# The Classification and Pathological Analysis of Tumors in Slaughtered Cattle in Miyazaki Prefecture (Mesothelioma in Cattle)

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# 1. PREFACE

The purpose of meat inspection is to maintain food sanitation standards in order to protect consumers from the risk connected with meats and other food products. Thus, accurate inspection methods are required to cope with diverse diseases. At meat inspection sites, many scientifically interesting cases can be found related to pathological materials. Miyazaki prefecture is one of the eminent livestock industry prefectures in Japan. And Miyazaki, the number of beef cattle slaughtered annually is about 60,000 beef cattle and inspections discover pathologic tumors in many cases. As part of a study concerning classification of cattle tumors and pathology analyses in Miyazaki prefecture, we investigated the situation related to the annual incidence of tumors at individual facilities among beef cattle slaughtered and dressed at meat-processing facilities in Miyazaki prefecture. Cases of mesothelioma, which had been detected frequently within the jurisdiction of the Miyakonojo Meat Inspection Office, were investigated.

Mesothelioma is a tumor, which occurs in the serous membrane within the thoracic and peritoneal cavities. This disease has been found in many animals, including livestock. In humans, Wagner et al. in South Africa reported in 1960 that asbestos is a cause of mesothelioma. Since then, there have been many reports in which asbestos has been involved. Recently, human mesothelioma cases related to a history of exposure of the body to asbestos have been reported from various places in Japan.

Chapter 1 contains the results for compound cases of malignant mesothelioma and ovarian granule cell tumor in cattle that were investigated histopathologically and immuno-histochemically. As for malignant mesothelioma of thoracic tumor, the cells were cultured to conduct histopathlogical and immunohistochemical examinations. In addition, we also investigated 328 tumor cases detected among 139,556 cattle in the jurisdiction of the

Miyakonojo Meet Inspection Office during a 21-year period from April, 1974 to March, 1995 and discussed the etiologies of the compound tumors encountered.

There have been only a few reports on long-term statistical investigations of livestock tumors, taking regional features into consideration. In Chapter 2, the annual variations in the detection and histopathological classification were described in the 377 tumor cases among 162,328 cattle examined within the jurisdiction of Miyakonojo Meat Inspection Office for 23 years from April, 1974 to March, 1997.

In Chapter 3, the annual variations involved in the situation of the detection and histopathological classification are described in 542 tumor cases at six Meat Inspection Offices in Miyazaki Prefecture over a period of 16 years and the findings of these epidemiological surveys, including breed, sex and production sites are indicated. .

In Chapter 4, the findings of surveys concerning breed, sex, age, and production site for mesothelioma cases occurring especially within the jurisdiction of Miyakonojo Meat Inspection Office and those related to histopathological and immunohistochemical examinations of individual lesion sites and histological types are presented. A comparison of incidence levels was conducted between nationwide Meat Inspection Offices and Miyakonojo Meat Inspection Office. We also surveyed the use of volcanic ash containing similar chemical components to asbestos, specific to Southern Kyushu, found in cattle barns, and discuss the relationship of use of this material with the occurrence of cattle mesothelioma.

Finally, in Chapter 5, rare malignant aortic body tumor cases in cattle are described in connection with histopathological and immunohistochemical examinations.

# 2. CHAPTER Ⅰ

## Malignant Mesothelioma and Granulosa Cell Tumor in a Japanese-Black Cow

### 2.1 *INTRODUCTION*

Mesothelioma is a rare mesothermal neoplasm that arises from the mesothelial cells lining the serous of the pericardial, pleural and peritoneal cavities [5,12,22,30,31,36, 52,54,60,62]. In domestic animals, mesothelioma has been reported most frequently in cattle because they are known to arise as a congenital tumor in fetal or young cattle [22,31, 36]. However, the incidence of the tumor might be relatively low in other animal species such as dogs [22, 36]. In medical fields, Wagner et al. [60] had been proposed the close relationship between the histories of asbestos exposure and the incidence of mesotheliomas [42], while such epidemiological evidences of the tumors in domestic animals still remain unclear [12, 22,36].

On the other hand, ovary granulosa cell tumor is one of the most common tumors in cattle and they have tended to appear unilateral and usually non-malignant tumors. These biological features of the tumors are most likely consistent in any species [22,31, 36]. Granulosa cell tumors sometimes show intra-peritoneal metastasis resulting multiple serous seeding of the tumors. Pathologically, these features sometimes make it difficult to give complete diagnosis for the tumor and as the differential diagnoses, metastastic carcinomas from the stomach, intestines, and adrenal glands, and mesotheliomas might be possible.

Although both mesothelioma and granulosa tumors can occur in calves forming multiple tumors, malignant mesothelioma also frequently involved the pleural cavities and

their morphological features were somewhat different from those of the granulosa cell tumors. The present paper describes pathological features of a Japanese black cattle involved in both malignant mesothelioma and ovarian granulosa cell tumor. The different pathological features of these tumors are also discussed.

## 2.2 *MATERIALS AND METHODS*

***Histopathology:*** Tissue samples of the pleural and left ovarian masses, diaphragm, lung, heart, liver, spleen, kidney, adrenal, premediastinal lymph nodes, and right ovary were fixed with 10% neutral buffered formalin, and embedded in paraffin. Paraffin sections of 4 μm, were stained with hematoxylin and eosin (HE), Periodic acid-Schiff (PAS), Alcian blue (pH 2.5), and Toluidin blue (pH 2.5 and 7.0, respectively), and Azan methods.

***Immunohistochemistry:*** Immunostaining was carried out by the labelled streptavidin-biotin (LSAB) method using a kit (Dako, Carpinteria, CA, USA.). The primary antibodies were rabbit sera against Cytokeratin (prediluted, Dako), monoclonal antibodies for vimentin (1:20, Dako), carcino-enbryonal antigens (CEA, prediluted, Dako). The secondary antibodies were biotinylated goat anti-sera against rabbit or mouse immunoglobulins (prediluted, Dako). The reaction products were visualized using 3,3'-diaminobenzidine (Sigma, St. Louis, MO, USA) counter-stained with Mayer's hematoxylin.

***Tissue culture:*** The tumor tissue was minced, and digested with 4 mg/ml collagenase (232 U/mg, Wako, Tokyo, Japan) in Dulbecco's modified Eagle's medium (DMEM, Sigma) and Ham's nutrient mixture F-12 (Sigma) containing 10% fetal calf serum (FCS), 100 IU/ml penicillin, and 100 μg/ml streptomycin, for 6 hours at 37℃ in a humidified atmosphere of 5% carbon dioxide in air. The digested tissue was filtered

through nylon mesh cloth (80 μm), centrifuged at 1,000 rpm for 10 min and cultured according to Hiratsuka *et al.* [19]. Coverslips with cultured tumor cells were washed in PBS, fixed in cold acetone, and stored at -20℃ for 30 min. The cells were incubated with one or other of the primary antibodies overnight at 4℃. The reacted antibodies were visualized by the LSAB method.

## 2.3 *RESIULTS*

***Gross findings:*** A 3-year-old female JBC, weighing about 650 kg, was slaughtered at Miyakonojo Meat Inspection Office. No clinical symptoms were noted by the general physical examinations. Grossly, multiple pleural masses, about 1 to 5 cm in diameter, from gray-white to yellow colored nodules, were observed (Fig.1). Some superficial nodules were also found in the superficial parts of the lungs and diaphragm. Cut surface of the pleural mass were grayish-white (Fig.2). They were consisted of hard tissues like fibrous peduncles. The premediastinal lymph nodes were grossly intact. There were no neoplastic nodules in the parenchyma of the lungs and diagram. The left ovary was enlarged to approximately $20 \times 15 \times 10$ cm in size, and its surface was reddish-white and coarsely nodular. Cut surface of the ovary mass was dark reddishbrown, serous fluid lucent and their walls were fibrous and hard with hemorrhage and necrosis (Fig.12). The right ovary and the others samples were grossly intact.

***Histopathological findings:*** Histopathological examinations of the tumors of the pleura, diaphragm, and lungs revealed the wide-dissemination of the neoplastic lesions. The neoplastic cells proliferated at the superficial layer of the serosa, and some neoplastic foci were hard to distinguish from hyperplastic lesions of normal mesothelial cells. The histological features of the serosa were almost consistent to those in tubular pattern of the epithelial form of mesotheliomas. Most parts of the pleural tumor exhibited the lesions

of mesotheliomas of the epithelial type with tubular and papillary patterns (Fig.3). The neoplastic cells were cuboidal or round in shape with moderate cellular atypia and pleomorphism (Fig.4). The neoplastic cells had moderate amount of eosinophilic cytoplasm and round to ovoid nuclei with the defined chromatin and a distinct nucleolus. Mitotic figures of the neoplastic cells were less frequent. The cytoplasm of the neoplastic cells was positively stained with toluidn blue (pH 7.0) (Fig.5) and negative for that (pH2.5), alucian blue and PAS (Fig.6), while those in stamp specimens were stained by PAS. Metastastic lesions of the neoplastic cells were found in the premediastinal lymph nodes. In the trabecular sins, there were a small number of cell clusters consisting of abundant collagen fibers and flattened neoplastic cells. Some clusters composed of less collagen fibers and cuboidal neoplastic cells.

Besides, the left ovary was entirely replaced by the proliferation of neoplastic cells forming insular and/or follicular structures mimicking the Graafian follicle. The follicular structures sometimes contained a small amount of clear to eosinophilic fluid mimicking Call-Exner bodies that occasionally found in granulosa cell tumors (Fig.12). The tumor cells in the left ovary are round or ovoid and resemble normal follicular cells (Fig.13), and the nuclei are round or ovoid and hyperchromatic. Mitotic figures were relatively frequent as compared to those in the pleural tumors. The cytoplasm contained PAS and toluidin blue (pH 7.0 and pH 2.5)-positive materials (Fig.14).

***Immunohistochemical features:*** Immunohistochemically, the neoplastic cells of the pleural tumors were intensely positive for cytokeratin (Fig.7), and were negative for vimentin and CEA. Although, the cultured cells of the pleural tumor were proliferated in bundles or fascicular patterns to form the meshwork architecture (Fig.8), those were positive for cytokeratin (Fig.9) and vimentin, and were negative for CEA (Fig.11). On the other hands, the neoplastic cells from ovarian tumor were positive for vimentin, and

were negative for cytokeratin and CEA (Fig.15). The results of several special-stainings and immunohistochemical-stainings of pleural and ovarian tumors were summarized in Table 1.

## 2.4 *DISCUSSION*

Based on the gross and histopathological findings, the present case was considered as to be involved in diffuse malignant mesothelioma of the pleural cavities and concurrent ovarian granulosa cell tumor. The morphological features of both tumors had their own specific features, respectively. The immunohistochemical and special staining results of these tumors might reflect the different natures of these tumors. There are a few possibilities that the multiple tumors in the pleural cavities appeared as the metastastic lesions of the ovarian granulosa cell tumor. However, the different morphological features may support our diagnosis for the present case.

The definite relationship between the asbestos exposure and the occurrence of mesothelioma has been established epidemiologically and experimentally [5,30,31,36], but the mechanisms of tumoregenesis associated to asbestos still remain unclear [42], especially in domestic animals [12,22,36]. In the present case, asbestos fibers were never found in the neoplastic lesions of malignant mesothelioma or the respiratory systems by routine histological examinations. In humans, malignant mesothelioma is a rare tumor, approximately 0.34-3.5 per million per year in frequency [62]. It has been estimated that the incidence of this tumor is one to two per million in the general population, although among the populations exposed occupationally to asbestos, the incidence has been increasing at a high rate [52].

In cattle, this tumor is also rare, approximately 0.22-1.4% incidence in bovine tumors [5,34]. In our experiences, tumors were detected in 328 of 139,556 of

slaughtered cattle examined during 1974-1994 at Miyakonojo Meat Inspection Office in Miyazaki prefecture (Table 2). Mesotheliomas of 60 cases (18.3%) that was approximately 4.3 per 10 thousands were observed most frequently as the previous examinations by Miyazaki University that 10 cases (18.2%) of 55 tumors in the cattle were mesotheliomas [44]. The incidence of mesotheliomas in cattle tented to be high at Southern Kyushu, especially around Miyakonojo city. In this area, many small farmers of Japanese black cow were usually used "Shirasu" for calves instead of sawdusts. The chemical composition of "Shirasu" which is volcanic ashes very likes asbestos in a main component of $SiO_2$ [60]. This fact may indicate the possibility of carcinogenicity of mesothelioma by "Shirasu".

On the other hand, although multiple primary tumors may be related to hereditary or iatrogenic diseases in man [25], and those are rare in cattle. The mechanisms of the occurrence of multiple primary tumors are still unclear. In our examination, highly, susceptible tumors were in the order of bovine mesothelioma and ovarian granulosa cell tumor in Miyakonojo and surrounding areas. Therefore, it is assumed that this case of multiple primary tumors is almost spontaneous generation with coincidence.

## 2.5 *ABSTRACT*

A 3-year-old female Japanese black cow had malignant mesothelioma on the pleura and granulosa cell tumor of the left ovary. The pleural surface was disseminated with numerous small neoplastic nodules, and the tumor cells were immunohistochemically positive for cytokeratin and negative for vimentin and CEA, while cultured epithelioid cells derived from the tumor were positive for vimentin. The left ovary was entirely replaced by the proliferation of neoplastic cells with insular and follicular patterns, and the neoplastic cell clusters with some inter cellular spaces were separated by connective tissue septa. The tumor cells were positive for vimentin and negative for cytokeratin and CEA.

**KEY WORDS** : granulosa cell tumor, mesothelioma, multiple tumor.

**Table 1. The results of special staining for the tumors**

| Staining for | Pleura | Ovary |
|---|---|---|
| Perioidic acid-Schiff | - ( + ) | + |
| Perioidic acid-Schiff(stamp) | + | N D |
| Toluidin Blue (pH2.5) | - | - |
| Toluidin Blue (pH7.0) | + | - |
| Alcian blue | - | - |
| **【Immunohistochemistry】** | | |
| Cytokeratin (Prediluted, Dako) | + | - |
| Vimentin (1:20, Dako) | - ( + ) | + |
| CEA (Prediluted, Dako) | - | - |
| **【Cultured Immunohistochemistry】** | | |
| Cytokeratin (Prediluted, Dako) | + | N D |
| Vimentin (1:20, Dako) | + | N D |
| CEA (Prediluted, Dako) | - | N D |

+ : Positive - : Negative N D : Not done ( ) : Stroma

## Table 2. Incidence of tumors among spesies of cattle durig 21 years

| Type of tumor | cases | Type of tumor | cases | Type of tumor | cases |
|---|---|---|---|---|---|
| ***Hematopoietic system*** | ***44*** | ***Digestive system*** | ***56*** | ***Genital system*** | ***60*** |
| lymphoma | 41 | hepatoma | 18 | malignant granulosa cell tumor | 8 |
| (adult type) | ⑰ | (hepatocellulor carcinoma) | ⑪ | granulosa cell tumor | 40 |
| (thymic type) | ③ | (cholangiocarcinoma) | ④ | leiomyoma of the uterus | 6 |
| (lymphosarucoma) | ⑤ | liver papilloma | 1 | leiomyosarcoma of the uterus | 2 |
| (reticulosarcoma) | ⑥ | liver cell adnoma | 11 | squmous cell tumor | 2 |
| hyperplasia of the spleen | 3 | hemangioma | 2 | adenoma, adenocarcinoma | 2 |
| | | fibrosarcoma | 1 | | |
| ***Cardiovascular system*** | ***1*** | fibroma | 1 | ***Skeletal system*** | ***6*** |
| aotic body tumor | 1 | squmous cell tumor | 1 | rhabdomyosarcoma | 3 |
| | | papilloma of the gallbladder | 4 | rhabdomyoma | 1 |
| ***Respiratory system*** | ***21*** | myxoma | 1 | schwannoma | 1 |
| lung cancer | 19 | intestinal adenoma | 4 | fibroma | 1 |
| (squmous cell tumor) | ⑥ | papilloma of the stomach | 1 | | |
| (epithelioid sarcoma) | ① | stomac hyperplastic polyp | 3 | ***Others*** | ***121*** |
| fibrosarcoma | 2 | hyperplasia of the pancreas | 4 | malignant mesothelioma | 54 |
| | | pancreatic carcinoma | 1 | mesothelioma | 6 |
| ***Cutaneous system*** | ***12*** | adenocarcinoma | 3 | adrenocortical adenoma | 10 |
| malignant melanoma | 2 | | | pheochromocytoma | 1 |
| melanoma | 2 | | | cancer of the adrenal gland | 1 |
| fibrosarcoma | 3 | ***Urinary system*** | *7* | thyroid adenoma | 4 |
| fiborma | 2 | nephrobalstoma | 3 | fibrosarcoma | 1 |
| lipoma | 1 | renal carcinoma | 1 | ameloblastoma | 1 |
| squamous cell carcinoma | 1 | papilloma of the urinary bladder | 2 | mesenchymal chondrosarcoma | 1 |
| mixed tumor | 1 | rhabdomyosarcoma | 1 | adenocarcinoma | 6 |
| | | | | unknown | 36 |

○ include cases

Fig.1

Fig.2

**Fig.1** The masses of pleura 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.2** Cut surface of the mass were grayish-white.

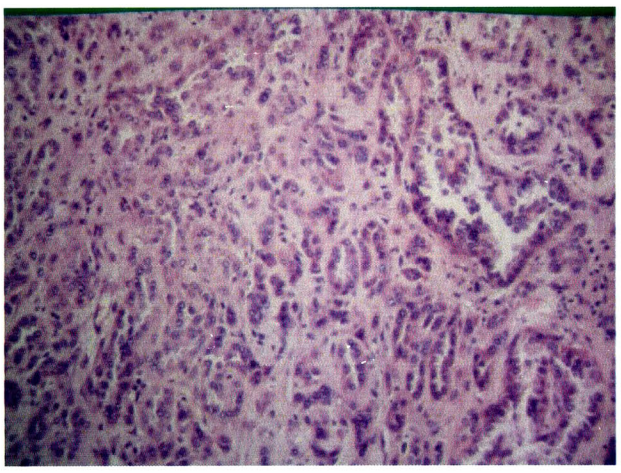


Fig.3

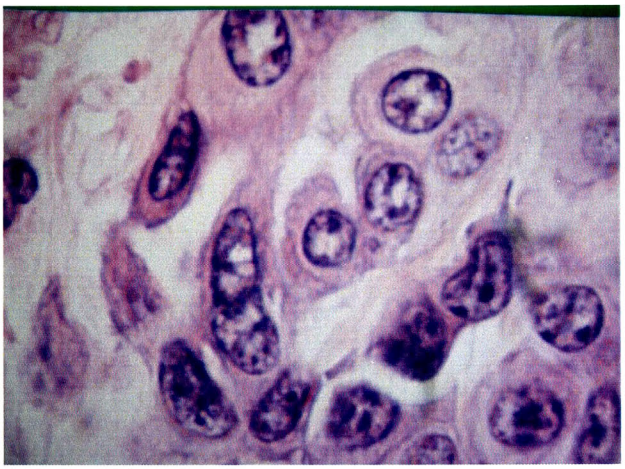


Fig.4

**Fig.3** The pleura tumor cells showing tubular pattern of the epithelial form. HE stain. ×200.

**Fig.4** The tumor cells were cuboidal or round in shape with moderate cellular atypia and pleomorphism. The round to ovoid nuclei had the defined chromatin and a distinct nucleolus. HE stain. ×1000.

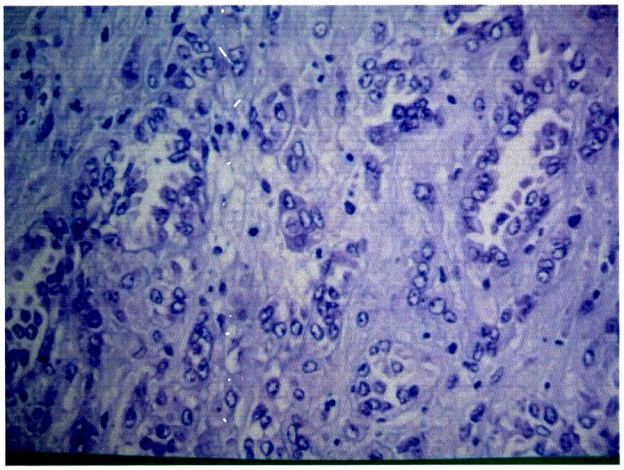


Fig.5

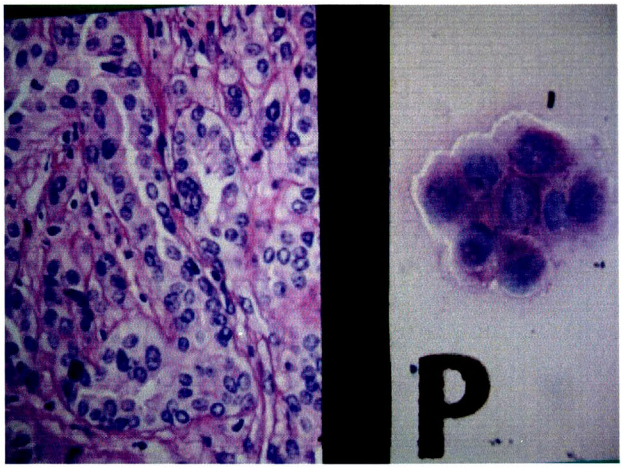



Fig.6

**Fig.5** The cytoplasm of the neoplastic cells was positively stained with toluidin blue (pH 7.0). Toluidin blue stain. ×400.

**Fig.6** The neoplastic cells negative for that (pH2.5), alucian blue and PAS, while those in stamp specimens (P) were stained by PAS. PAS stain ×400. ×1000.

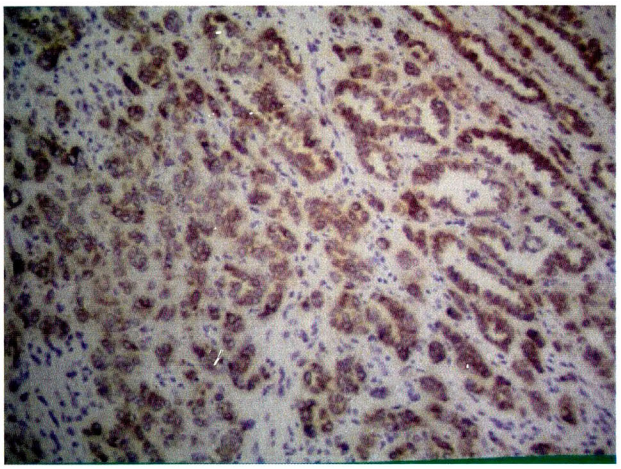


Fig.7

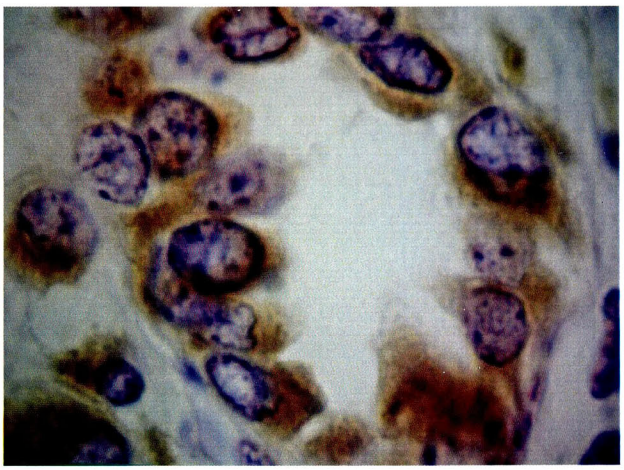


Fig.8

**Fig.7 and Fig.8** The masses of pleura tumor showing tubular pattern of the epithelial form. Immunohistochemical staining for Cytokeratin. Note the stronger staining pattern in the epithelial form. Cytoketatin immunohistochemistry ×200. ×1000.

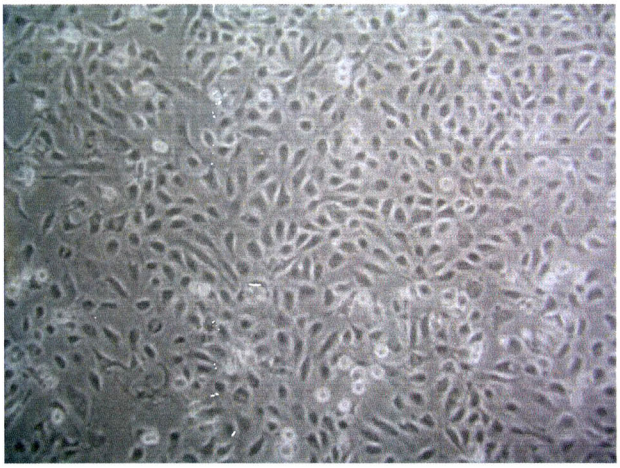


Fig.9.

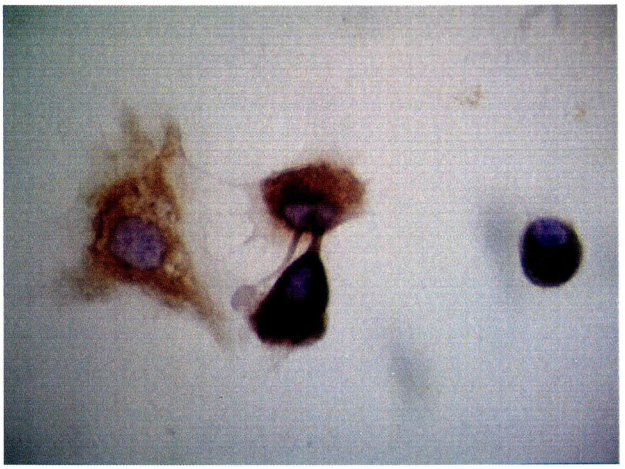


Fig.10.

**Fig.9** The cultured cells were proliferated in bundles or fascicular patterns to form a meshwork architecture. Phase-contrast microscopy. × 126.

**Fig.10** The cultured cells showing positive reaction for antibody against cytokeratin positive. Cytokeratin immunohistochemistry. × 1000.

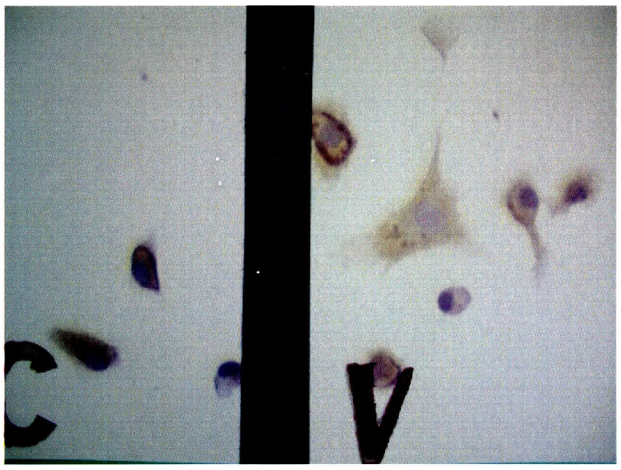



Fig.11

**Fig.11** The cultured cells showing positive reaction for antibody against cytokeratin (C) and vimentin (V), and were negative for CEA. Immunohistochemistry. × 400.

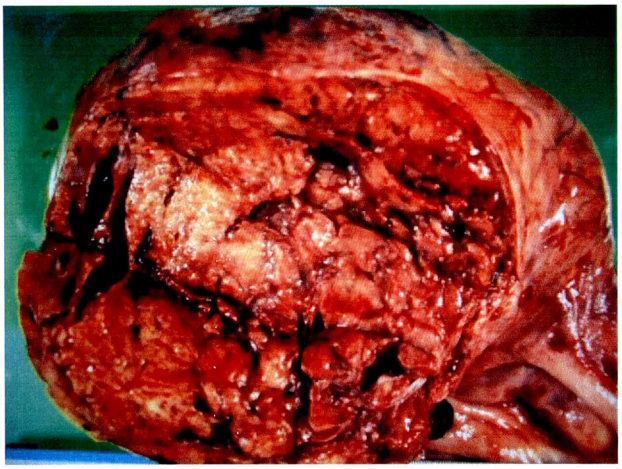


Fig.12

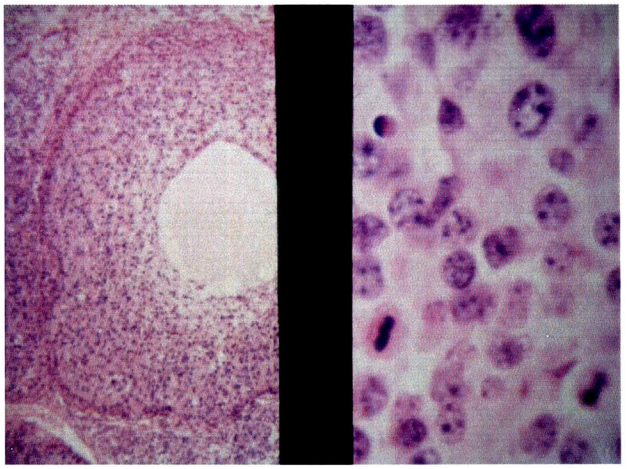


Fig.13

**Fig.12** The left ovary was enlarged to approximately 15 cm in diameter the surface and cut surface was reddish-white and coarsely nodular.

**Fig.13** The neoplastic cells were separated by connective tissue septa and folliclelike structure with Call-Exer body was seen in center. H.E. stain ×40. The tumor cells showing round or ovoid and and the nuclei showing round ovoid, and hyperchromatin. HE stain. ×400.

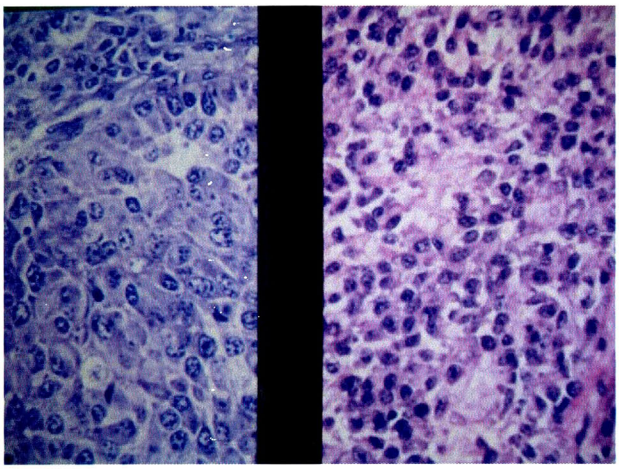


Fig.14

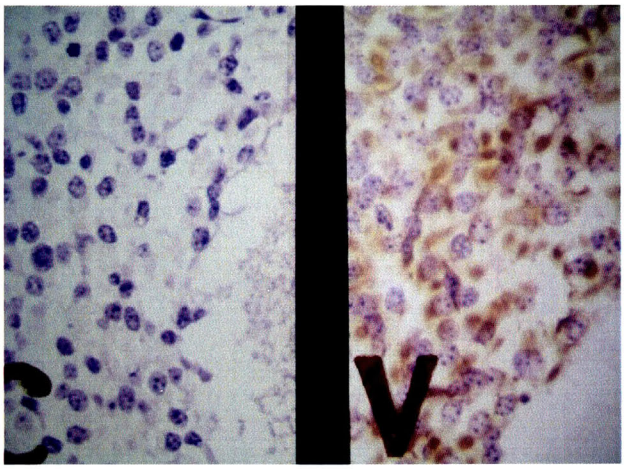



Fig.15

**Fig.14** The ovarian tumor cytoplasm contained PAS(×200) and toluidin blue (pH 7.0 and pH 2.5 ×200)

**Fig.15** The cells from ovarian tumor positive for vimentin (V) , and negative for cytokeratin immunohistochemistry (C). ×200.

# 3. Chapter II

## Bovine Tumors Detected at Miyakonojo Meat Inspection Office of Miyazaki Prefecture during 1974-1996

### 3.1 *INTRODUCTION*

Several long-term surveillance data on tumors in domestic animals have been reported not only in Europe and North American countries but also in Japan [21,23, 40,44,45,55,56]. Although Kanagawa Meat Inspection Office reported a survey on the incidence of porcine tumors, only a few surveys on cattle tumors including a regional study were reported in Japan.

A national survey on the incidence of tumors in cattle started in 1995 by National Conference of Meat inspection offices. This prompted us to conduct epidemiological and pathological surveys on the incidence of tumors in cattle slaughtered at Miyakonojo Meat Inspection Office. Our goal of this study is to compare the data examined among municipal and prefectures meat inspection offices in Kyushu.

### 3.2 *MATERIALS AND METHODS*

***Sampling:*** A total of 377 tumors detected from 162,328 slaughtered cattle during 23 years (1974 to 1966) at Miyakonojo Meat Inspection Office, Miyazaki Prefecture, were examined. The cattle examined were transported from Miyakonojo city and Nishimorokata-gun in Miyazaki prefecture, and Satsunan area in Kagoshima prefecture. The number of cattle with any tumors in each year, breed, gender, and the location of the tumors were recorded, respectively. The cattle examined were divided into four groups; (1) cattle used for reproduction such as Japanese black beef cattle aged more than four

years old, (2) dairy cattle such as Holstein aged more than four years old, (3) beef cattle less than three years old, and (4) calves less than one year old.

***Pathological examinations:*** The tumor tissues were fixed in 10% neutral buffered formalin or methanol-Carnoy's solution and embedded in paraffin. Then, paraffin sections of 4 μm were stained with hematoxylin and eosin (HE). Some selected sections were also stained with several specific stainings including periodic acid Schiff (PAS) and Azan stains. Immunostaining for the detection of antigens *in situ* was performed by avidin-biotin peroxidase complex (ABC) methods using a system kit (Vector Lab., Burlingame, CA, USA). Based on these histopathological examinations, diagnoses of the tumors were determined.

***Data analysis:*** To compare the incidence of tumors in cattle in Kyushu, we collected the data concerning the incidence of tumors in cattle from additional five meat inspection offices in Miyazaki prefecture and 17 meat inspection offices out of Miyazaki prefecture. However, we excluded papillomas and associated tumors from the present data, since a few information were available.

## 3.3 *RESULTS*

***Change of the incidence of tumors in cattle during 23 years examined:*** Tumors were detected in 377 of 162,328 (232 per 100,000) slaughtered cattle examined during 23 years (1974 to 1996) at Miyakonojo Meat Inspection Office, Miyazaki prefecture. The high incidences of tumors were recoded in 1979 (485 per 100,000), 1988 (445 per 100,000) and 1980 (441 per 100,000), respectively. In contrast, the bottoms were in 1990 (41 per 100,000) and 1984 (75 per 100,000) (Fig. 16).

***Incidence of tumors among species of cattle:*** A total of 118,438 slaughtered cattle were inspected during 14 years (1983~1996). The details of four groups examined

were as follows; 78,843 cattle used for reproduction (66.6%), 20,313 dairy cattle (17.2%), 17,720 beef cattle (15.0%), and 1,562 calves (1.3%). The incidence of tumors detected in each four groups was as follows; 182 cattle used for reproduction (230 per 100,000), 29 dairy cattle (143 per 100,000), 12 beef cattle (68 per 100,000), and 6 calves (384 per 100,000), and 21 unrecorded cases.

***Details of tumors detected***: Tumors were detected in 377 of slaughtered cattle examined during 23 years including 64 mesotheliomas (Fig.17,18,19,20), 56 granulosa cell tumors (Fig.21,22,23,24), 48 bovine leukosis (Fig.25,26,27,28,29,30,31,32), 20 lung cancers (Fig.33,34,35,36), 19 liver carcinomas (Fig.37,38,39), and 17 adrenocortical adenomas (Fig.40,41,42,43) (Table 3). These six tumors occupied approximately 60% among all tumors observed.

In the figures, several autopsy cases suffering from tumors examined in the present study were introduced. Figure 44, 45 and 46 represented pheochromocytoma, figure 47, 48, 49 represented malignant melanoma and figure 50, 51, 52, 53 represented rhabdomyosarcoma and figure 54, 55 represented schwannoma.

Mesothelioma was observed in 61 Japanese black beef cattle and 4 Holstein species (mean age; 11-years-old). Among them, 43 cases were classified into epithelial type, which was characterized with tube formation and/or papillomatous proliferation, 13 cases were fibrous type with remarkable proliferation of basal body, and 8 were the mixed type.

The locations of granulosa cell tumors in the ovary were examined among 56 cases. Two cases were observed bilaterally. Sixteen were observed laterally. Although the location of the tumor was not currently recorded, 19 cases had lateral ovarian tumors. Moreover, the location of the tumors was not available in 3 cases. These 25 cases diagnosed histopathologically were classified into middle ovary follicle type (13 cases) and strip type (12 cases).

Fifty-eight bovine leukosis were 33 adult multi-centric type, 5 thymic type, and 10 unknown, respectively.

***Comparative study of the incidence of tumors in cattle observed among meat inspection offices in Miyazaki prefecture***: Tumors were detected in 277 of 359,416 (77 per 100,000) slaughtered cattle examined during 16 years (1978～1993) at five meat inspection offices in Miyazaki prefecture except for Miyakonojo. The incidence of tumors observed in each meat inspection office was ranging 50-159 per 100,000 heads (Table 4). Compared to the incidence of tumors at these meat inspection offices within the same period, that at Miyakonojo Meat Inspection Office was the highest (247 per 100,000). Interestingly, when the frequency of tumors at Miyakonojo and Takasaki Meat Inspection Office, a meat processing plant authorized for exportation to the USA and processing mainly beef cattle, was compared, the incidence at the former was approximately four fold higher than that at the latter. The incidence of mesotheliomas among the five meat inspection offices other than Miyakonojo was 4.73 per 100,000.

***Comparative study of the incidence of tumors in cattle observed among meat inspection offices in Kyushu:*** Tumors were detected in 1,186 of 2,175,645 (55 per 100,000) slaughtered cattle examined during 16 years (1978～1993) at 17 meat inspection offices including municipal and prefectural facilities other than Miyazaki prefecture in Kyusyu. The highest incidence was bovine leukosis (304 cases, 13.97 per 100,000). Mesothelioma was observed in 20 cattle (0.92 per 100,000). It should be noted that the incidence of mesothelioma at Miyakonojo Meat Inspection Office during the same period was 57 cases (53.13 per 100,000).

## 3.4 *DISCUSSION*

According to the "Report of Hygiene Administration" edited by the Division of

Statistical Analyses in the Ministry of Health and Welfare, which includes the total number of tumors observed in meat inspection offices in Japan, tumors were detected in ranging 74 to 591 (the mean 259 cases) per 100,000 slaughtered cattle examined during 20 years (1975 to 1994). Similar to that of the government data, tumors were also detected in ranging 41 to 485 (the mean 232 cases) per 100,000 slaughtered cattle in the present study. In Miyakonojo Meat Inspection Office, the incidence of tumor cases tended to alter every 6-7 years. The real reasons is unclear, however, we cannot rule out the possibility of the personal factors that were in charge of the tumor inspection.

There was higher incidence in the older breeding cattle with more times of giving birth, while in the cattle with a short breeding period the incidence was comparatively low, with no relative with the breed. We thought that older cattle commonly have the higher incidence of tumors. However, it was found that the cattle under 1 year old also showed a high incidence, which may be the result that these cattle were transported to the meat inspection office because of some diseases.

According to the research of the Miyakonojo Meat Inspection Office, tumors with the highest incidence were of mesotheliomas (64 cases, 17%), granulose cell tumor (56 cases), and bovine leukosis (48 cases), respectively. The high incidence of mesotheliomas was in accordance with the reports from Miyazaki University [44,56]. These results including our data might indicate that the incidence of mesotheliomas is higher in South Kyoshu compared to that in the other areas.

Generally, the incidence of mesotheliomas was approximately 0.22 to 1.4% of all bovine tumors, and it was also found in fetus of later pregnant period and in young calves [12,22,36]. In other adult domestic animals, mesotheliomas were also observed with a low frequency [12,22,36]. In this study, we found that the average age was 11 years old in the diseased cattle with mesotheliomas, showing a possible of postnatal cause. In

human, cases of breast mesotheliomas are frequently observed in patients with an inhaling exposure to asbestos [52]. From the present study and the data of the nationwide meat inspection office, it was found that abdominal mesotheliomas were of a high incidence in cattle. This may be because those cattle have much more frequent abdominal exposure to some carcinogens. The relationship between human mesotheliomas and asbestos has been proofed [52,60], while the tumoregenesis of bovine mesotheliomas is still unknown [22,36]. It is necessary to study the possible relationship between mesotheliomas and some substance with a similar chemical structure with asbestos, for example, the characteristic ash in South Kyushu.

Among ovary tumors, ovarian granulosa cell tumor was found to have a highest incidence in this study, which was in accordance with the reports from Saeki et al. [45]. Of which, most of the cases was of single side, with only two bilateral cases. In cattle, the inhibition of the left ovary action by the giant first stomach was reported [20], however, we did not find that this has some effects on the incidence of either the left or right ovary tumors.

Although bovine leukosis showed the highest incidence among tumors observed by the meat inspection offices in Japan [20,21], it was found to be the third one in Miyakonojo Meat Inspection. Among which, the adult multicentric type was the most one, and mostly found in Holstein breed, similar to the report of Onuma et al. [39]. Furthermore, in the research of bovine tumors in the prefectures in Kyushu during last 16 years, 304 cases of bovine leukosis were detected, among which 244 cases were found in one prefecture (260 per 100,000) (data not shown), suggesting that it is likely an endemic disease [39] occurring frequently in some specific area in Kyusyu.

## 3.5 *ABSTRACT*

Tumors were detected in 377 of 162,328 (232 per 100,000) slaughtered cattle examined during 23 years (1974～1996) at Miyakonojo Meat Inspection Office, Miyazaki Prefecture, including 64 mesotheliomas, 56 granulosa cell tumors, 48 bovine leukosis, 20 lung cancers,19 liver carcinomas and 17 adrenocortical adenomas. Mesothelioma was most frequently observed in Japanese black breed (11-years-old on average) and the incidence seemed to be much higher in Southern Kyushu including Miyakonojo areas than others areas of Kyushu.

**Key words**: cattle, meat inspection, mesothelioma, tumor

**Fig.16 Change of the incidence tumors in cattle during 23 years examined**

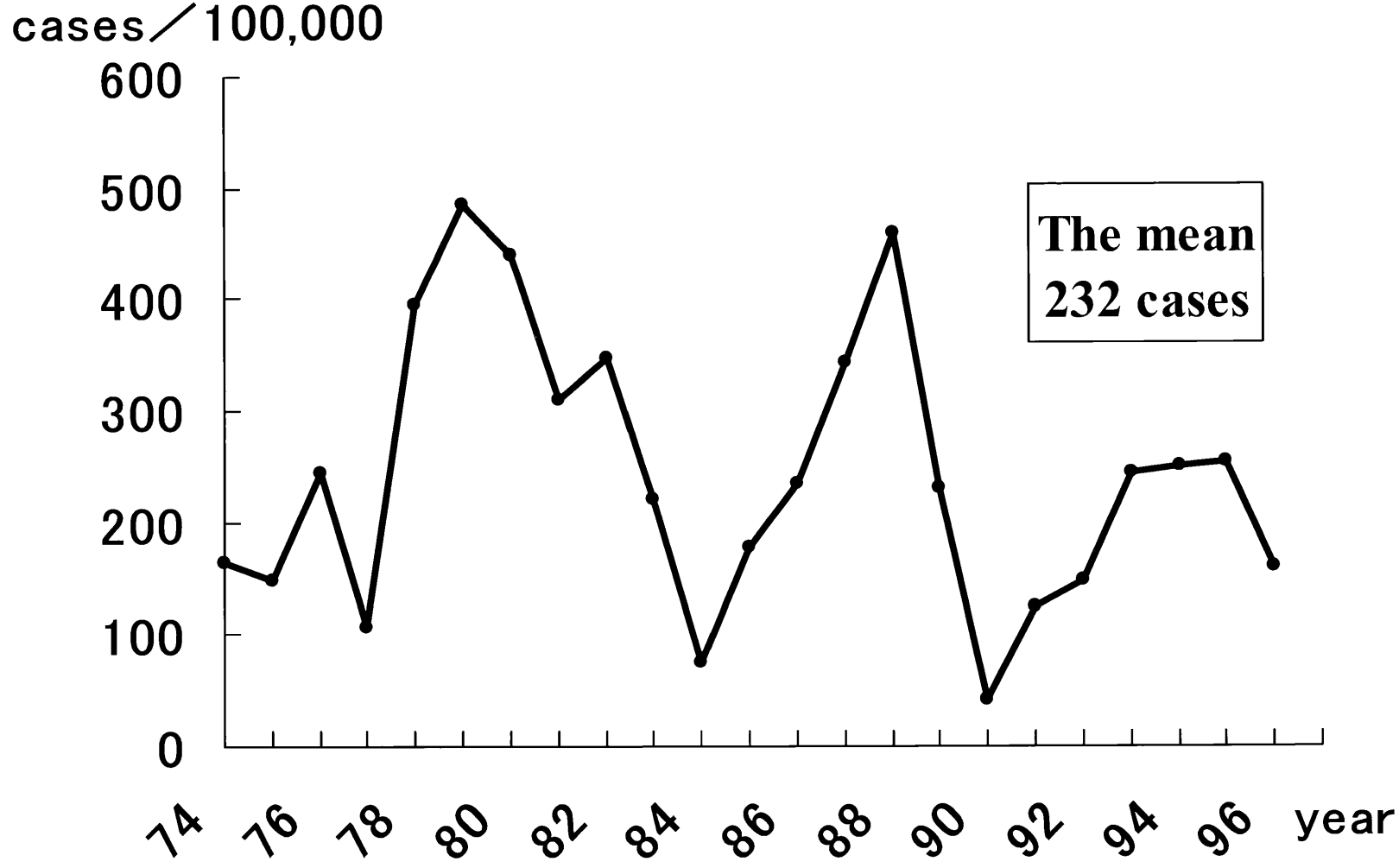


**Table 3. Incidence of tumors in cattle observed among Miyakonojo Meat Inspection Office during 23 years**

| Classification | cases |
|---|---|
| ***Cutaneous system*** | ***14*** |
| malignant melanoma | 3 |
| melanoma | 2 |
| fibrosarcoma | 3 |
| fiborma | 2 |
| lipoma | 1 |
| squamous cell carcinoma | 1 |
| mixed tumor | 1 |
| other | 1 |
| ***Hematopoietic system*** | ***51*** |
| lyphoma | 48 |
| (adult type) | ㉝ |
| (thymic type) | ⑤ |
| hyperplasia of the spleen | 3 |
| ***Cardiovascular system*** | ***1*** |
| aortic body tumor | 1 |
| ***Respiratory system*** | ***22*** |
| lung cancer | 20 |
| (squmous cell tumor) | ⑦ |
| fibrosarcoma | 2 |

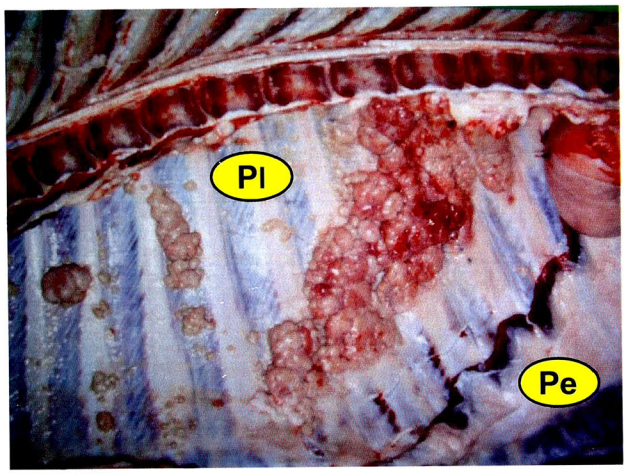
| Classification | cases |
|---|---|
| ***Digestive system*** | ***63*** |
| hepatoma | 19 |
| (hepatocellulor carcinoma) | ⑪ |
| (cholangiocarcinoma) | ④ |
| liver papilloma | 1 |
| liver cell adnoma | 12 |
| hemangioma | 2 |
| fibrosarcoma | 1 |
| fibroma | 2 |
| squmous cell tumor | 1 |
| papilloma of the gall bladder | 4 |
| myxoma | 1 |
| intestinal adenoma | 4 |
| papilloma of the stomach | 2 |
| stomac hyperplastic polyp | 5 |
| hyperplasia of the pancreas | 5 |
| pancreatic carcinoma | 1 |
| adenocarcinoma | 3 |
| ***Urinary system*** | ***8*** |
| nephrobalstoma | 3 |
| renal carcinoma | 1 |
| papilloma | 3 |
| rhabdomyosarcoma | 1 |

| Classification | cases |
|---|---|
| ***Genital system*** | ***71*** |
| malignant granulosa cell tumor | 9 |
| granulosa cell tumor | 47 |
| luteoma | 1 |
| fibroma | 1 |
| leiomyoma of the uterus | 7 |
| leiomyosarcoma of the uterus | 2 |
| squmous cell tumor | 2 |
| adenoma, adenocarcinoma | 2 |
| ***Skeletal system*** | ***6*** |
| rhabdomyosarcoma | 3 |
| rhabdomyoma | 1 |
| schwannoma | 1 |
| fibroma | 1 |
| ***Others*** | ***141*** |
| malignant mesothelioma | 58 |
| mesothelioma | 6 |
| adrenocartical adenoma | 17 |
| pheochormaocytoma | 4 |
| cancer of the adrenal gland | 1 |
| thyroid adenoma | 4 |
| fibrosarcoma | 1 |
| ameloblastoma | 1 |
| mesenchymal chondrosarcoma | 1 |
| adenocarcinoma | 9 |
| unknown | 39 |

○ include cases

**Table 4. Comparsion of tumor occurrence situation with 6 meat inspection offices in Miyazaki prefecture（1978～1993）**

| Meat Inspection Office | meat inspection cases | tumor cases | cases per 100,000 |
|---|---|---|---|
| Miyakonojo | 107,283 | 265 | 247.0 |
| Takasaki | 123,182 | 83 | 67.4 |
| Miyazaki | 37,624 | 60 | 159.5 |
| Kobayshi | 41,633 | 51 | 122.5 |
| Tsuno | 113,898 | 57 | 50.0 |
| Hyuga | 43,079 | 26 | 60.4 |
| Total | 359,416 | 277 | 77.0 |




Pl :Pleura Pe :Peritoneum

Fig.17

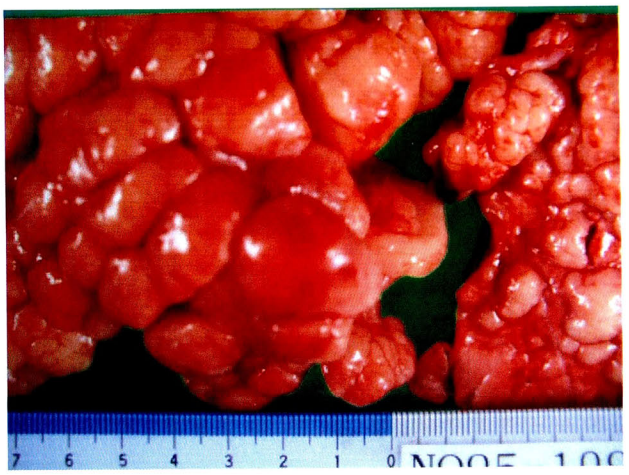


Fig.18

**Fig.17** Malignant mesothelioma in a 3-year-old female Japanese black cattle, weighing about 545 kg. The neoplasm showing extend to the pleura.

**Fig.18** Tumor nodules showing from gray-white to reddish-yellow colored nodules like fibrous peduncles.

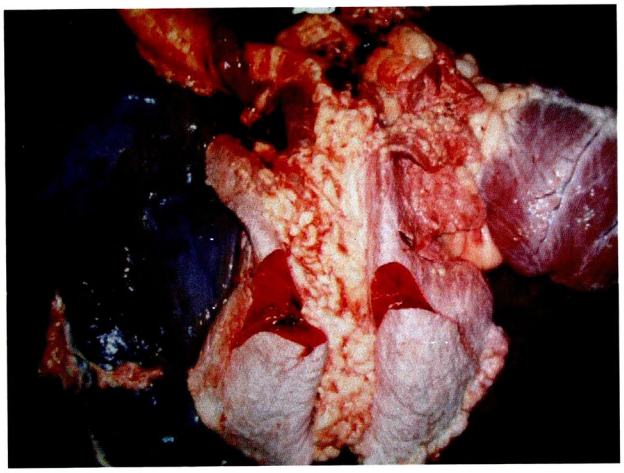


Fig.19

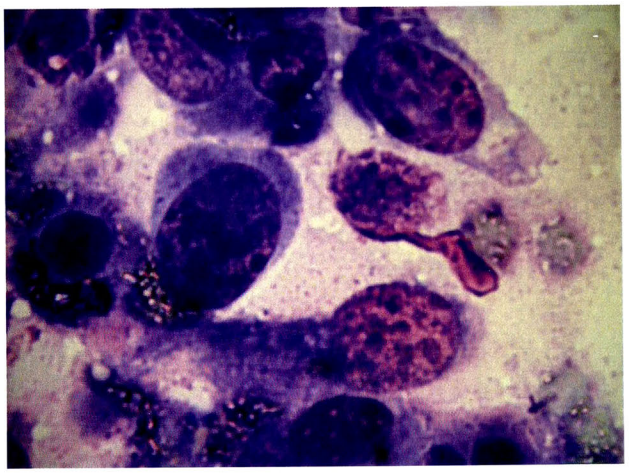


Fig.20

**Fig.19** Some superficial nodules found in the parts of the diaphragm and lungs.

**Fig.20** The tumor cells had moderate amount of eosinophilic cytoplasm and round to ovoid nuclei with the defined chromatin and a distinct nucleolus. Diff Quick stain. × 1000.

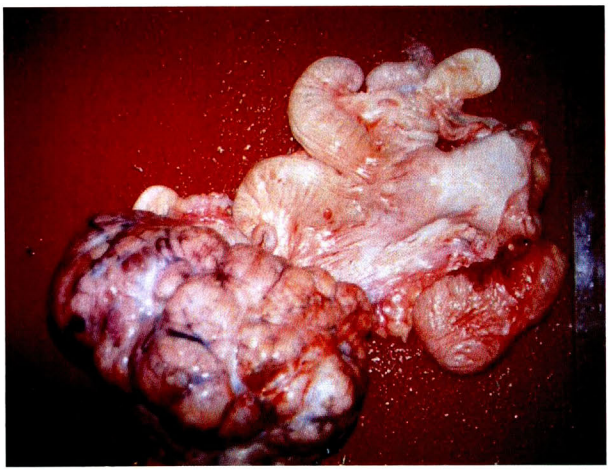


Fig.21

Fig.22

**Fig.21** Granulose cell tumor in a 8-year-old female Japanese black cattle, weighing about 570kg. Surface view of granulosa cell tumor in the left ovary.
**Fig.22** The left ovary was enlarged to approximately 30 × 17 × 13cm in size. The cut surface showing gray-white and subdividing irregular lobules.

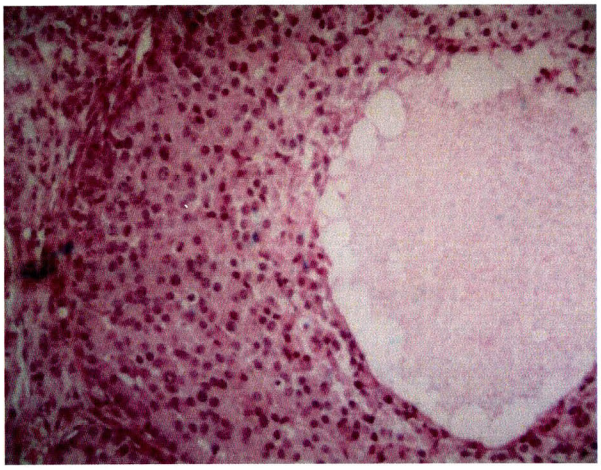


Fig.23

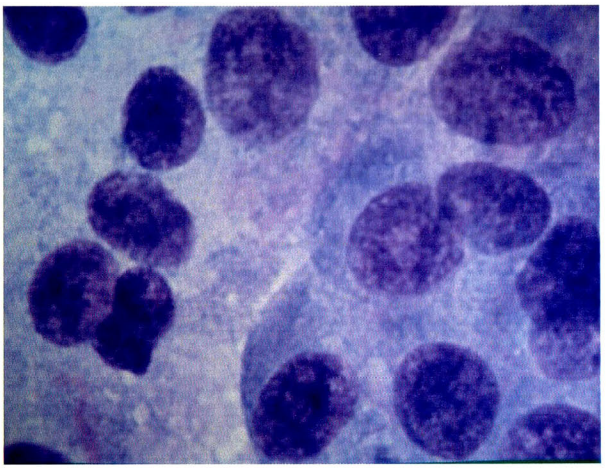


Fig.24

**Fig.23** The neopastic cells were separated by connective tissue septa and follicular structure with Call-Exer body was seen in center. HE stain. × 100.
**Fig.24** The tumor cells showing round or ovoid and resemble normal follicular cells, and the nuclei showing round ovoid. Diff Quick stain. × 1000.

Fig.25

Fig.26

**Fig.25 and Fig.26** Bovine leukemia in a 9-year-old female Japanese black cattle, weighing about 450kg. Massive lymph node enlargements in the mesentery.

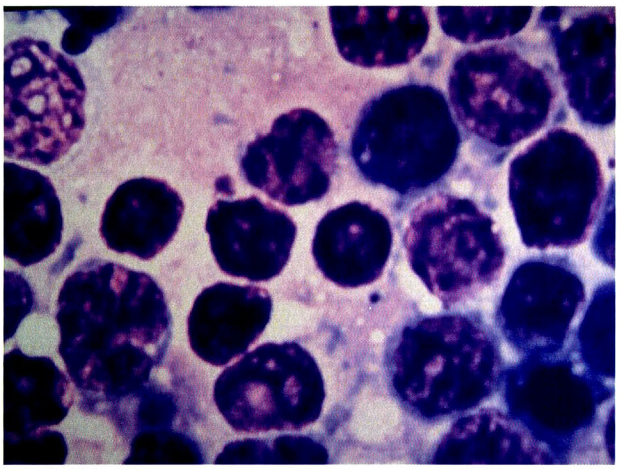


Fig.27

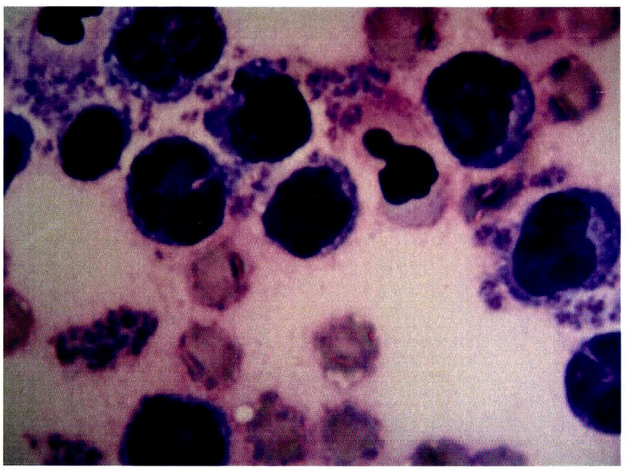


Fig.28

**Fig.27** Tumor mass touch impression. Diff Quick stain. × 1000.
**Fig.28** Blood aspirate. Observe the lymphoid cells are large with fine chromatin and obvious nucleoli. Giemsa stain. × 1000.

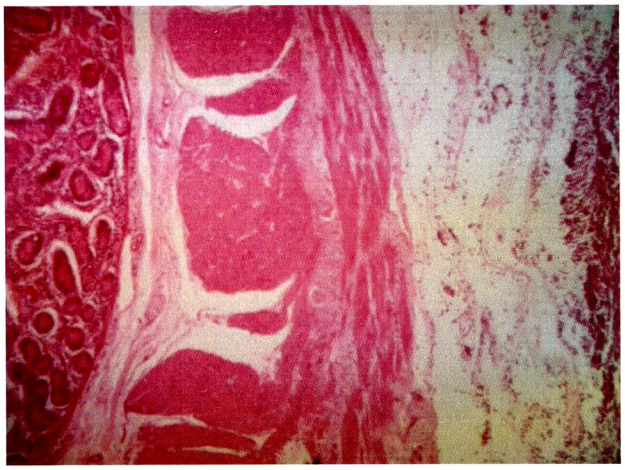


Fig.29

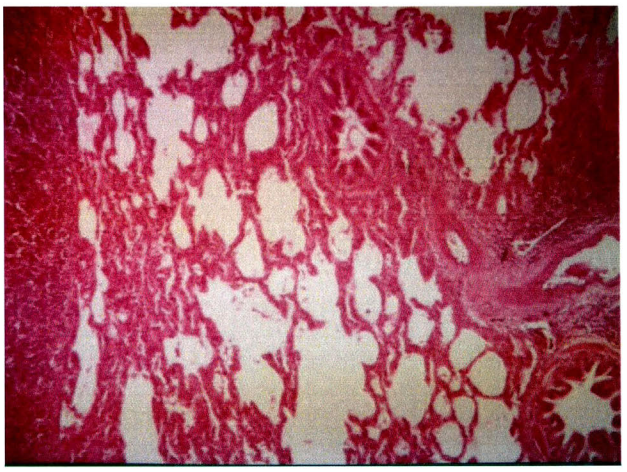


Fig.30

**Fig.29** Bovine leukemia. Infiltration of tumor cells in the small intestine. HE Stain. × 40.
**Fig.30** Infiltration of tumor cells in the lung. HE Stain. × 40.

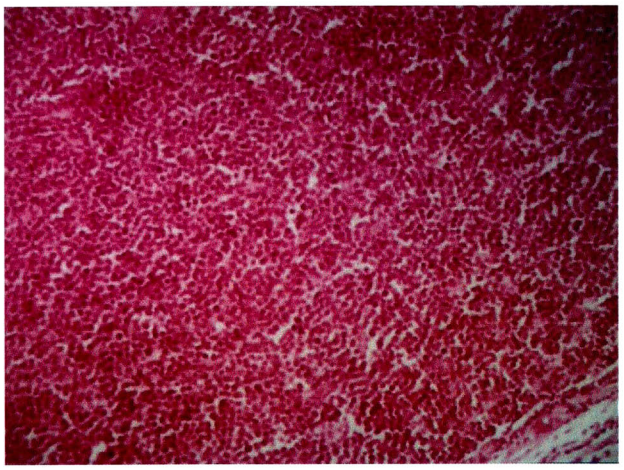


Fig.31

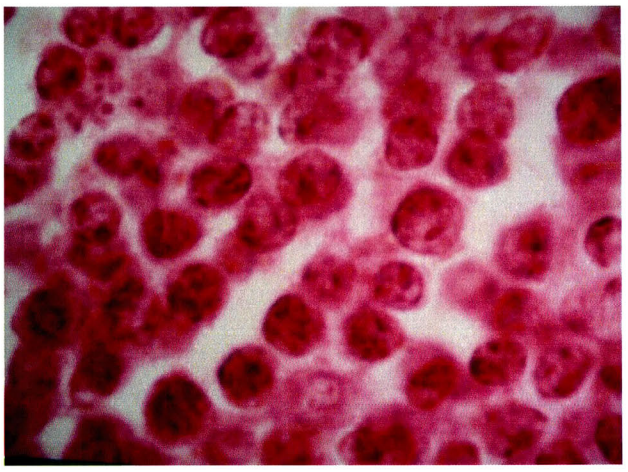


Fig.32

**Fig.31** Infiltration of tumor cells in the pulmonary lymph node. HE Stain. × 100.
**Fig.32** The neoplasmaes of pulmonary node. HE Stain. × 1000.

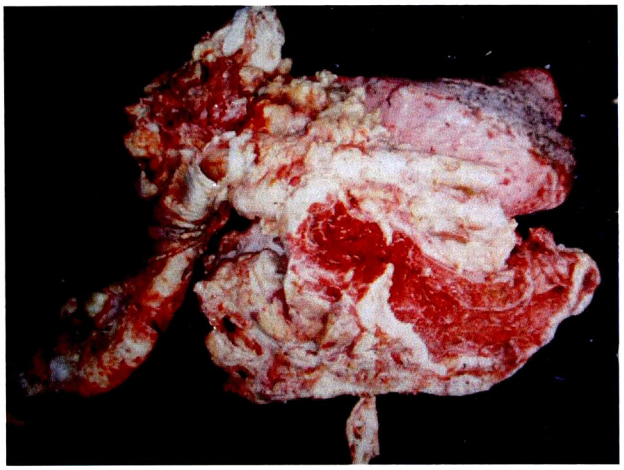


Fig.33

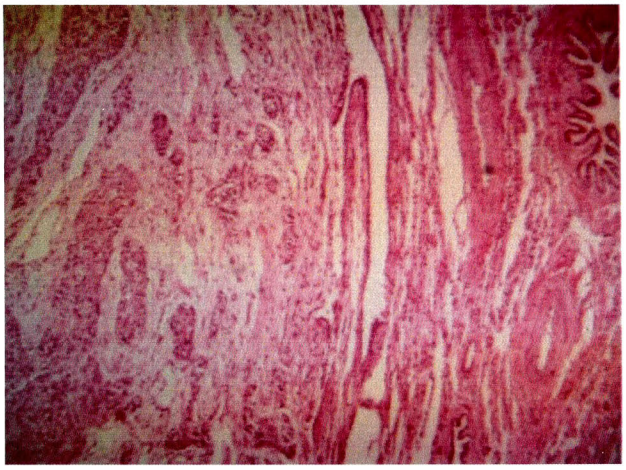


Fig.34

**Fig.33** Lung cancer in a 9-year-old female Japanese black cattle, weighing about 400kg. Diffuse mass of carcinoma in the right and left lungs. Metastastic lesions of the neoplastic cells were found in the premediastinal lymph nodes.

**Fig.34** The tumor separating by connective tissue from normal lung tissue. HE stain. ×40.

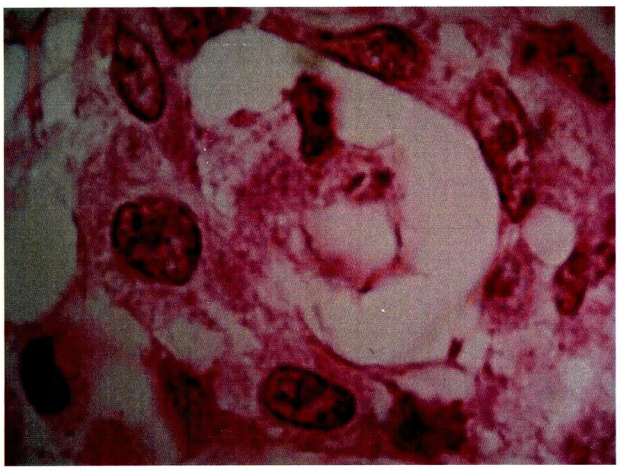


Fig.35

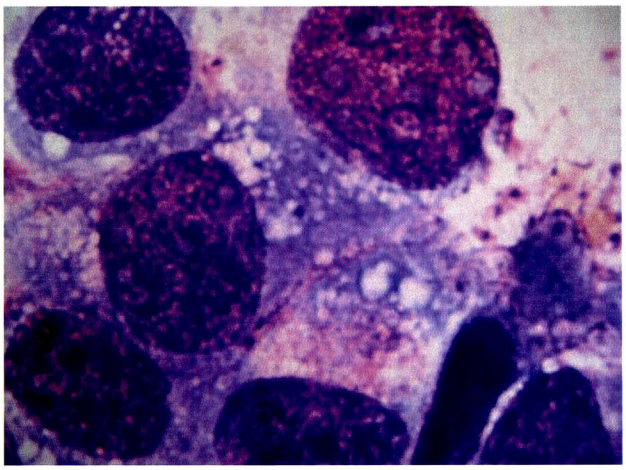


Fig.36

**Fig.35** The neoplastic tissue showing cuboidal cells form alvolar like structures. HE stain. ×1000.

**Fig.36** The tumor cells showing large, and obvious or ovoid, and tumor nuclei showing round or ovoid. The tumor mass touch impression. Diff Quick stain. ×1000.


Fig.37

Fig.38

**Fig.37 and Fig.38** Hepatocellulor carcinoma in a 10-year-old female Japanese black cattle, weighing about 450kg. Diffuse mass in the liver. The cut surface showing distinguished from the surrounding liver because of their pale color.

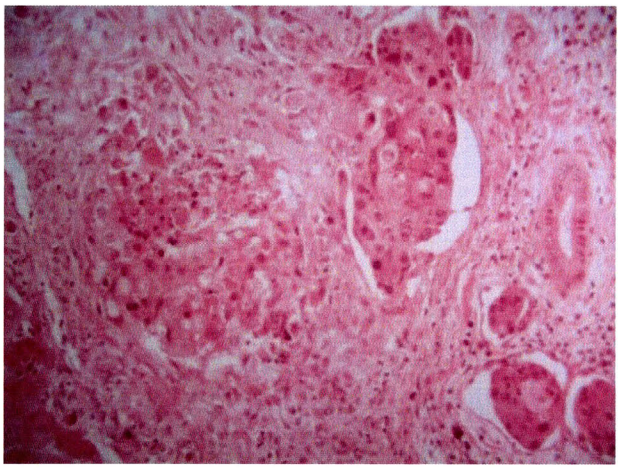


Fig.39

**Fig.39** The neoplastic tissue showing form pseudoglandular structures. The tumor cells had moderate amount of eosinophilicc cytoplasm and round nuclei with the defined chromatin. HE stain. × 100.

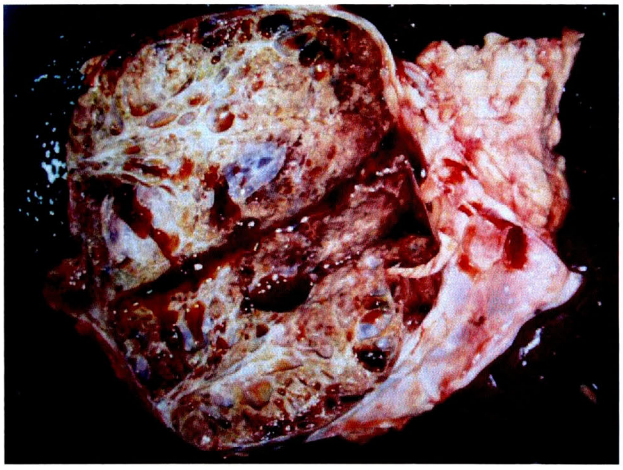


Fig.40

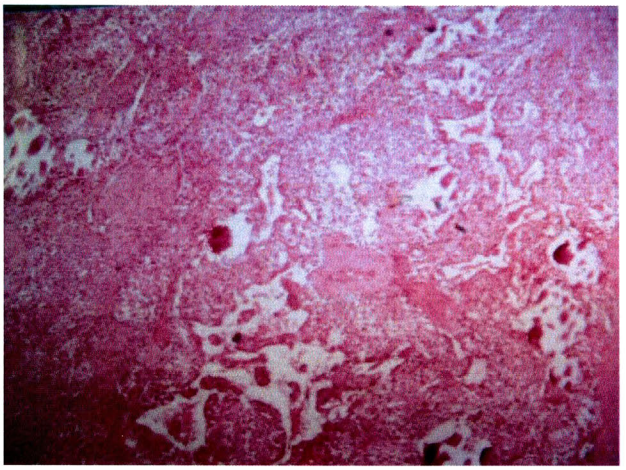


Fig.41

**Fig.40** Adrenocortical adenoma in a 5-year-old female Japanese-black cattle, weighing about 450kg. Surface view in the right side of the adrenal gland. The tumor was enlarged to approximately 34 × 23 × 11cm in size, and cut surface showing dark red collared .

**Fig.41** The tumor have some calcification. HE stain. × 40.

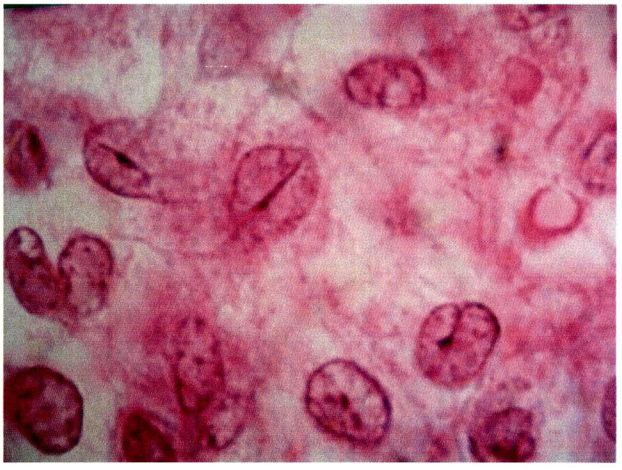


Fig.42

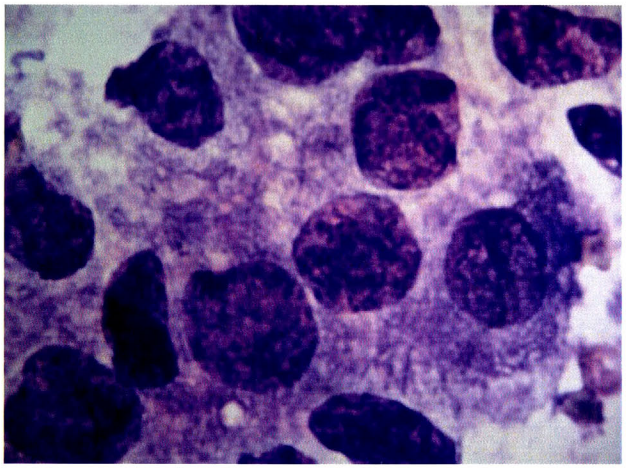


Fig.43

**Fig.42** The tumor cells had moderate amount of cytoplasm, and showing like coffee bean. HE stain. × 1000.

**Fig.43** The smear of the tumor mass. Diff Quick stain. × 1000.

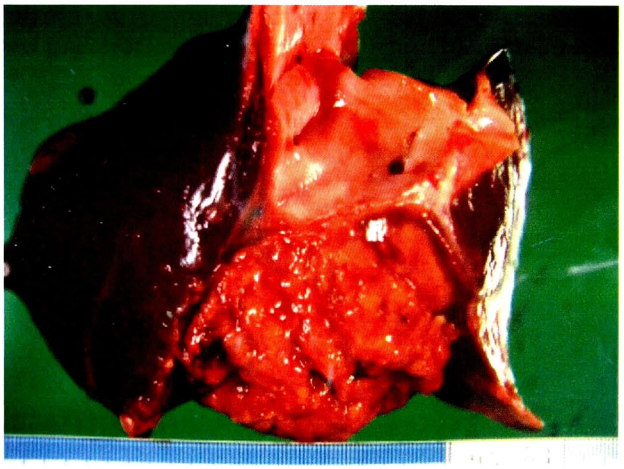


Fig.44


Fig.45

**Fig.44** Pheochromocytoma in a 8-year-old female Holstein cattle, weighing about 550kg. Surface view in the right side of the adrenal gland The tumor was enlarged to approximately 6 × 5 × 5cm in size, and cut surface showing yellow brown.
**Fig.45** The formalin liquid changes to yellow brown from clean color.

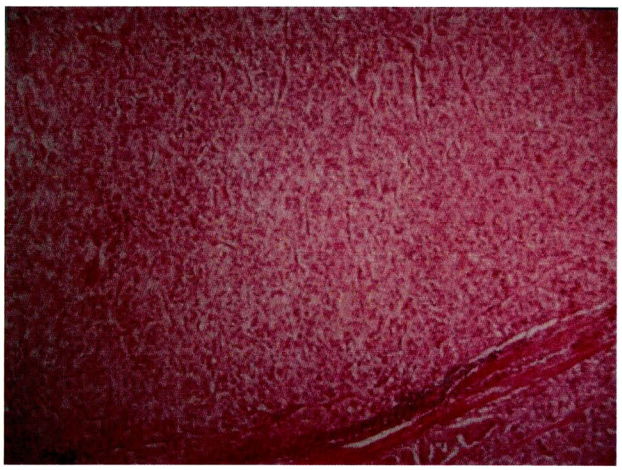


Fig.46

**Fig.46** The tumor cells showing vary cells from small cuboidal to polyhedral, similar to those in normal adrenal medulla.
The tumor cells showing positive for Grimelius' silver stain. HE stain. × 40.

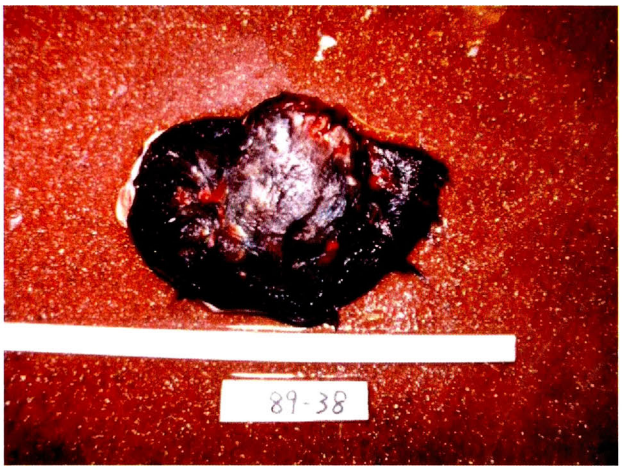



Fig.47

Fig.48

**Fig.47** Malignant melanoma in left shoulder skin of a 5-year-old female Holstein cattle, weighing about 540kg. The tumor was enlarged to approximately 7 × 7 × 4cm in size.

**Fig.48** Metastasis of the malignant melanoma to right lung showing multiple black nodules.

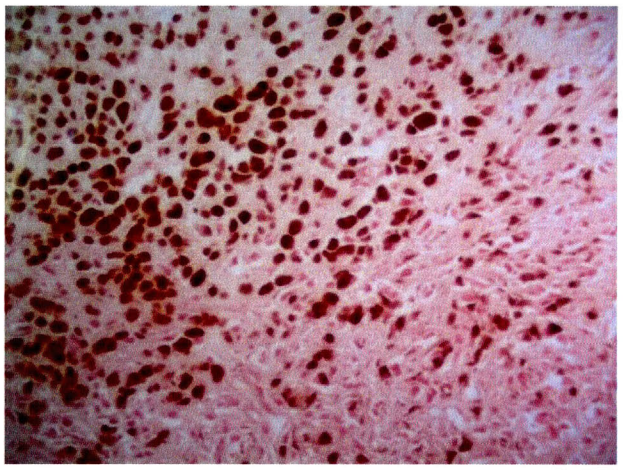


Fig.49

**Fig.49** Junctional melanocytoma showing nests of melanocyotes at the lung. HE stain. × 40.

Fig.50

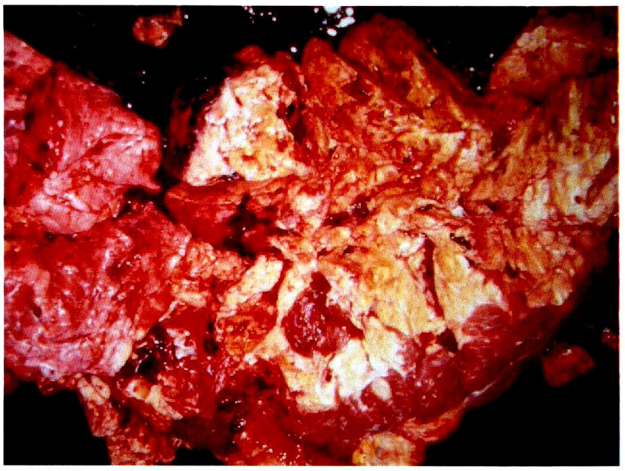


Fig.51

**Fig.50** Rhabdomyosarcoma in the neck muscles of a 10-year-old female Japanese black cattle, weighing about 380kg.
**Fig.51** The neoplasm shape line demarcation from normal skeleton maculae the cut surface showing gray-white.

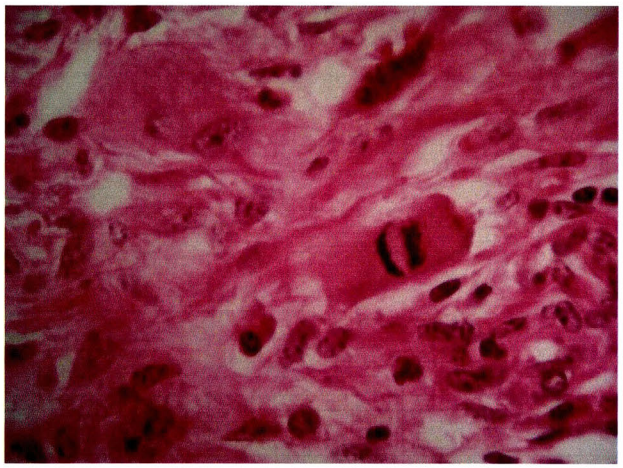


Fig.52

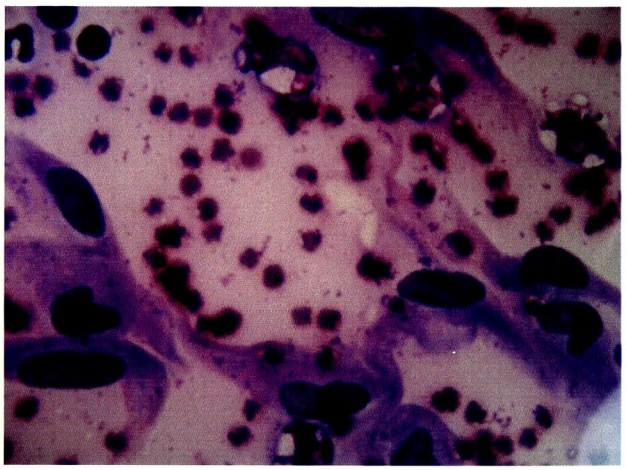


Fig.53

**Fig.52** Scattered mitotic figures were present. HE stain. × 400.
**Fig.53** Impression smear of the neoplasm showing elongated muscle fibers. Diff Quick stain. × 400.

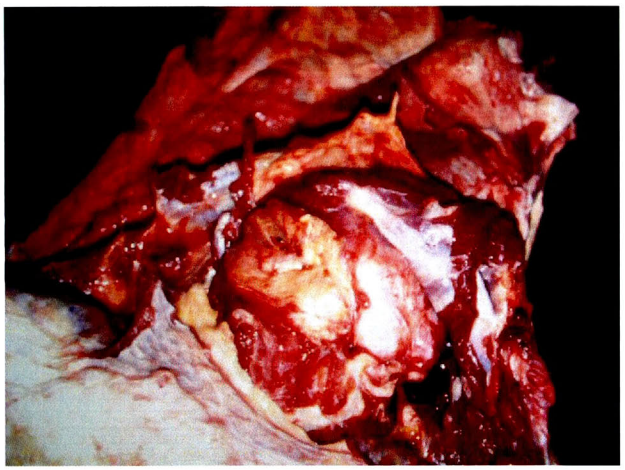


Fig.54

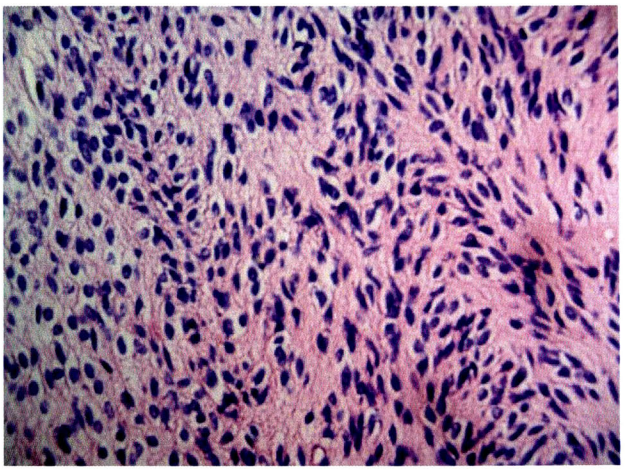


Fig.55

**Fig.54** Schwannoma in a 13-year-old female Japanese black cattle, weighing about 450kg. The neoplasm showing skeletal muscles.
The tumor was enlarged to approximately 20 × 15 × 15cm in size and cut surface showing gray-white collar.

**Fig.55** The neoplastic cells showing antoni type-A tissue patterns.
HE stain. × 200.

# 4. CHAPTER Ⅲ

## Bovine Tumors Detected at 6 Meat Inspection Offices in Miyazaki Prefecture during 1978-1993

### 4.1 *INTRODUCITON*

On the long-term statistics for cattle tumors in Japan, some reports have been described by several universities [3,44,47,56], and by certain meat inspection offices [14, 21,23,26]. Recently, Kanagawa Meat Inspection Office has reported the results concerning the occurrence of swine tumors in the past 21 years [23]. The national scale statistical survey on tumor occurrence situation at the meat inspection offices was also carried out as of the end of 1995. However, few reports can be found for systematic analysis of tumors, which is unique to respective area as well as the reports on the comparative researches of bovine tumors between neighborhood prefectures.

We have examined the detected situation and pathological search of bovine tumors by chronological trends, breeding habitat, cattle breeding and gender in the vicinities of 6 meat inspection offices in Miyazaki prefecture. In addition, we investigated the differences of tumor incidence among 17 meat inspection offices in respective prefectures and cities in Kyushu.

### 4.2 *MATERIALS AND METHODS*

Out of 466,699 slaughtered cattle at 6 meat inspection offices in Miyazaki prefecture during 16 years form 1978 to 1993, 542 tumor cases were examined by chronological trends, breeding styles, gender, and organs, and followed by histopathological search. The breeds of cattle are broadly classified into Holstein

including other foreign cattle and Japanese black cattle. To know the regional peculiarities, the incidence of cattle tumors at 17 meat inspection offices in respective prefectures and cities in Kyushu during the past 16 years, were compared.

Histopathological examinations were performed on paraffin sections stained with hematoxylin and eosin (HE) staining and other various special stainings. Some selected sections were employed for immunohistochemistry using an avidin-biotin-peroxidase complex (ABC) method (Vectastain PK-400, Vector Laboratories, Burlingame, CA, U.S.A.). Papillomas, which often occur as a change to a morbid state, are excluded from this study because of incomplete records.

## 4.3 *RESULTS*

***Chronological trend:*** Total number of slaughtered cattle in Miyazaki prefecture from 1978 to 1993 was 466,699 with average annual slaughtered number at 29,169. Among the chronological trend of detected tumors amounted to 542 cases in 16 years (0.116%), 58 cases in 1988 was the highest rate and 12 cases in 1990 was the lowest rate with average annual occurrence rate at 33.9 cases. Tumors occurrence rate per 10,000 cattle was 11.6 cases, with 22.9 cases in 1979 was the highest, and yearly decreasing trend can be seen thereafter (Fig. 56).

***Detected tumors situations by breed, gender and region*:** The incidence of tumors by cattle breed and gender for 481 cases with sustaining record files, out of total 542 cases, were 329 cases for Japanese black cow (68.3%), 17 cases for steer (3.5%), 4 cases for sex unknown Japanese black, 77 cases for Holstein cow (16.0%), 19 cases for steer (3.9%), 5 cases for sex unknown Holstein, and 30 cases for unknown cattle breeding (6.2%). The incidence of tumors in each organs were 92 cases for the hematopoietic system (19.1%), 80 cases for the digestive system (16.6%), 76 cases for the genital system

(15.8%), 29 cases for the cutaneous system (6.0%), 24 cases for the respiratory system (4.9%), 23 cases for the urinary system (4.8%), 23 cases for the endocrine system (4.8%), 15 cases for the skeletal system (3.1%), and 119 cases for the other systems (24.7%, Table 5). The total number of cattle slaughtered during 11 years from 1983 when the year when Miyazaki prefecture started to collect records according to cattle breeds, to 1993, was 391,134, and they were composed of 114,682 Japanese black cow (29.3%), 106,602 steer (27.3%), 51,769 Holstein cow (13.2%), 114,208 steer (29.2%) and 3,873 the others (1.0%). Statistically slaughtered result of these data showed that tumor occurrence rate per 10,000 cattle was the highest at 23.8 cases with Japanese black cow followed by 12.7 cases with Holstein, 1.4 cases with Holstein steer, and 1.3 cases with Japanese black steer. By the occurrence of each organ, most tumors in Japanese black cattle were found in the genital and digestive systems other than 70 cases for mesothelioma and most tumors in Holstein cattle were detected for the hematopoietic system (Table 6).

***Histopathological classification*:** Histopathological classifications of 481 cases were 87 cases for lymphoma (18.1%), 74 cases for mesothelioma (15.4%), 52 cases for ovarian granulosa cell tumors (10.8%), 29 cases for hepatoma (6.0%), 18 cases for lung cancer (3.7%) and 12 cases for adrenal gland tumor (2.5%). Approximately 57% of tumors were these six tumors. The most frequently recorded tumors were 69 cases of mesothelioma in Japanese black cow (5.0 cases/10,000) followed by 49 cases of ovary granulosa cell tumor, 29 cases of bovine leukosis, 24 cases of hepatoma, 14 cases of lung cancer (including squamous cell carcinoma) and 8 cases of adrenal cortex gland tumor. In Holstein cow, 29 cases of bovine leukosis (4.7 cases/10,000) followed by 5 cases of squamous cell carcinoma, 4 cases of mesothelioma, and 4 cases of hepatoma. Then, bovine leukosis was found in 9 cases in Japanese black and 8 cases in Holstein steer (Table 6).

***Comparison of tumor occurrence rates by meat inspection offices in Miyazaki prefecture*:** The incidence of bovine tumors at 6 meat inspection offices in Miyazaki prefecture is represented in Figure 57. The highest occurrence rate of 24.7 cases/10,000, which was about 2 times higher than the other meat inspection offices, was seen at Miyakonojo Meat Inspection Office where older Japanese black and milking cows have been slaughtered. Especially, big difference of about 4 times was seen by comparing its rate with that of Takasaki Meat Inspection Office where more fattening cows were slaughtered at the plants certified to export meat to the United States (Fig. 57).

***Comparison of tumor incidence with other meat inspection offices in respective prefectures and cities in Kyushu*:** The results of tumor occurrence during 16 years from 1978 to 1993 at respective prefectures and cities are represented in Table 3. Among 1,186 cases (0.055%) out of total 2,175,645 cattle, 304 cases were bovine leukosis, and 20 cases were mesotheliomas. Of 304 bovine leukosis cases, 244 were found in Oita prefecture (26 cases/10,000). At 6 meat inspection offices in Miyazaki prefecture, 542 cases (0.116%) were detected, which is about 2 times higher than those in other prefectures and cites in Kyushu. Of which, 87 cases were bovine leukosis and 74 cases were mesothelioma (Table 7).

## 4.4 *DISCUSSION*

The incidence of tumors during17 years from 1975 to 1991 in Japan, according to the operational report for Sanitary Administration of the Welfare Ministry, varied from 7.1 to 53.3 cases per 100,000 swine (mean 27.7 cases) [20]. However, the number was varied in previous reports. Those were 7.3 cases per 100,000 by Kashima et al. [23], 3.3 cases by Brandly and Migaki et al.[14] in United States, and 3.8 cases by Anderson et al. [3] in England. While it is said that tumor occurrence rate in cattle is higher than that in

swine as their breeding terms are far much longer than 6 months for swine, the tumor incidence in cattle during 19 years from1975 to 1993, as reported in the operational report for Sanitary Administration of the Welfare Ministry, were varied from 7.4 to 59.1 cases per 10,000 cattle (mean 26.3 cases). The results in this study at same period were from 3.4 to 22.9 cases (mean 11.6 cases) per 10,000 cattle, which was about half rate in cattle covering nationwide meat inspection offices investigated by the Welfare Ministry. Reasons for the lower incidence might be because Miyazaki prefecture is one of the stock breeding prefectures, 57% of slaughtered cattle is accounted by fattening cattle with shorter breeding terms. In addition, the incidence of papilloma has been excluded from the records. While the average ages by breed and gender of cattle with tumors was 11.1 years old in Japanese black cow and 2.7 years old in steer, and 6.3 years old in Holstein cow and 2.2 years old in steer. High tumor occurrence was found in higher age of Japanese black cow (23.8 cases/10,000) followed by 12.6 cases/10,000 in Holstein steer. The phenomena might be due to longer breeding period in Japanese black cow to obtain more reproduction opportunities.

Highly susceptible tumors were in bovine leukosis, mesothelioma and ovary granulosa cell tumor, respectively. Bovine leukosis is considered as highest incidence of bovine tumors according to the statistics of nationwide meat inspection offices [20]. The similar trend has been confirmed in this study and the incidental ratio of this tumor is concluded as 87 cases out of 542 samples (1.8 cases/10,000). Almost of these 87 cases were lymphocytic lymphoma, adult, and polycentric type with high incidence in Holstein cow, which accord with the reports of the meat inspection offices at most areas in Japan, with the incidental rate (mean 4.7 case/10,000). Similarly, the occurrence rate is higher in cattle with ages of 5 to 8 years (mean 6.0 years old) [59]. Of 304 cases of bovine leukosis in each prefecture or city in Kyushu, highest occurrence was found in Ohita

prefecture; 244 cases (26.3 cases/10,000) [39], suggesting wide spreading of bovine leukosis virus in this area.

Bovine mesothelioma, which is disseminated tumor in the pleura and peritoneum, rarely occurs in Japan. The tumors were found in 74 cases (1.5 cases/10,000) following to bovine leukosis. As noted in the report of Miyazaki University [44], more mesothelioma can be found in southern Kyushu at 12 cases (18.2%) out of 55 bovine tumors. The high occurrence of mesothelioma was also found (57 cases out of 74 cases) in the prefecture including the vicinities of Miyakonojo Meat Inspection Office and other surrounding areas. These findings may indicate that the tumor has peculiar occurrence in this area. Although bovine mesothelioma is said as congenial tumor and is more susceptible in calf [22,63], the result showed that much of the tumor was seen in older Japanese black cattle at mean ages of more than 10 years. Also, while pleural mesothelioma can be often found in humans [52,62], peritoneum mesothelioma was seen more frequently in cattle. The difference between humans and cattle may suggest that some exposures through digestive organ system than through respiratory system might be closely associated to the tumoregenesis of bovine peritoneal mesotheliomas. Histopathologically, as similar with Ackerman's classification [2], epithelial types with tube and cavity formation and papillary hyperplasia were seen more frequently than sarcomatous types with conspicuous substrate hyperplasia and mixed/biphasic types. While the cause of human mesothelioma has been reported for asbestos participation [52, 62] since after Wagner's report [60], the pathogenesis of bovine mesotheliomas is still unknown [63]. We have previously investigated that many cow houses in southern Kyushu used 'Shirasu' [50] instead of sawdust because of low cost, availability, and high water absorption. People in Miyakonojo and surrounding areas have kept a traditional event to scatter 'Shirasu' (volcanic ash erupted by Kirishima Big Eruption, about 20,000

years ago, similar with volcanic glass having similar chemical structure with asbestos) in the garden. We assume that 'Shirasu' might have some roles on the tumoregenesis of bovine mesotheliomas in these areas.

The third highest incidence was noted in ovarian granulosa cell tumor at 52 cases (1.1 cases/10,000), which accounted for the major ovarian tumors as in previous reports [20]. The ovary of cattle is restrained its activity due to the compression of the paunch located at left abdominal region [20]. Previously, we examined the relationship between the incidence of ovarian tumors and its regions grossly (Data not shown). In the studies, only a few cases had ovarian tumors in both sides and most tumors laterally appeared, while there was no significant difference between the left and right sides.

Concerning adrenal gland tumors, there is a report describing that the tumors in the cortex and medulla occurred in almost same frequency in 327 bovine cases with adrenal gland tumors by Wright [20]. In this study, 10 cases of all 12 adrenal gland tumors were cortical adenomas and those were found in Japanese black older cattle. Since bovine adrenocartical adenomas has been described to occur dominantly in older cattle [20], the results might be that a number of meat cattle and milking cattle were slaughtered in some of the meat inspection offices with higher occurrence rates.

In conclusion, present study has conducted the researches on the actual conditions of 6 meat inspection offices in Miyazaki prefecture and could find the characteristics of occurrence situation of cattle tumors during the 16 years in Miyazaki prefecture.

## 4.5 *ABSTRACT*

Tumors were detected in 542 cases (0.116%) of 466,699 slaughtered cattle examined during 16 years at 6 meat inspection offices in Miyazaki prefecture during 16 years from 1978 to 1993, including 87 cases of bovine leukosis, 78 cases of mesothelioma, 52 cases of ovary granulosa cell tumor, 29 cases of hepatoma, 18 cases of lung cancer, and 12 cases of adrenal grand tumor etc. By cattle breeding and by distinction of sex, Japanese black cows were detected at 23.8 cases per 10,000 with the highest rates including mesothelioma and ovary granulosa cell tumor. Holstein dairy cows were at 12.7 cases including bovine leukosis and mesothelioma. Japanese black steer and Holstein steer were only 1.3 cases and 1.4 cases respectively including bovine leukosis in both classifications. By habitat, in Miyakonojo area, more older cattles have been slaughtered, was detected with 24.7 cases per 10,000 which was about 4 times more than Takasaki, where they keep the plants certified to export meat to the United States and more fattening cattles have been slaughtered, and it shown greater differences by cattle breeding and by sexes of the slaughtered cattle. Bovine leukosis that has broken out with the highest rate at the meat inspection offices in all parts of Japan was found at equally high rate, and followed by mesothelioma which has been regarded as rate cases to arise. Especially, within the vicinities of Miyakonojo Meat Inspection Office, occurrence of mesothelioma showed very high rate at 5.3 cases of per 10,000 and gave suggestions that mesothelioma is tumors specifically susceptible to break out in Southern Kyushu.

KEY WORDS: cattle tumors, cattle breed, meat inspection, mesothelioma, Southern Kyushu.

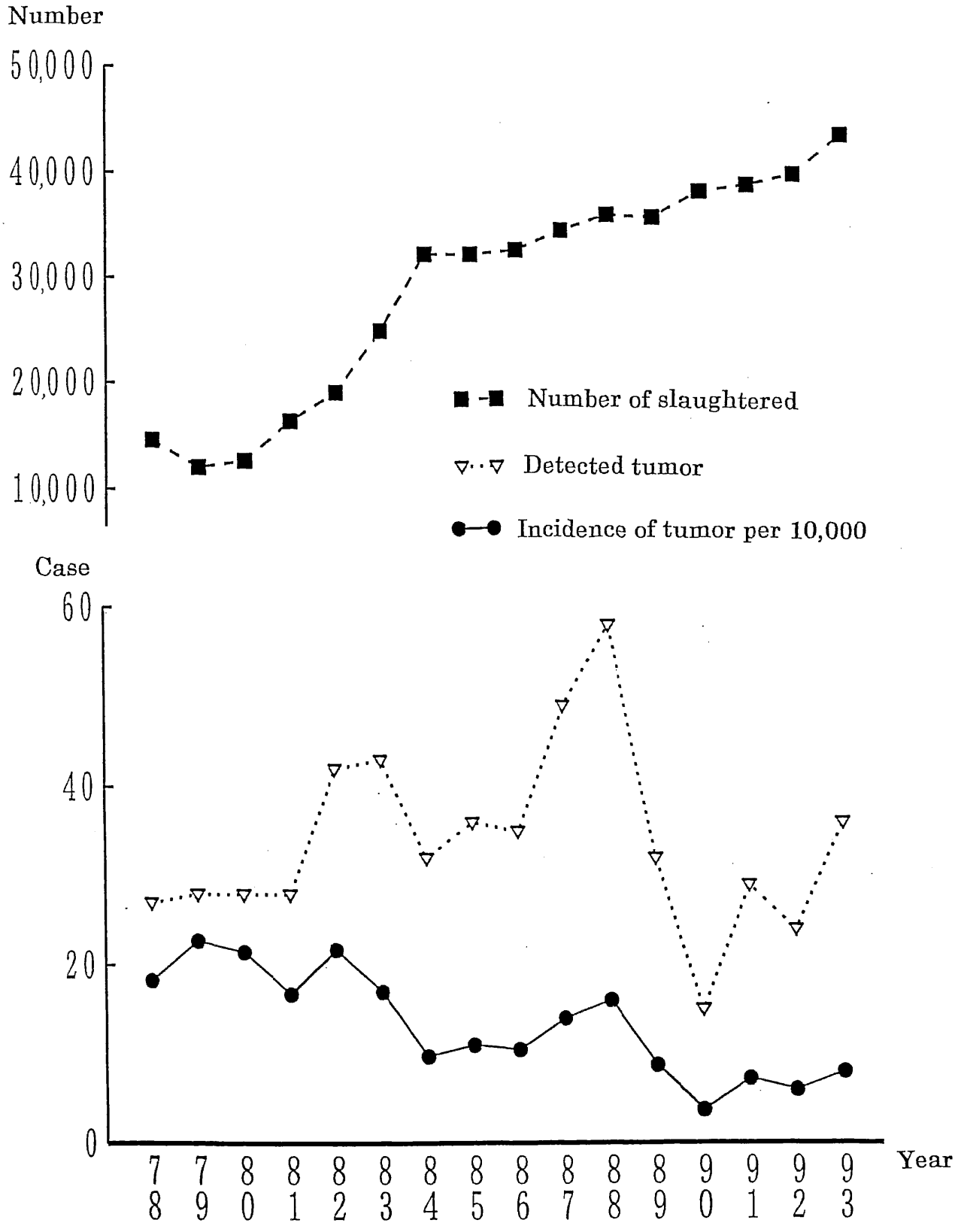



Fig. 56 Chronological trend

**Table 5. Location and region of tumors detection on breeding cattles, sex**

| Breeding, sex | | Japanese black | | | Holstein | | | Unknown |
|---|---|---|---|---|---|---|---|---|
| System | Cases | ♀ | ♂ | U N | ♀ | ♂ | U N | breeding |
| Hematopoietic system | 92 | 31 | 9 | 2 | 30 | 9 | 1 | 10 |
| Respiratory system | 24 | 20 | 1 | - | 2 | - | - | 1 |
| Digestive system | 80 | 61 | - | 1 | 9 | 2 | 2 | 5 |
| Urinary system | 23 | 15 | 1 | - | 4 | 1 | 1 | 1 |
| Genital system | 76 | 69 | - | - | 5 | - | - | 2 |
| Cutaneous system | 29 | 14 | 3 | 1 | 9 | 1 | 1 | - |
| Skeletal system | 15 | 7 | 2 | - | 4 | 1 | - | 1 |
| Endocrine system | 23 | 13 | - | - | 4 | 3 | - | 3 |
| Others | 119 | 99 | 1 | - | 10 | 2 | - | 7 |
| **Total** | **481** | **329** | **17** | **4** | **77** | **19** | **5** | **30** |

U N : Unknwon sex

## Table 6. Histopathological classification of tumors found

| System | cases | Japanese Black ♀ | | Japanese Black ♂ | | Japanese Black UN | | Holstein ♀ | | Holstein ♂ | | Holstein UN | | Unknown breeding | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Total** | **481** | | **329** | | **17** | | **4** | | **77** | | **19** | | **5** | | **30** |
| Hematopoietic system | 92 | lymphoma | 29 | | | lymphoma | 9 | lymphoma | 2 | lymphoma | 29 | lymphoma | 8 | lymphoma | 1 | lymphoma | 9 |
| | | other(lymph, spleen) | 2 | | | | | | | other(spleen) | 1 | other(spleen) | 1 | | | other(spleen) | 1 |
| Respiratory system | 24 | lung cancer | 8 | fibroma | 2 | lung cancer | 1 | | | lung cancer | 2 | | | | | squqmous cell carcinoma | 1 |
| | | squamous cell carcinoma | 6 | fibrosarcoma | 1 | | | | | | | | | | | | |
| | | other(lung, trachea) | 3 | | | | | | | | | | | | | | |
| Digestive syatem | 80 | hepatocellulor carcinoma | 18 | liver cell adenoma | 6 | | | other(liver) | 1 | hepatocellulor carcinoma | 4 | lipoma | 2 | fibroma | 1 | hepatocellulor carcinoma | 1 |
| | | cholangiocarcinoma | 6 | hyperplastic polyp | 5 | | | | | | | | | other (liver) | 1 | adenoma | 1 |
| | | adenocarcinoma | 4 | fibroma | 2 | | | | | liver cell adenoma | 1 | | | | | fibroma | 1 |
| | | adenoma | 3 | fibrosarcoma | 1 | | | | | pancreatic carcinoma | 2 | | | | | papilloma | 1 |
| | | papilloma | 3 | hemagioma | 1 | | | | | papilloma | 2 | | | | | other | 1 |
| | | squamous cell carcinoma | 1 | other(liver,intestine) | 11 | | | | | | | | | | | | |
| Urinary system | 23 | nephroblastoma | 2 | renal carcinoma | 1 | papilloma | 1 | | | nephroblastoma | 1 | papilloma | 1 | other (bladder) | 1 | renal carcinoma | 1 |
| | | papilloma | 4 | rhabdomyosarcoma | 1 | | | | | renal carcinoma | 1 | | | | | | |
| | | hemangioma | 3 | other(renal,bladder) | 4 | | | | | other(renal,bladder) | 2 | | | | | | |
| Genital system | 76 | granulosa cell tumor | 49 | hemangioma | 3 | | | | | granulosa cell tumor | 1 | | | | | granulosa cell tumor | 2 |
| | | luteoma | 3 | thecoma | 1 | | | | | leioma | 1 | | | | | | |
| | | leioma | 8 | leiosarcoma | 2 | | | | | other(ovary) | 3 | | | | | | |
| | | squamous cell carcinoma | 1 | adenocarcinoma | 1 | | | | | | | | | | | | |
| | | other(ovary) | 1 | | | | | | | | | | | | | | |
| Cutaneous system | 29 | melanoma | 3 | fibroma | 3 | fibroma | 1 | melanoma | 1 | squamous cell carcinoma | 5 | melanoma | 1 | melanoma | 1 | | |
| | | squamous cell carcinoma | 2 | fibrosarcoma | 2 | fibrosarcoma | 1 | | | melnoma | 2 | | | | | | |
| | | mixed tumor | 1 | liposarcoma | 1 | melanoma | 1 | | | other(eye,skin) | 2 | | | | | | |
| | | other(tongue) | 2 | | | | | | | | | | | | | | |
| Skeletal system | 15 | rhabdomyosarcoma | 3 | leiosarcoma | 1 | rhabdomyoma | 1 | | | rhabdomyosarcoma | 2 | fibroma | 1 | | | other(muscle) | 1 |
| | | rhabdomyoma | 2 | osteoma | 1 | other(muscle) | 1 | | | other(muscle) | 2 | | | | | | |
| Endocrine system | 23 | adrenocartical adenoma | 8 | thyroid adenoma | 1 | | | | | thyroid adenoma | 2 | thyroid adenoma | 1 | | | adrenocartical adenoma | 1 |
| | | pheochromaocytoma | 2 | other(adrenal) | 2 | | | | | adrenocartical adenoma | 1 | | | | | other(adrenal) | 2 |
| | | | | | | | | | | other(adrenal) | 1 | other(adrenal) | 2 | | | | |
| Others | 119 | mesothelioma | 69 | adenocarcinoma | 13 | mesothelioma | 1 | | | mesothelioma | 4 | other (peritoneum) | 2 | | | | |
| | | leiomyoma | 1 | ameloblastoma | 1 | | | | | adenocarcinoma | 2 | | | | | other(peritoneum) | 7 |
| | | other(peritoneum) | 15 | | | | | | | other(peritoneum) | 4 | | | | | | |

UN : Unknwon sex  no file case 61

Table 7. Comparison of tumor occurrence situations with other meat inspection offices in respective prefectures, cities in Kyushu (1978-1993)

| Disorders | meat inspection cases | tumor cases(%) | mesothelioma(%) | lymphoma(%) |
|---|---|---|---|---|
| 6 Meat inspection offices in Miyazaki prefecture | 466,699 | 542(0.116) | 74(0.0159) | 87(0.0186) |
| 17 Meat inspection offices in respective prefecture, cities in Kyushu | 2,175,645 | 1,186(0.055) | 20(0.00092) | 304(0.01397) |

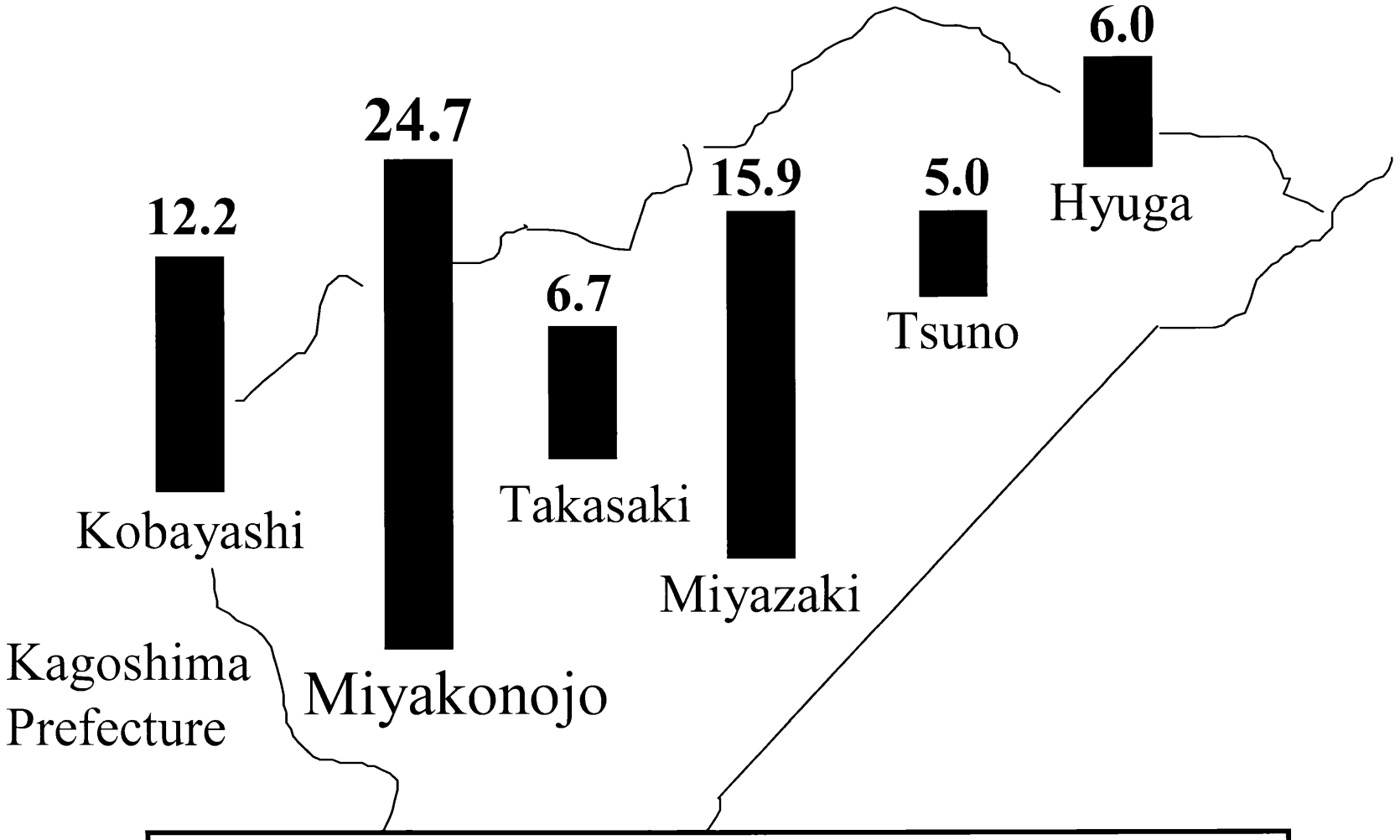



**Fig. 57 Tumors occurrence rate per 10,000 cattles at 6 meat inspection offices in Miyazaki prefecture.**

# 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 123$^{rd}$ 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 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 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 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)

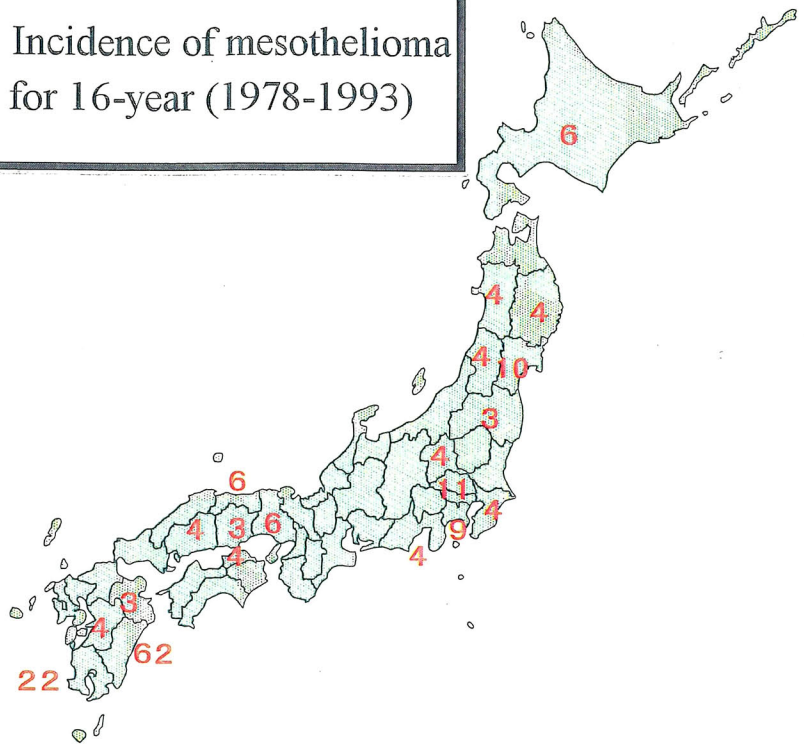



Fig.59

**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

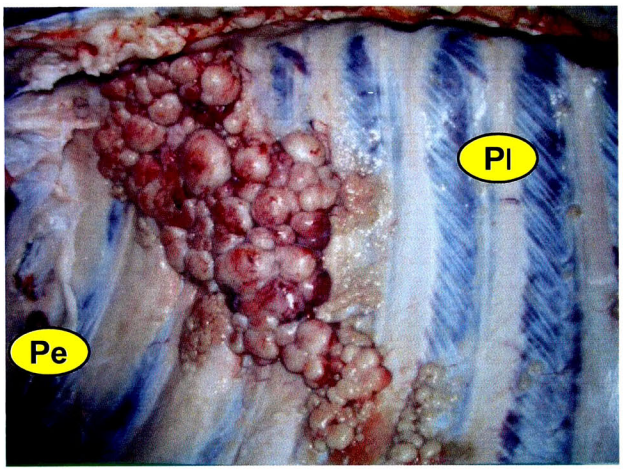
**Fig.62** Shirasu at the garden of the livestock farmer in Miyakonojo area.
**Fig.63** Many livestock farmers in Miyakonojo area use the water permeability of Shirasu for cattle barn bedding.

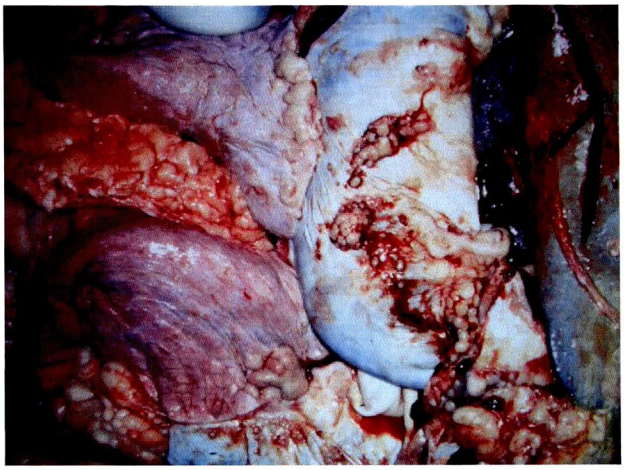
Fig.64

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.

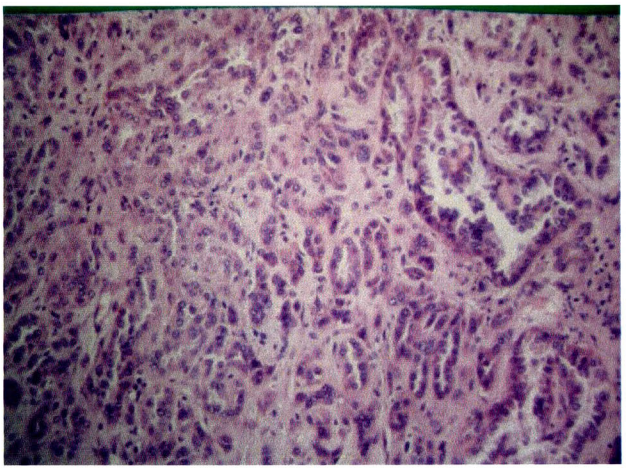
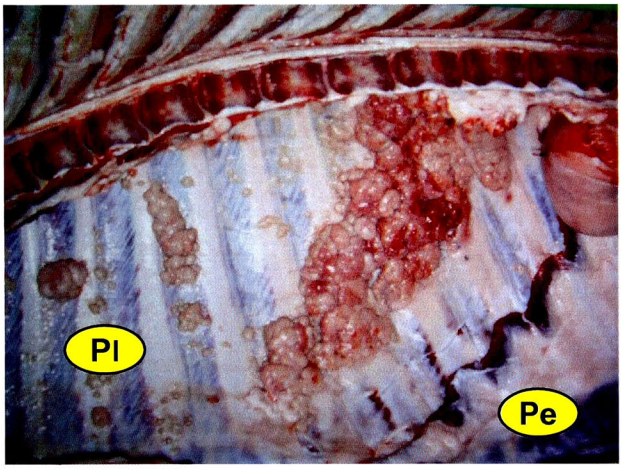


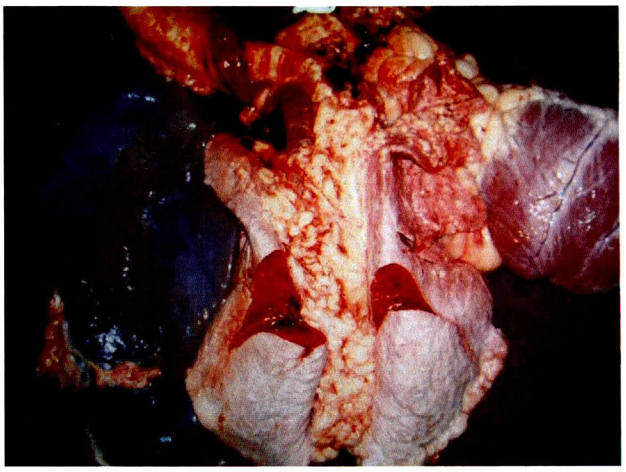
Fig.68

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)



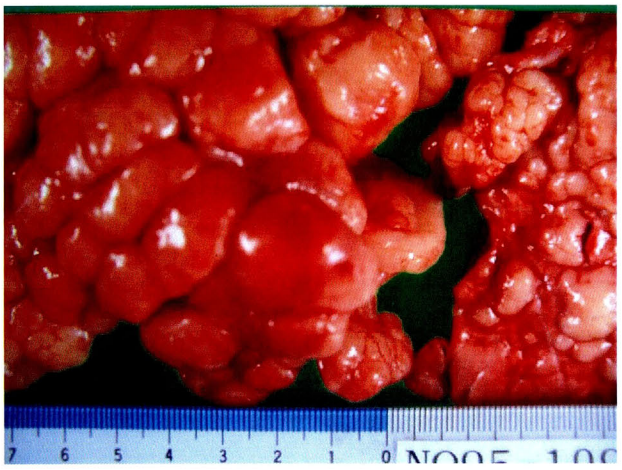
Pl :Pleura Pe :Peritoneum

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.



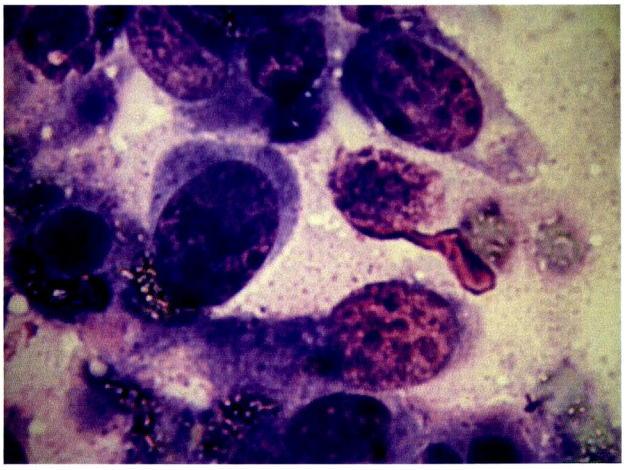


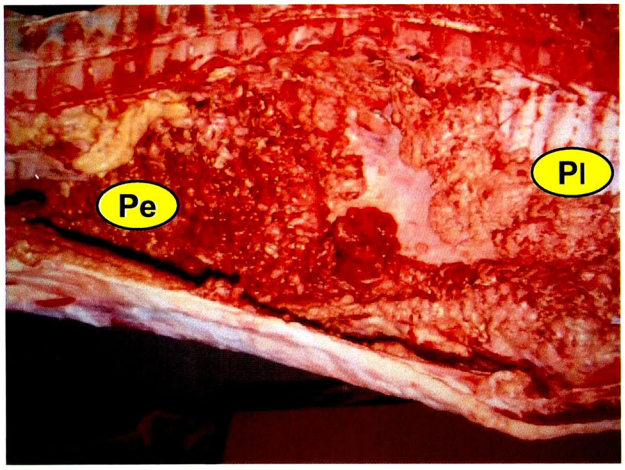
Fig.72

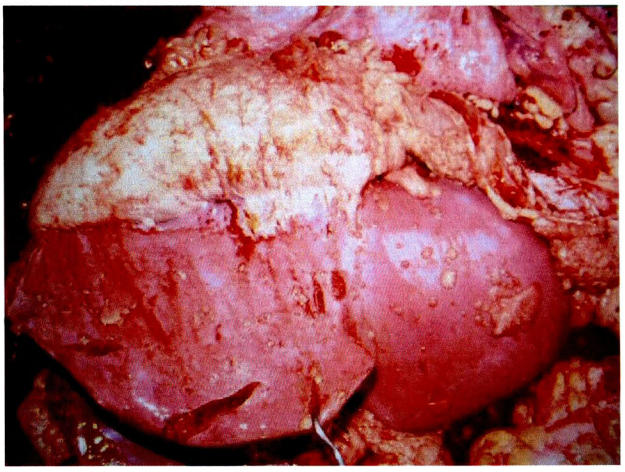
Fig.73

**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)



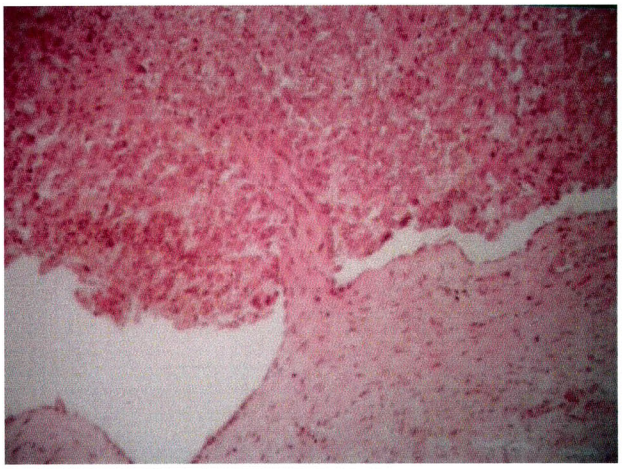



Pl :Pleura Pe :Peritoneum

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.



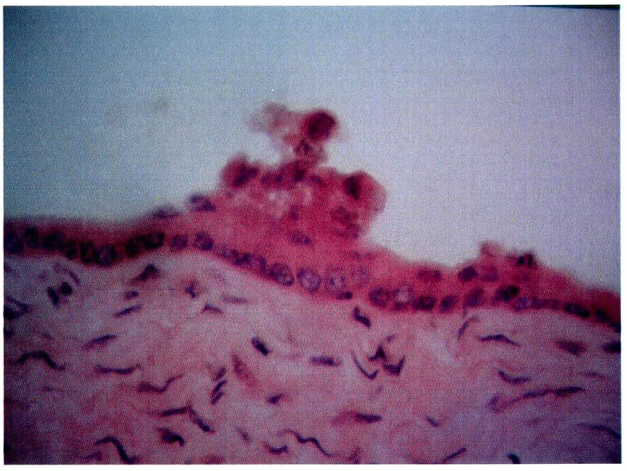
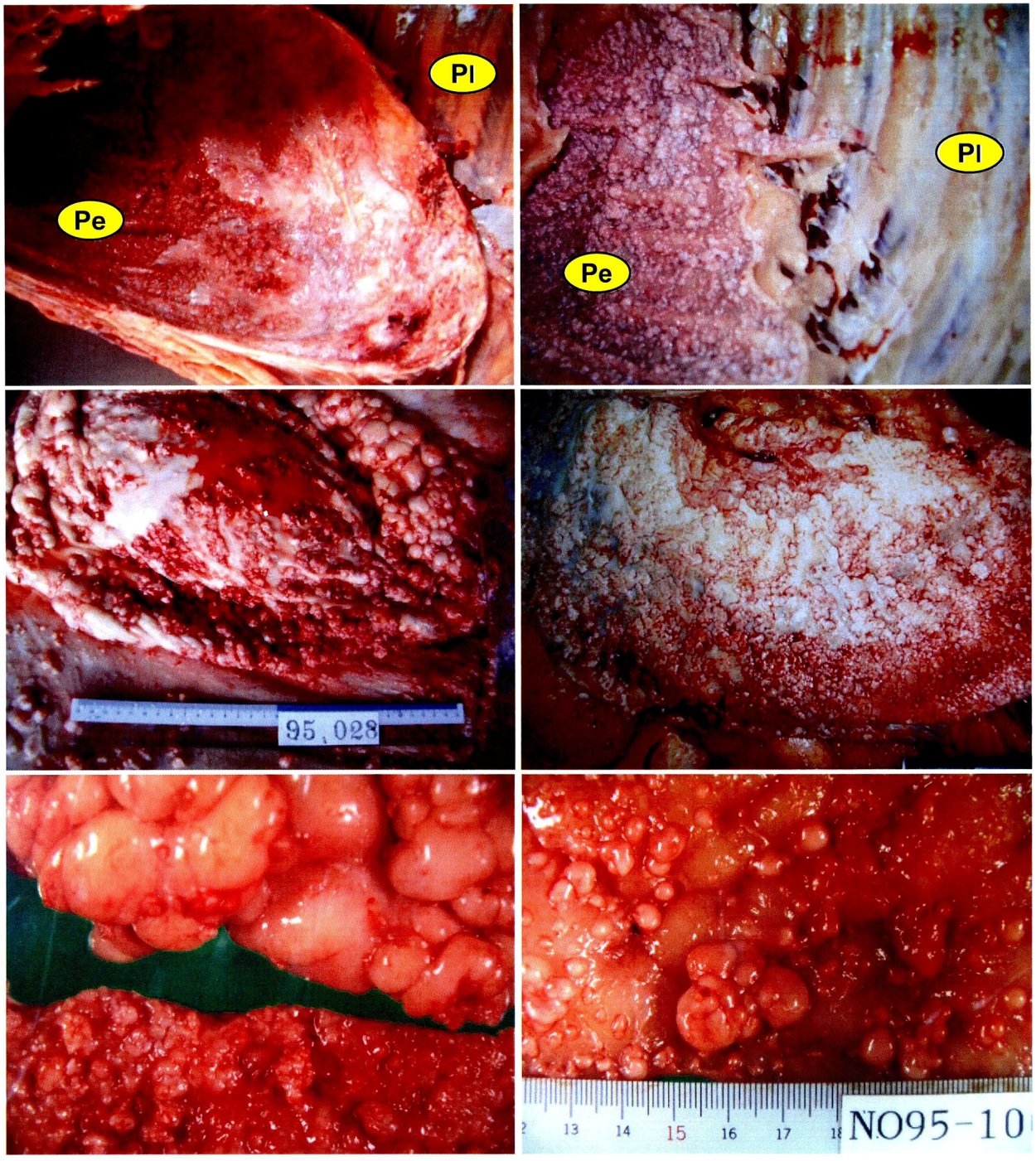


Fig.76

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)



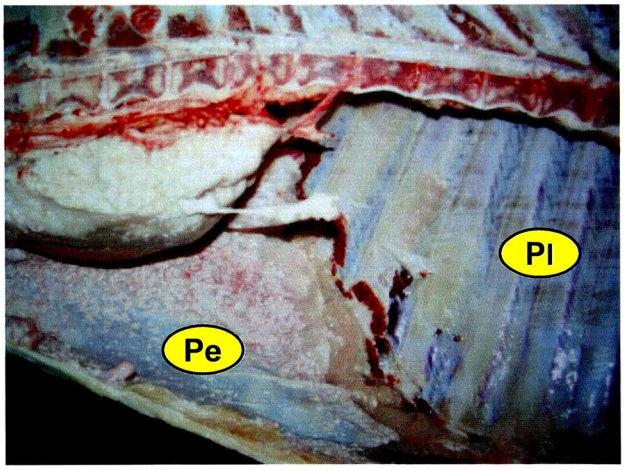

Pl :Pleura Pe :Peritoneum Fig.78

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.

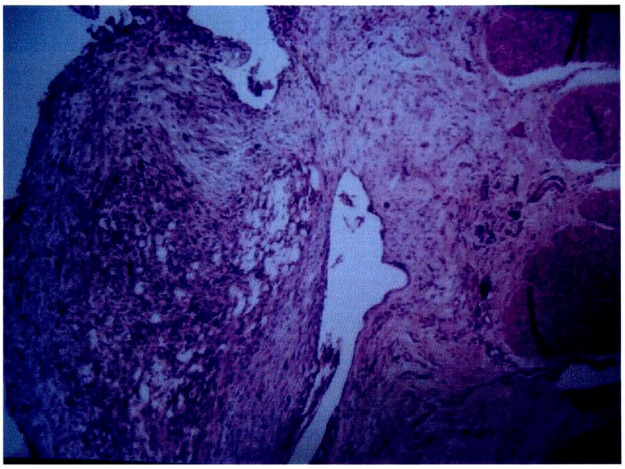


Fig.82

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.

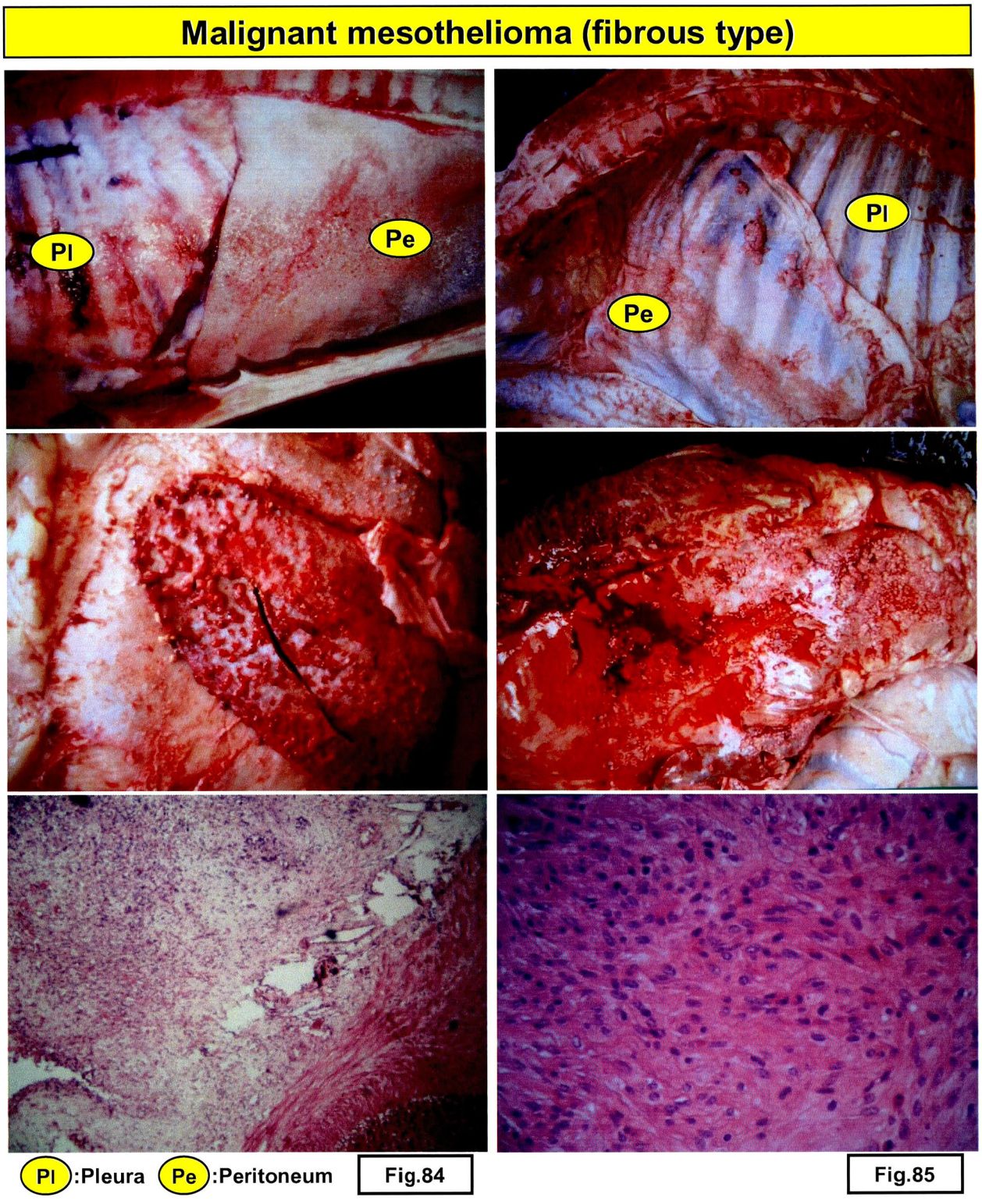



**Fig.84** Fibrous form of fuse malignant melanoma, No37, a-13-year-old female Japanese black cattle, weighing about 400kg. The neopastic cells are spindle-shaped. HE stain. ×40.

**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

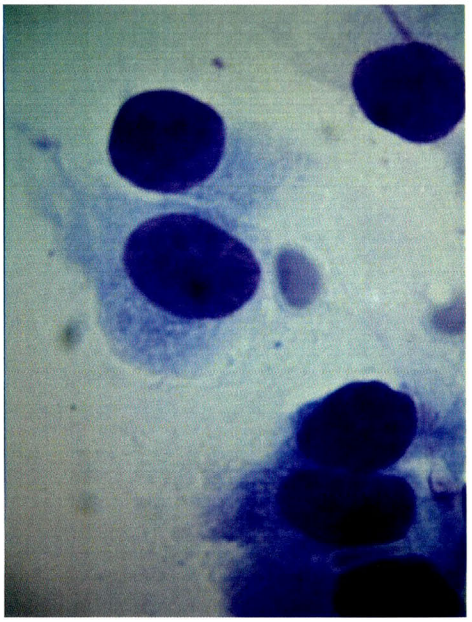


Fig.87

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

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

Fig.88

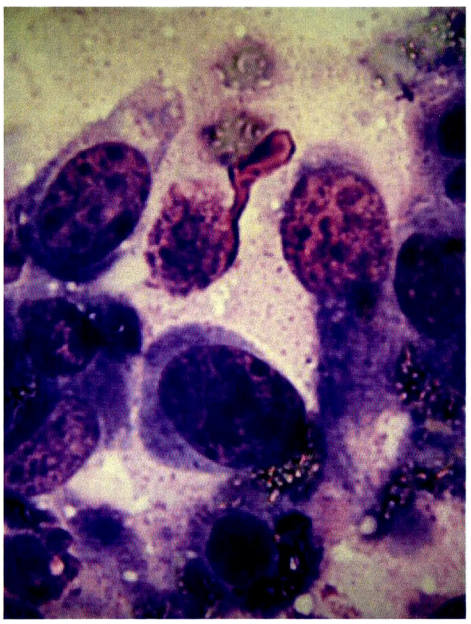


Fig.89

**Fig.88** The Malignant mesothelioma, neopastic cells. HE stain. ×1000.
**Fig.89** The neoplastic cells. Diff Quick. stain. ×1000.

## Disseminated peritoneal masses : Adenocarcinoma

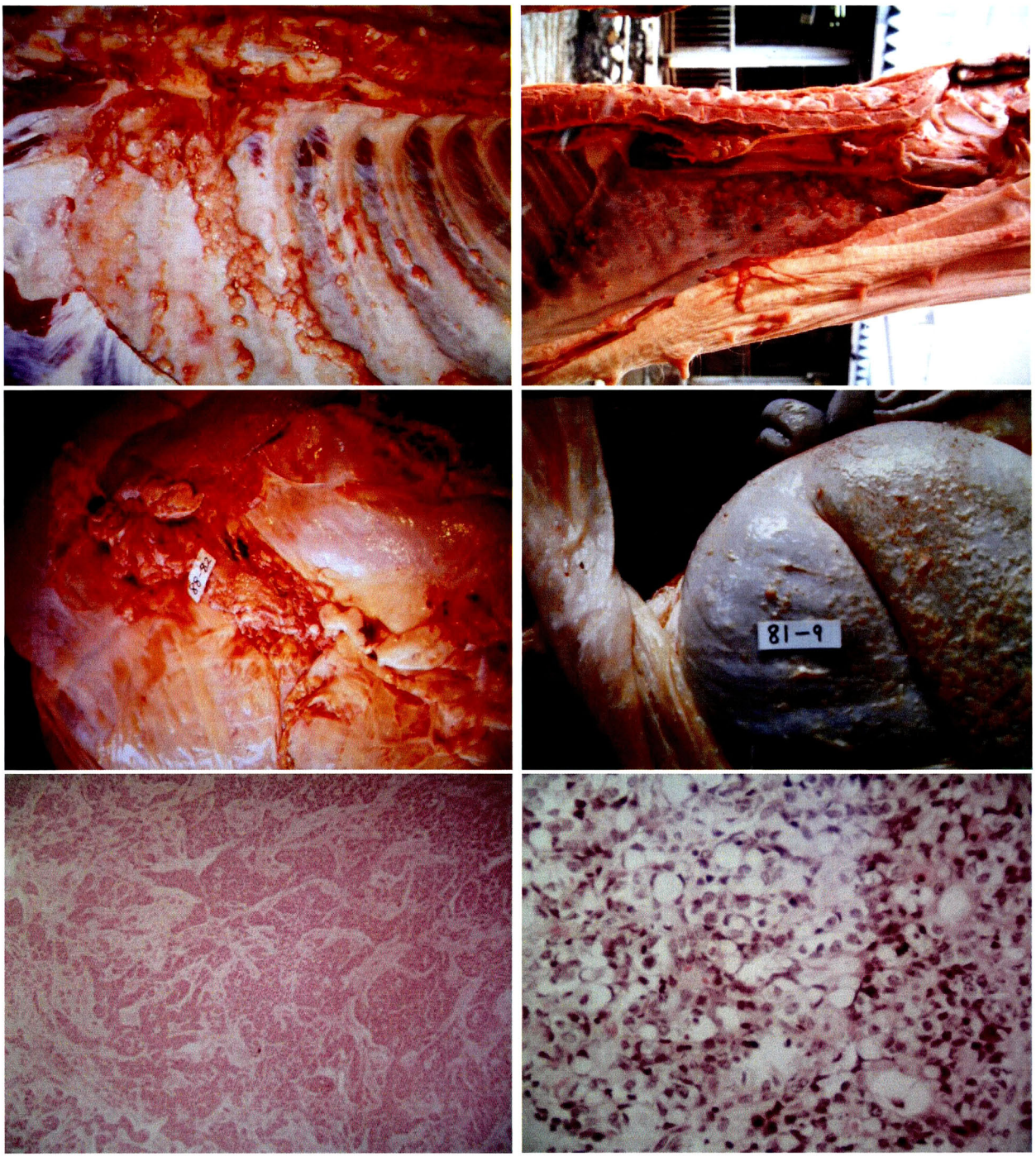



Fig.90

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

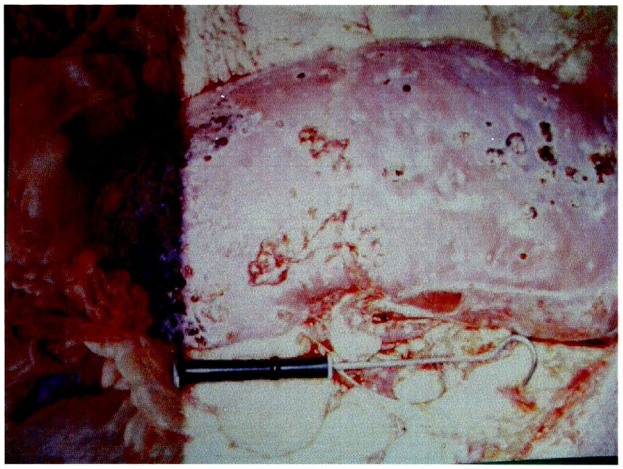


Fig.92

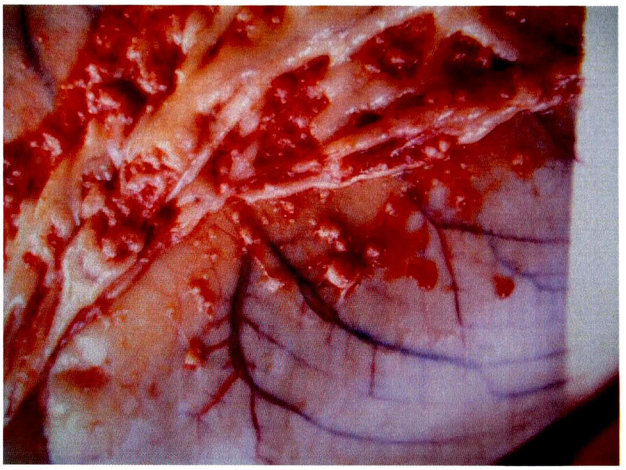


Fig.93

**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.

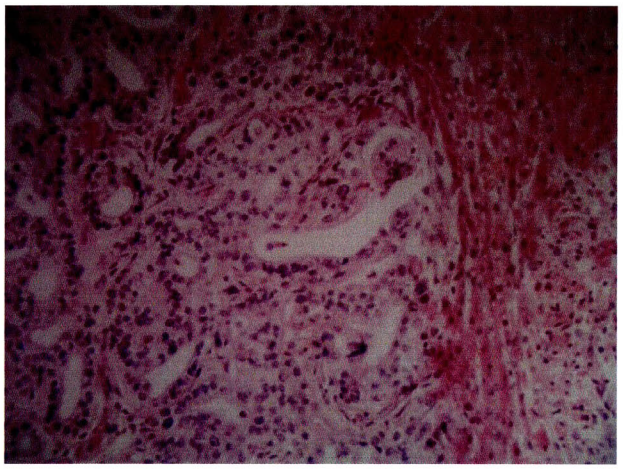


Fig. 94

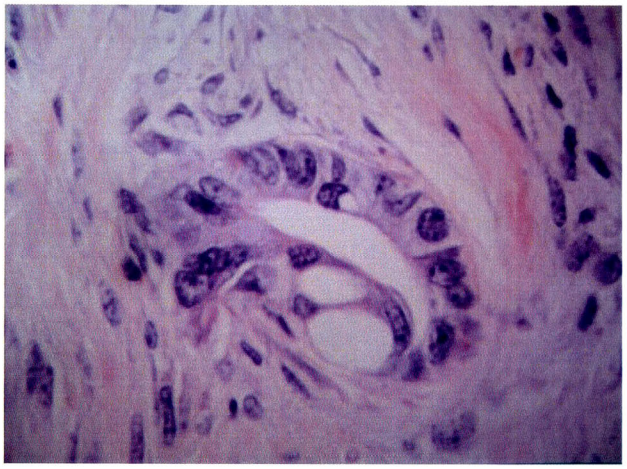


Fig. 95

**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)

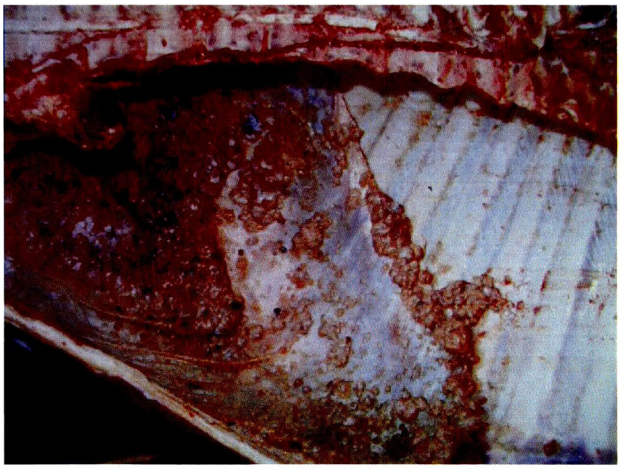


Fig.96

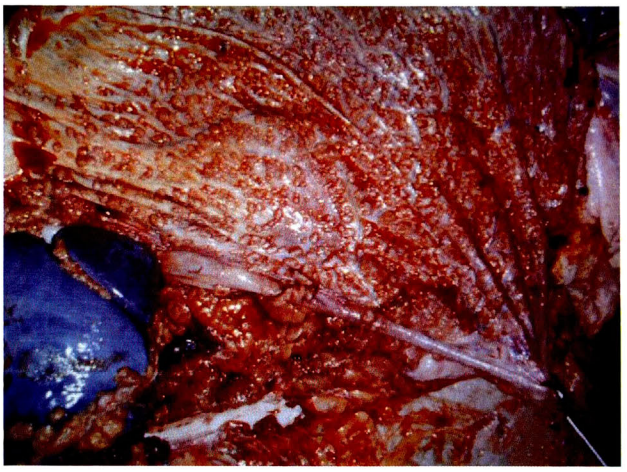


Fig.97

**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.

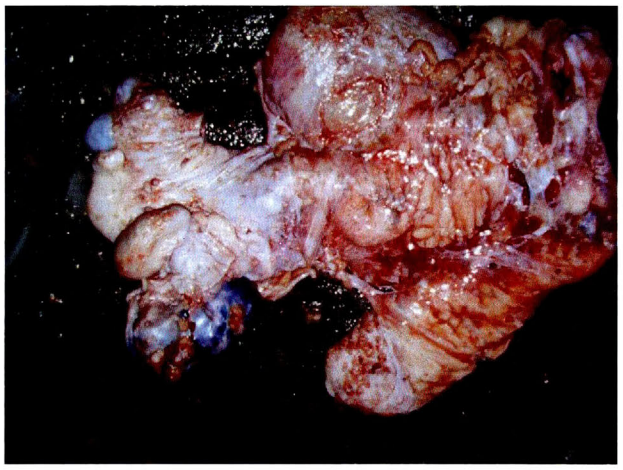


Fig.98

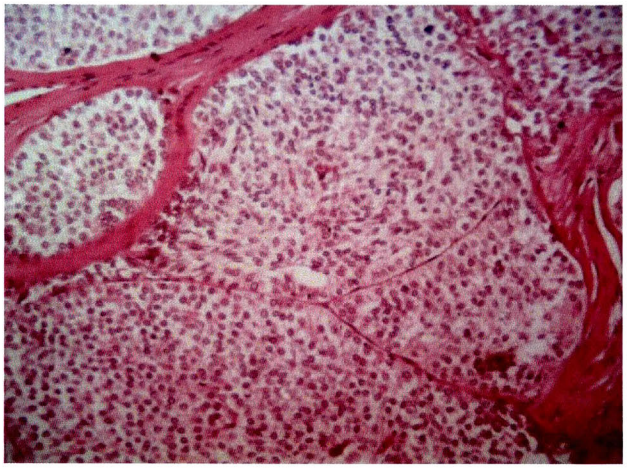


Fig.99

**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.

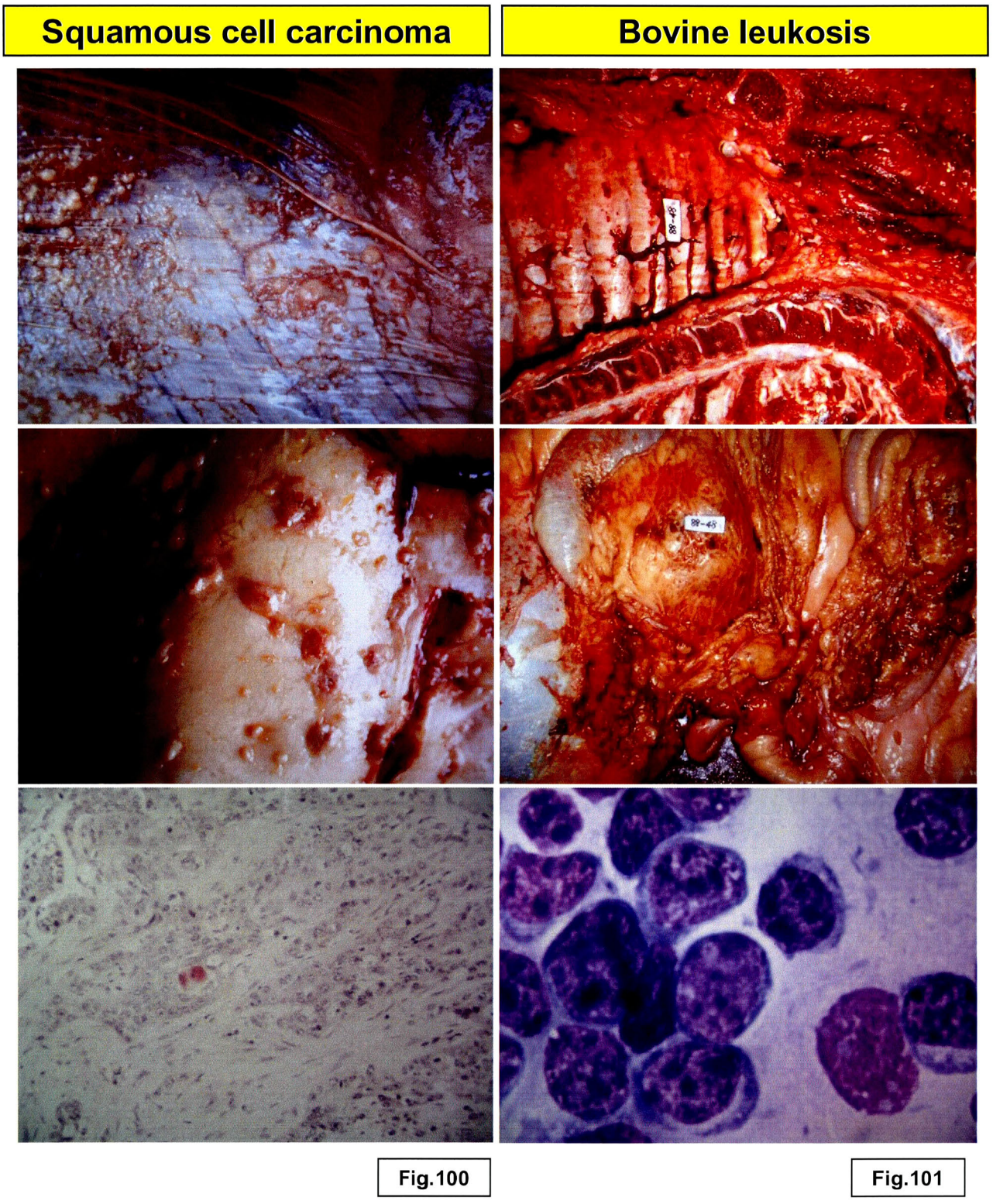



Fig.100

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

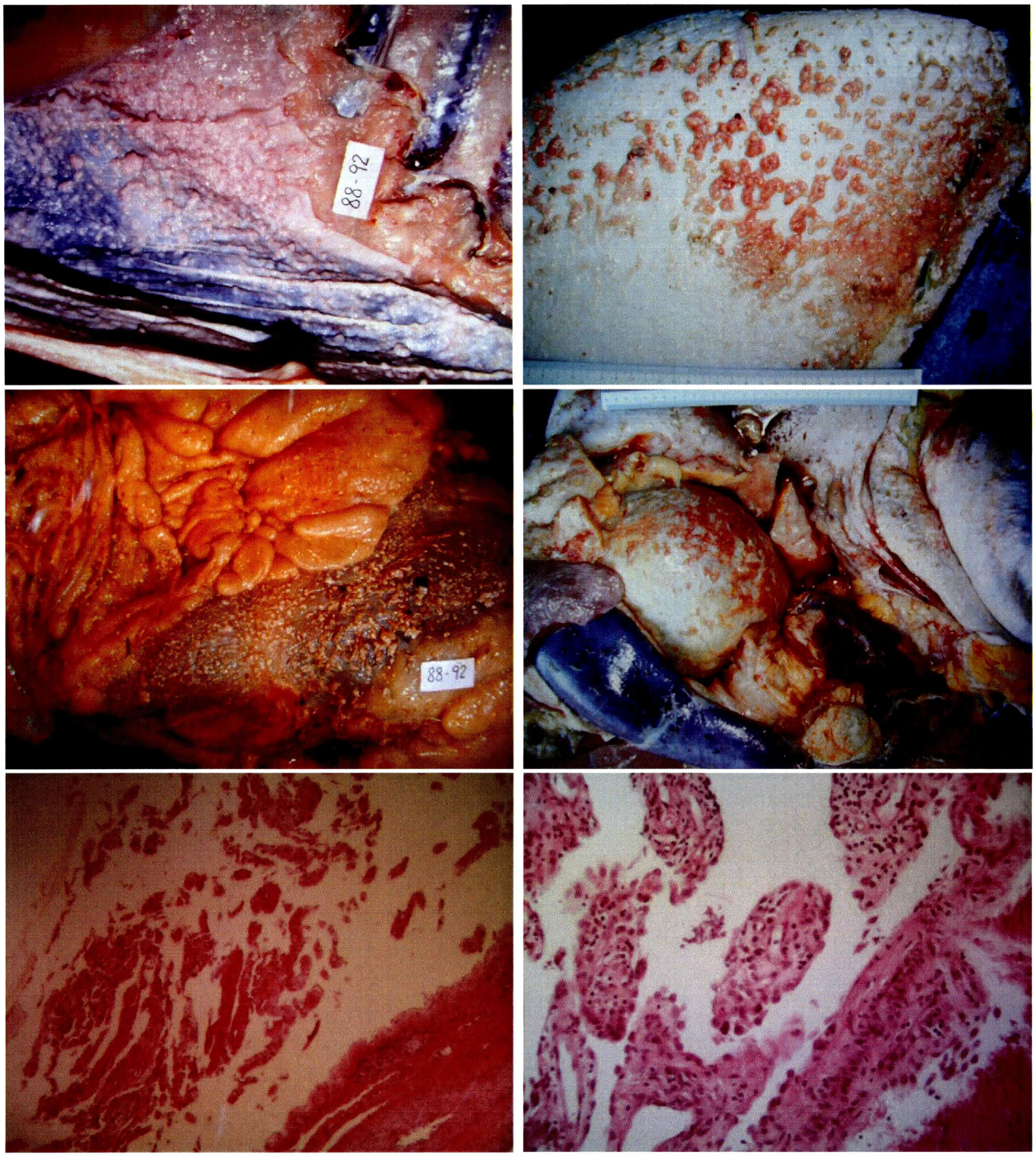



Fig.102

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.

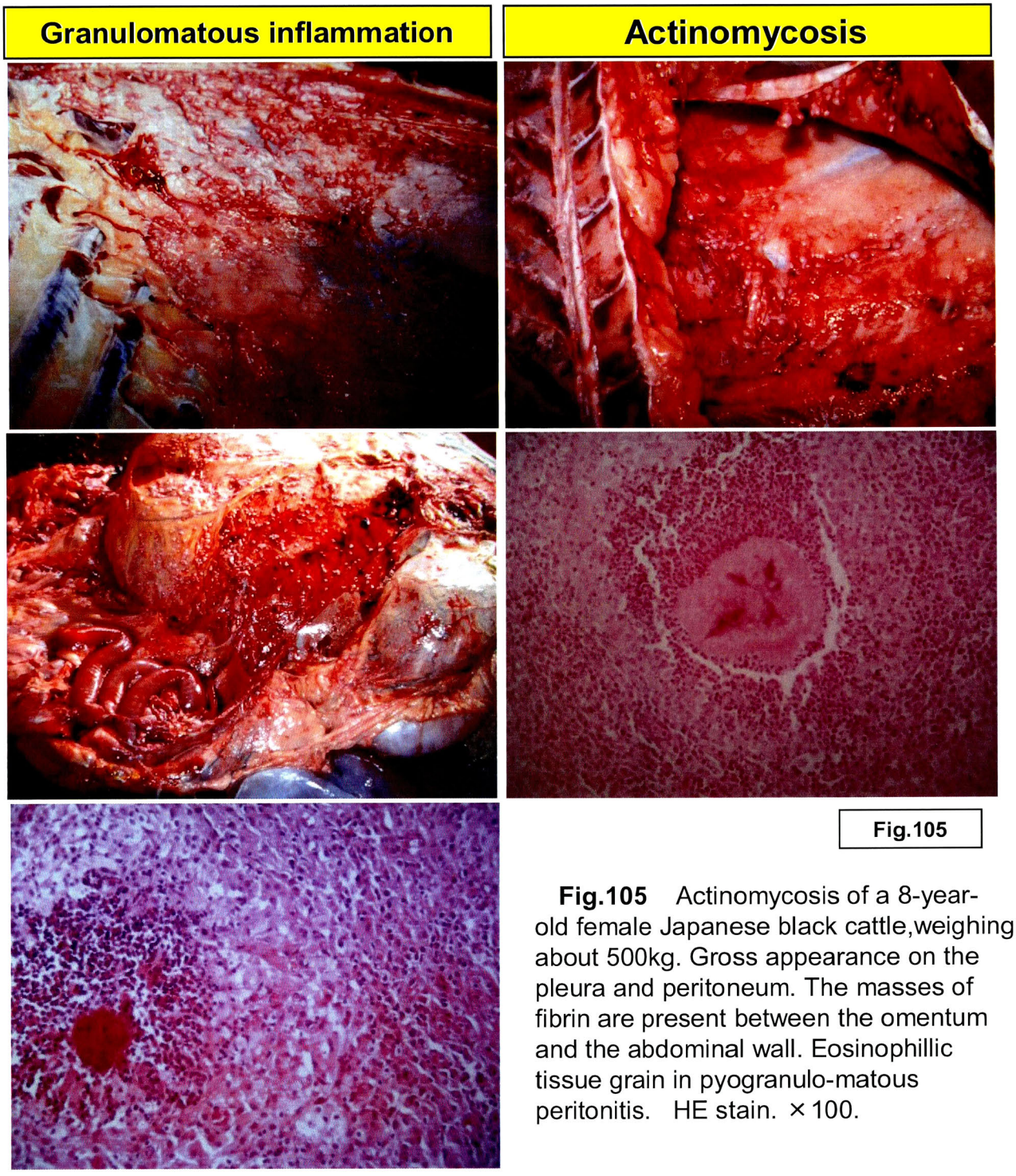



Fig.105

**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.

# 6. CHAPTER Ⅴ

## Malignant Aortic Body Tumor in a Holstein Cow

### 6.1 *INTRODUCTION*

Primary tumors of the heart are rare in either domestic animals and human beings. Rhabdomyoma, hemangioma, schwannoma, and mesothelioma have been described in the bovine heart [4,48]. The tumors that arise from the aortic bulb are called as aortic body tumor, chemodectoma or heart base tumor [15, 24, 37, 43, 48], and fairly common in dogs [4, 15, 24, 41,43,46,53], but rare in the other animals. There are only a few reports on aortic body tumor in cattle [15]. The present paper describes a case of bovine malignant aortic body tumor and the nature of cultured tumor cells, *in vitro*.

### 6.2 *MATERIALS AND METHODS*

A 5-year-old female Holstein cow, weighing about 500 kg, was slaughtered. No clinical symptoms were noted in the physical examination. The neoplastic mass, of 22×17×15 cm in size, was located at the base of the left atrium. Premediastinal lymph node showed slight swelling. Tissue samples of the atrial tumor mass, lung, liver, spleen, kidney, premediastinal lymph node, and intestinal lymph node were fixed with 10% neutral buffered formalin, embedded in paraffin wax, and cut at 4μm. Deparaffinized sections were stained with hematoxylin and eosin (HE), Azan, periodic acid-Schiff (PAS), phosphotungstic acid-hematoxylin (PTAH), Watanabe's silver impregnation for reticulin, and Grimelius' silver stain. Samples from the tumor mass were fixed with methanol-Carnoy's solution for immunohistochemical evaluation. Immunostaining was

carried out using the avidin-biotin-peroxidase complex (ABC) method using a kit (Vectastain PK-400, Vector Laboratories, Burlingame, CA, U.S.A.). Following primary antibodies were used: rabbit sera against keratin (prediluted, Dako, Carpinteria, CA, U.S.A.), vimetin (1:100, Dako), desmin (prediluted, Dako), S-100 (1:400, Dako), α-smooth muscle actin (1:40, Dako), sarcomeric actin (1:20,Dako), myoglobin (prediluted, Dako), glial fibrillary acidic protein (GFAP, prediluted, Dako), neuron-specific enolase (NSE, prediluted, Dako), synaptopshysin (1:10, Dako), and chromogranin A (prediluted, Dako). Six different types of antibody against neurofilament were also employed. The source and working dilution of six antibodies were given in Table 15. The secondary antibodies were biotinylated goat antisera against rabbit or mouse immunoglobulins (1:200, Dako). The reaction products were visualized using 3,3'-diaminobenzidine (Sigma, St. Louis, MO, U.S.A.) counter-stained with Mayer's hematoxylin. Epoxy-resin-embedded sections were also made to allow ultrastructual examination. Ultrathin sections were stained with uranyl acetate and lead citrate, and observed under a transmission electron microscope (H-800, Hitachi, Tokyo, Japan), at 80 kV. The tumor tissue was minced, and digested with 4 mg/ml collagenase (232 U/mg, Wako, Tokyo, Japan) in Dulbecco's modified Eagle's medium (DMEM, Sigma) and Ham's nutrient mixture F-12 (Sigma) containing 10% fetal calf serum (FCS), 100 IU/ml penicillin, and 100μg/ml streptomycin, for 6 hours at 37℃ in a humidified atmosphere of 5% carbon dioxide in air. The digested tissue was filtered through nylon mesh cloth (80μm), centrifuged at 1,000 rpm for 10 min and cultured according to Hiratsuka *et al.*[3]. Coverslips with cultured tumor cells were washed in PBS, fixed in cold acetone, and stored at -20℃ for 30 min. The cells were incubated with one or other of the primary antibodies overnight at 4℃. The reacted antibodies were visualized by the ABC method.

## 6.3 *RESULTS*

At necropsy, the grayish-white mass was encapsulated with fibrous tissue. Cut surface were grayish-white to milk-white in color, and irregularly lobulated (Fig. 106, 107). The tumor was divided into irregular lobules by connective tissue septa containing many capillaries. The tumor was composed of spindle-shaped and polyhedral cells, and the tumor cells had slightly eosinophilic cytoplasm. The nuclei were round-to-oval, and usually placed centrally in the cell. The chromatin pattern was finely granular, and mitotic figures were infrequent (Fig. 108, 109). These cells also lacked cytoplasmic argyrophil granules. The neoplastic cells invaded the myocardium. Metastasis was only present in the premediastinal lymph node.

The results of immunohistochemistry of the tumor cells were summarized in Table 15. The tumor cells showed intense immunoreactivity for NSE, and synaptophysin (Fig. 110). Some tumor cells were also moderately-positive for chromogranin A (Fig. 111). Tumor cells were negative for keratin, vimentin, desmin, S-100, α-smooth muscle actin, sarcomeric actin, myoglobin, and NF. Electron microscopy revealed dilated cisternae including electron-dense, membrane-limited secretory granules in the cytoplasm (Fig. 112).

The cells proliferated in DME/F12 medium with 10% FCS, were passaged till 6 times. Under phase-contrast microscope, the cultured tumor cells were spindle in shape and some cells having cytoplasmic projection (Fig. 113). The immunohistochemical findings of these cultured cells after for 4 passages were summarized in Table1. The cultured cells showed immunoreactivity for NSE, and synaptophysin, and were moderately-positive for chromogranin A. Moreover, the cells showed intense immunoreactivity for NF (200 kD) and vimentin, while the original tumor cells were not such reactivity.

## 6.4 *DISCUSSION*

From the findings described above, this bovine tumor was diagnosed as a malignant aortic body tumor. The immunoreactivity for NSE, synaptophsin, and chromoglanin A, and the presence of electron-dense-core membrane-bound granules strongly suggested that this tumor arose from a paraganglion in the parasympathetic nervous system [15, 57]. In general, the majority of bovine aortic body tumors are believed to be benign [57]. Azuma *et al.* [4] reported a case of bovine malignant aortic body tumor with metastasis to the premediastinal lymph node. Immunohistochemical features were not examined in that case, however the morphological features and the metastastic lesion were similar to those seen in the present case. Moreover, since the tumor cells in present case had only a few electron-dense-core granules, and a small number of cells showed moderate-to-slight immunoreactivity for chromogranin A, the present case might be an undifferentiated aortic body tumor. This made it possible to culture the tumor cells *in vitro.* Although many reports described the morphological characteristics of aortic body tumors *in vivo* [46], there is little information on the nature of this tumor, *in vitro.* In the present study, the cultured tumor cells showed different morphology and immunoreactivity *in vitro* compared with those of the original tumor. In particular, the additional expression of vimentin and NF in the cultured cells was quite unique, but was not a surprising phenomenon, since cultured cells have been known to exhibit vimentin in various degrees of differentiation [29]. Therefore the origin of this cell type might not be mesencymal. Further attempts to culture these cells, and transplant them into nude or skid mice will confirm this point.

## 6.5 *ABSTRACT*

A malignant aortic body tumor was observed in a 5-year-old female Holstein cow. The neoplastic mass of 22×17×15 cm in size was located at the base of the left atrium, having irregular lobular structures. The tumor cells had slightly eosinophilic cytoplasm, and a round or oval nucleus. Metastasis was only present in the premediastinal lymph node. The tumor cells exhibited intense immunoreactivity for neuron-specific enolase (NSE) and synaptophysin, and were moderately positive for chromogranin A. Electron-microscopy revealed membrane-limited granules in the cytoplasm. The cultured cells were spindle in shape, and having projectional cytoplasm. They were intensely positive for NSE, synaptophysin, chromogranin A, and neurofilament (200 kD). Consequently, this case was diagnosed as a malignant aortic body tumor from the neuroecrodermal origin.

KEY WORDS : arotic body tumor, cultured cell, Holstein cow.

**Table 15. The results of immunohistochemistry staining for the original tumor and the cultured cells.**

| Antibodies | Source | Dilution | Tumor | Cells |
|---|---|---|---|---|
| Keratin | Dako | Prediluted | − | − |
| Vimentin | Dako | 1:100 | − | ++ |
| Desmin | Dako | Prediluted | − | − |
| S-100 | Dako | 1:400 | − | − |
| Sarcomeric Actin | Dako | 1:20 | − | − |
| Smooth muscle acrtin | Dako | 1:40 | − | − |
| Myogolbin | Dako | Prediluted | − | ND |
| h-NF(200Da) | Sigma | 1:20 | − | ND |
| h-NF(160Da) | BioMakor | 1:200 | − | ND |
| h-NF( 68Da) | BioMakor | 1:200 | − | ++ |
| b-NF(200Da) | TFR* | 1:20 | − | − |
| b-NF(158Da) | TFR | 1:20 | − | − |
| b-NF( 68Da) | TFR | 1:20 | − | − |
| GFAP | Dako | Prediluted | − | − |
| NSE | Dako | Prediluted | ++ | + |
| Synaptophysin | Dako | 1:10 | ++ | + |
| Chromogranin A | Dako | Prediluted | + | + |

+ + : intensely positive + : moderately positive - : Negative
h: Human b: Bovine * : Transformation Reseach Inc ND : Not done

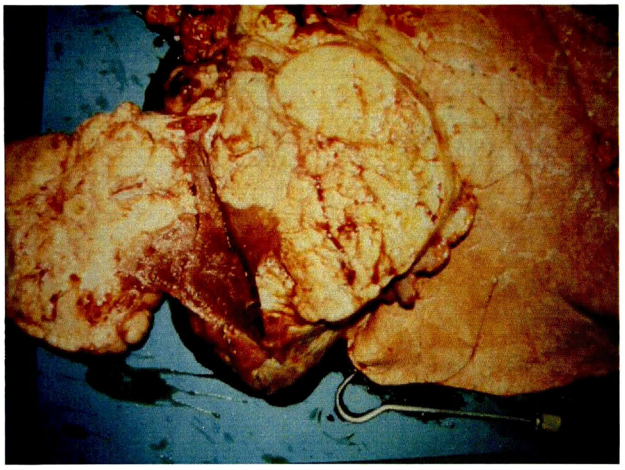


Fig.106

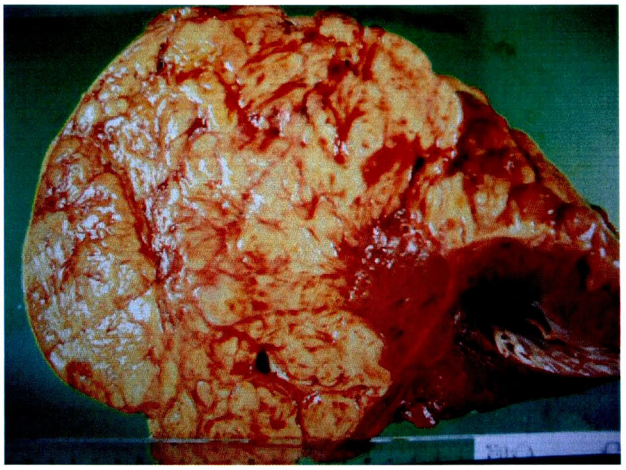


Fig.107

**Fig.106 and 107** The tumor mass replacing the base of the left atrium sinistrum and encapsulating by connective tissue and the grayish-white cut surface were subdivided into irregular lobules.

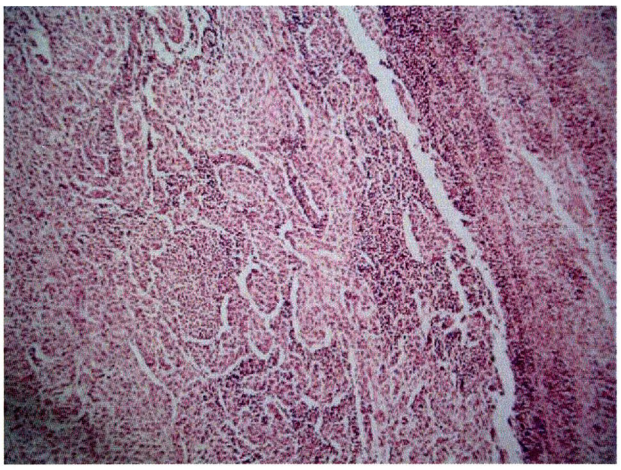


Fig.108

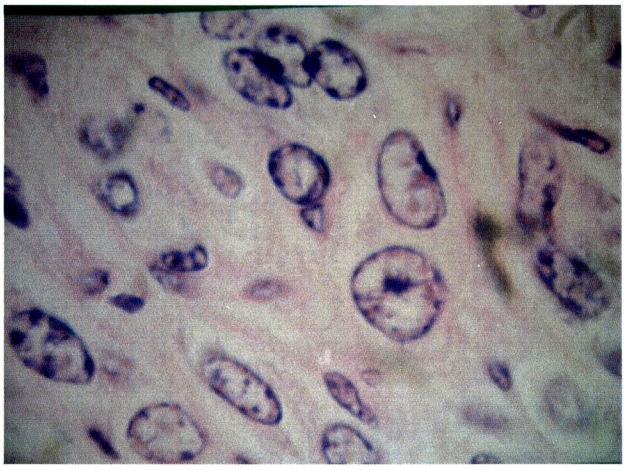


Fig.109

**Fig.108** Histological appearance of the proliferation tumor cells surrounding by connective tissue septa containing many capillaries. HE stain. ×40.

**Fig.109** The neclei were round-to-oval and the chromatin pattern was finely granular. HE stain. ×1000.

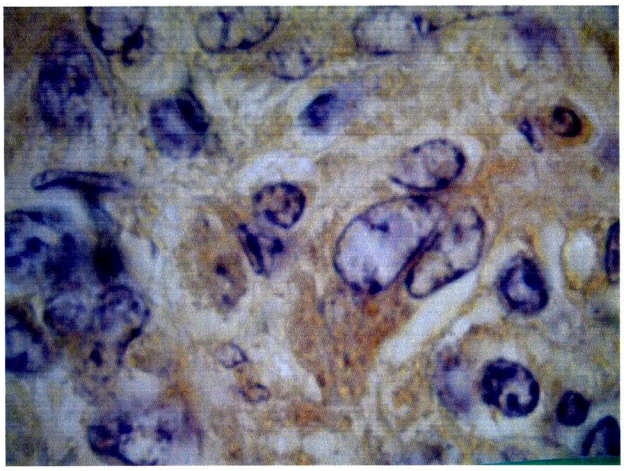


Fig.110

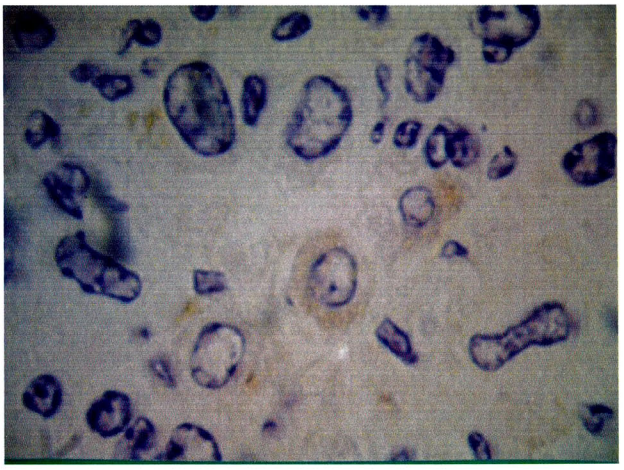


Fig.111

**Fig.110** The tumor cells showed intese immunoreactivity for synaptophysin. ×1000.
**Fig.111** Some tumor cells were also moderately-positive for chromogranin A. ×1000.

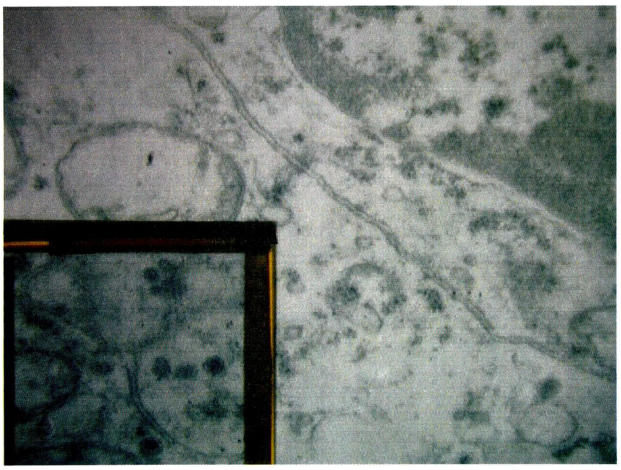


Fig.112

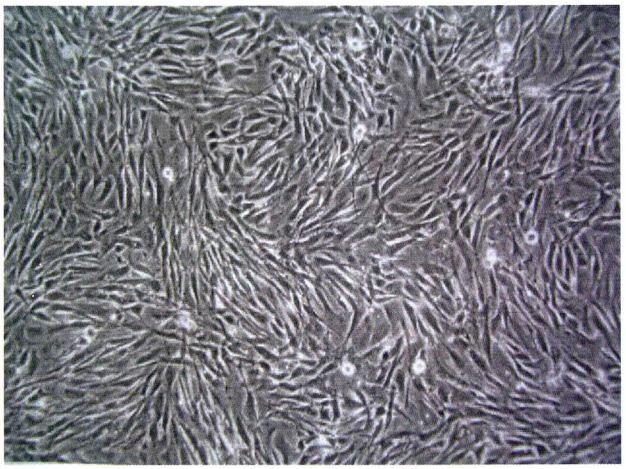


Fig.113

**Fig 112** Electron microscopy revealed dilated cisternae including electron-dense, membrane-limited secretory granules in the cytoplasm. ×6000.
**Fig.113** The cultured tumor cells were spindle in shape and some cells having cytoplasmic projection. Phase-contrast microscopy. ×126.

# 7. CONCLUSIONS

A study concerning classification of cattle tumors and pathological analyses in Miyazaki prefecture was conducted to investigate the situation concerning the annual incidence of cattle tumors at individual meat sanitary inspection centers in Miyazaki prefecture. Mesothelioma cases were detected especially frequently in the jurisdiction of the Miyakonojo Meat Inspection Office and were investigated in detail.

Chapter 1: Cases involving co-incidental malignant mesothelioma and ovarian granule cell tumor in cattle. There have been only a few reports on such simultaneous tumor cases in cattle. In a Japanese black cattle specimen (3 year-old female), co-incidental tumors were found at the thoracic walls and ovaries and histopathological and immuno-histochemical examinations were conducted. The cells of the thoracic tumor were cultured and stained with using various staining preparations. The tumors at the left and right thoracic walls consisted of a large number of grey tumors. The neoplastic cells were immunohistochemically positive for cytokeratin and negative for vimentin and CEA. The epitheloid cells cultured were positive for vimentin. The ovarian tumor was alveolar and the tumor cells proliferating in a solid mass were positive for vimentin and negative for cytokeratin and CEA. From those findings, the case was diagnosed a compound overlapped case of malignant mesothelioma and ovarian granule cell tumor.

Moreover, we investigated 328 tumor cases detected among 139, 556 cattle within the jurisdiction of the Miyakonojo Meat Inspection Office for the 21-year period from April, 1974 to March, 1995, and discussed the etiologies of such simultaneous tumors. In this case, it was speculated that the tumors seen in this area occurred incidentally in the same individual cattle.

Chapter 2: Surveys of cattle tumors at the Miyakonojo Meat Inspection Office over a 23-year period.

As for surveys concerning long-term statistics for livestock tumors, although there have been several reports, there have been only a few detailed reports which take regional factors into consideration. Tumors were found in 377 of 162, 328 cattle at Miyazaki Prefecture Miyakonojo Meat Inspection Office for the 23-year period from April, 1974 to March, 1997 (232 per 100,000 cattle). These included mesothelioma in 64, ovarian granule cell tumor in 56, leukemia in 48, lung cancer in 20, liver cancer in 19 and adrenal cortical adenoma in 17.

Mesothelioma was found in many Japanese black cattle being raised whose breeding period is long and the ages of the occurrence was an average 11 years. This case of multiple mesothelioma corresponded to a report on cattle tumors by Miyazaki University in 1994. The multiple mesotheliomas were thus considered to occur frequently in Southern Kyushu, including the jurisdiction of the Miyakonojo Meat Inspection Office.

Chapter 3: Survey of cattle tumors at six meat sanitary inspection center in Miyazaki Prefecture for 16 years

At six meat inspection offices in Miyazaki prefecture, tumors were detected in 542 (0.116%) among 446,699 for the 16-year period from April, 1978 to March, 1994. Main tumors included bovine leukosis in 87, mesothelioma in 74, ovarian granule cell tumor in 52, liver cancer in 31, lung cancer in 18 and adrenal tumors in 12. Female Japanese black cattle were the larger in number, 23.8 / 10,000 cattle, including mesothelioma and ovarian granule cell tumor. In female Holstein cattle, leukemia and mesothelioma were found in 12.7 / 10,000. There were 1.3 / 10,000 in male Japanese black cattle and 1.4 / 10,000 in male Holstein cattle, both of which were mainly leukemia. At individual

production sites, as an example, there were 24.7 / 10,000 cattle at Miyakonojo where quite older cattle are processed, which was about four times more than that at Takasaki where more fattening cattle are processed.

Cattle leukemia was found at the highest level reported from meat sanitary inspection centers all over Japan, including Kyushu, followed by mesothelioma, although the number was small. There were 5.3 mesothelioma cases / 10,000 within the jurisdiction of the Miyakonojo Meat Inspection Office. This indicated that tumors have been occurring frequently in Southern Kyushu, mainly in Miyakonojo area of Miyazaki Prefecture.

Chapter 4: Survey of cattle mesothelioma at Miyazaki Prefecture Miyakonojo Meat Inspection Office over a 23-year period

Mesothelioma was detected in 64 among 162,328 cattle at Miyazaki Prefecture Miyakonojo Meat Inspection Office for a 23-year period from April, 1997 to March, 1997. The incidence was 39.4 / 100,000.

Macroscopic findings in most of the cases were widely distributed nodal lesions with sizes similar to that of millet seed ~ rice grain ~ soybean ~ small finger tip ~ hen eggs and with white ~ milky white ~ yellow ~ dark red colors in the peritoneum, greater omentum, liver, spleen, diaphragm and pleura.

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

There have been only a few reports on the high incidence of cattle mesothelioma at specific areas in Japan. In Miyakonojo / some northern prefectural areas within the jurisdiction of Miyakonojo Meat Inspection Office, volcanic ash called "Shirasu" specific in Southern Kyushu has been used frequently for bedding of cattle barns and playgrounds. The 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 in the central area of Turkey is.

Chapter 5 Reports concerning malignant aortic body tumors in cattle

In a female Holstein cattle aged five, a grey tumor of 22×17×15cm in size was detected at the aortic starting base of the left atrium. The tumor surface was covered by fibrous membrane and the section presented a grey color. Histopathologically, the tumor cells had proliferated in a solid manner and were fractionated into irregular alveolar forms by fibroblasts and had a large number of capillary vessels. The cytoplasm of the tumor cell had circular to oviform nucleus, as detected by eosin light staining. Its metastasis was found only at the mediastinal lymph nodes. Immunohistochemically, the tumor cells were positive for NSE and synaptophysin and weakly positive for chromogranin. Electromicroscopic observation revealed clear granules on the border membranes in the tumor cytoplasm.

In addition, we attempted cell cultures by the method used by Hiratsuka et al. The cultured cells presented fusiforms with protruding cytoplasms and were positive for NSE, synaptophysin, chromogranin A, and NF (200kD). From those findings, this case was diagnosed as a malignant aortic body tumor.

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