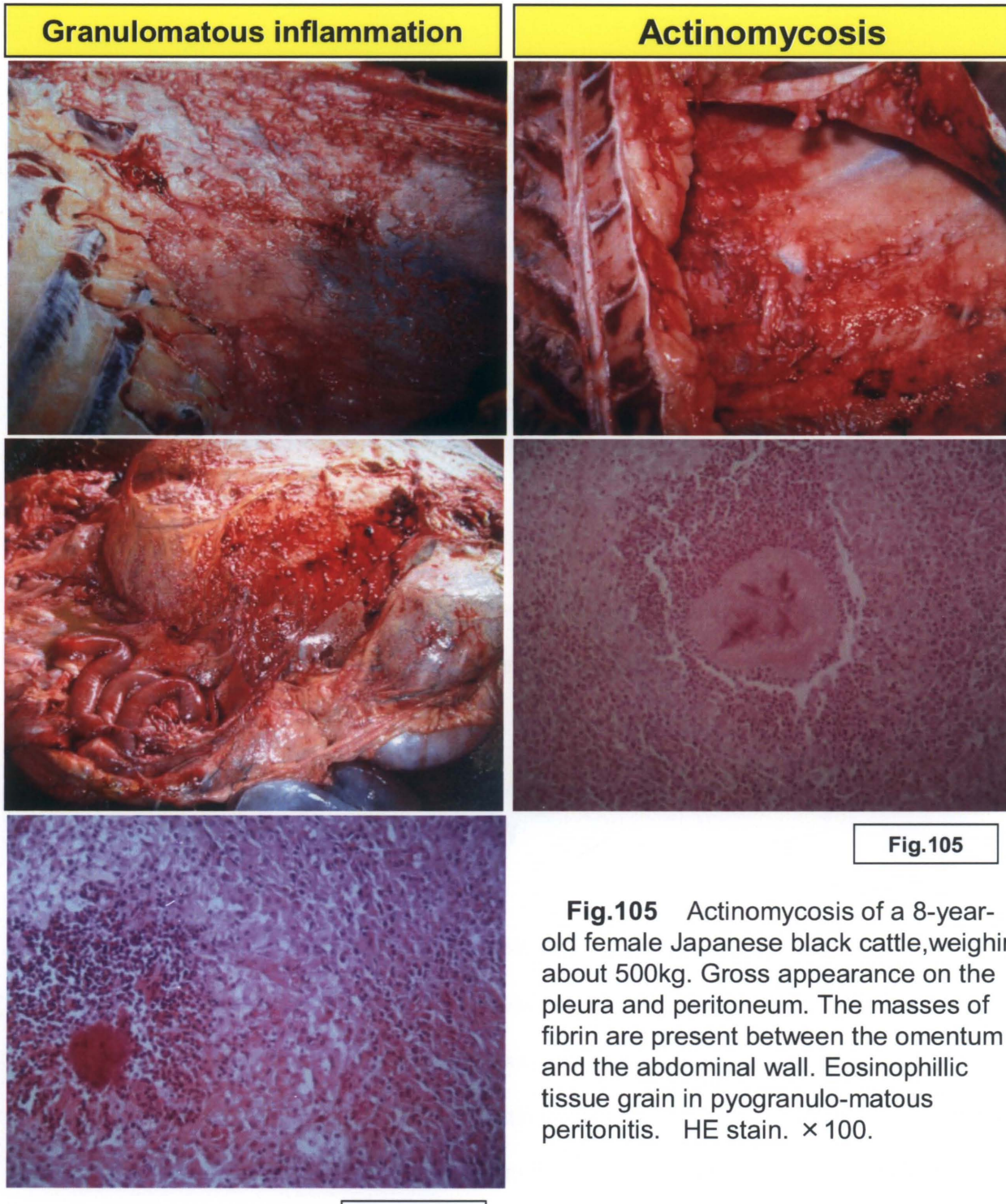


Fig.105

Fig.105 Actinomycosis of a 8-year-old female Japanese black cattle,weighing about 500kg. Gross appearance on the pleura and peritoneum. The masses of fibrin are present between the omentum and the abdominal wall. Eosinophillic tissue grain in pyogranulo-matous peritonitis. HE stain. × 100.

Fig.104

Fig.104 Diffuse fibronous peritonitis of a 6-year-old female Japanese black cattle, weighing about 450kg. Gross appearance of the pleura and peritoneum. Histopathologically, There is a nodular to diffuse peritnitis, with tissue granules surronuded by a granulomatous to pyogranulomatous infiltrate of histocytes, plasma, lymphocytes, neutrophils, and multinucleate histiocytic giant cells. The masses of fibrin are present between the omentum and the abdominal wall. HE stain. × 100.