STUDIES ON FELINE INFECTIOUS PERITONITIS (FIP)

I. OCCURRENCE AND EXPERIMENTAL TRANSMISSION OF THE DISEASE IN JAPAN

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Feline infectious peritonitis (FIP) is a recently reported disease entity which is characterized clinically by fever, anorexia, ascites, depression and leukocytosis, and pathologically by diffuse fibrinous peritonitis and focal hepatitis. It was first described in detail by WOLFE & GRIESEMER⁹⁾ in 1966. In the United States similar reports on the occurrence and experimental transmission of the disease have been published since then^{1,2,4,6,9)}. Besides, only one British worker encountered a similar disease in England very recently³⁾.

During the past 2 years, the authors have received 12 cats affected with copious ascites and fibrinous peritonitis. In this paper, for the first time in Japan, they deal with the description of the spontaneous case of FIP, a transmission experiment on such disease as giving rise to the condition identical with that described for FIP by the above-cited authors, and attempts to demonstrate an etiologic agent.

MATERIALS AND METHODS

Specimens from affected cats

Since April, 1969, the authors have obtained 12 cats, dead or alive, affected with cloudy, straw-colored ascitic fluid and diffuse peritonitis from the University of Tokyo Veterinary Hospital and several veterinary practitioners in the Tokyo area, and performed necropsy upon them. The ascitic fluid, liver and spleen were collected from each cat and stored at -20°C , until they were used for experimental transmission to domestic kittens and other microbiological investigations.

Experimental transmission

Fifteen domestic kittens, which were 4 weeks to 8 months of age, were inoculated intraperitoneally with ascitic fluid or an approximately 10% liver emulsion originated from the affected cats. One of them was inoculated specially with homogenized infective liver treated with 300 $\mu\text{g}/\text{ml}$ of kanamycin sulfate. Rectal temperatures were taken daily and white blood cells (WBC) counted at 1- or 2-day intervals after inoculation.

Microbiological examination

Blood agar plates were inoculated with the specimens of ascitic fluid, liver, spleen, kidney and lung from the affected cats, and incubated at 37°C for 48 hours aerobically and anaerobically. Attempts were made to isolate mycoplasmas both in a liquid medium (50 ml Hanks' solution, 30 ml Hartley's broth, 0.5 g lactalbumin hydrolysate (NBC),

20 ml inactivated swine serum, 1 ml 25% yeast extract, 100,000 units penicillin G potassium, 1 ml 2.5% thalium acetate) and in a solid medium (0.6 g Oxoid ion agar No. 2 added to the above-mentioned liquid medium).

An undiluted ascitic fluid from an affected cats was inoculated into the yolk sac of 7-day-old embryonated eggs and onto the chorio-allantoic membrane of 9-day-old eggs. The yolk sac and chorio-allantoic membrane were harvested on the 7th day postinoculation and stained by Giemsa's method.

Ten mice, 3 or 4 weeks of age, three guinea pigs and four hamsters were inoculated intraperitoneally with ascitic fluid from a naturally affected cat. At two weeks post-inoculation, the mice and guinea pigs were bled. Their serums were stored at -20°C for serologic examination, and their tissues stained with Giemsa's solution.

RESULTS

Occurrence of the disease

Of the 12 cats affected with the disease, nine were received from the University of Tokyo Veterinary Hospital and three from veterinary practitioners in Tokyo. The disease was most commonly seen among cats from 6 months to 7 years of age (Table 1). The breeds involved were Siamese, Persian, Himalayan and indigenous Japanese short-haired.

The clinical signs usually manifested were fever (ranging from 39.0 to 40.5°C), anorexia, depression and ascites. Leukocytosis as high as $30,000/\text{cmm}$ appeared in some cases. Death occurred in most cases in spite of any antibiotic and intensive fluid therapy. The duration of the disease varied considerably from 2 to 8 weeks.

Experimental disease

Serial transmission of the disease was successful in 12 of 15 conventional young kittens. Treatment of the inoculum with kanamycin sulfate didn't alter its infectivity (Table 3). The duration of the experimental disease ranged from 10 days to 8 weeks. The first clinical sign of the disease was a rise in body temperature up to 40.0 to 41.0°C , which usually appeared within 2 or 3 days after inoculation. The total WBC count was usually elevated shortly after the onset of fever. It sometimes reached as high as $40,000/\text{cmm}$ in 7 or 10 days (Chart 1). In some of the diseased cats the WBC count fell below normal limits within 2 or 3 days shortly after the inoculation. Following their

Table 1. Clinical Data on Natural Cases of Feline Infectious Peritonitis (FIP)

Cat No.	Age	Sex	Breed	Duration of illness (weeks)
1000	— ¹⁾	—	—	—
5000	8 mos.	male	Siamese	3
6000	6 mos.	female	Persian	4
7000	1 yr.	male	Himalayan	3
8000	2 yrs.	female	DSH ²⁾	5
9000	6 mos.	female	Siamese	6
10000	7 yrs.	male	Siamese	4
11000	4 yrs.	female	Siamese	5
12000	1.5 yrs.	female	Siamese	—
13000	7 yrs.	female	Siamese	6
14000	5 mos.	male	Persian	—
15000	4 mos.	male	Persian	—

1) unknown.

2) Domestic short hair.

Table 2. Gross Lesions of Cats Affected with FIP

Cat No.	Ascites	Fibrinous peritonitis	Hepatic necrosis
1000	+	ND	ND
5000	+	+	+
6000	+	+	+
7000	+	+	+
8000	+	+	+
9000	+	+	+
10000	+	+	+
11000	+	ND	ND
12000	+	+	+
13000	+	+	+
14000	+	ND	ND
15000	+	ND	ND

ND=not done.

Table 3. Clinical Data and Gross Lesions of Cats Affected with FIP Transmitted Experimentally

Cat No.	Age	Inoculum	Course of disease	Gross lesions		
				Ascites	Hepatic necrosis	Fibrinous peritonitis
1111	4 wks.	Ascitic fluid	48 days K ¹⁾	—	+	+
1112	4 wks.	Ascitic fluid	41 days K	+	+	+
1121	2 mos.	Ascitic fluid	55 days K	—	+	+
1122	2 mos.	Ascitic fluid	37 days K	—	—	—
1123	2 mos.	Ascitic fluid	44 days K	—	+	+
1211	2 mos.	Liver suspension	47 days K	—	+	+
1212	2 mos.	Liver suspension	47 days K	—	—	—
1213	2 mos.	Liver suspension	47 days K	—	—	—
7111	6 mos.	Ascitic fluid	11 days D ²⁾	+	+	+
7112	6 mos.	Ascitic fluid	13 days D	+	+	+
7113	6 mos.	Ascitic fluid	16 days D	—	+	+
7211	8 mos.	Liver suspension	16 days D	+	+	+
7212	4 mos.	Liver suspension ³⁾	12 days D	—	+	+
7311	4 wks.	Liver suspension	31 days D	+ ⁴⁾	+	+
14111	6 mos.	Ascitic fluid	58 days D	+	+	+

1) killed 2) died 3) treated with 300 µg/ml of kanamycin sulfate

4) tholacic fluid was also seen.

initial febrile response, the kittens usually became listless and rejected food and water. These experimental diseases could be serially transmitted for more than three passages.

Gross pathologic findings

All the cats infected with the disease spontaneously had diffuse fibrinous peritonitis characterized by the presence of grayish white or dark yellow fluid within the peritoneal cavity and the deposition of granular fibrinous exudate on the surface of abdominal viscera. Each infected cat had more than 100 ml, sometimes over a liter, of fluid in the peritoneal cavity. The fluid was partially clotted when exposed to air. Fibrinous masses covered the surface of the peritoneum and organs in the peritoneal cavity, especially the liver and the spleen.

Chart 1. Febrile and Leukocyte Responses in 2 Cats Affected with Experimental FIP

In most of the cats, discrete white necrotic foci 0.5 to 2 mm in diameter were seen obviously all over the liver and kidney (Table 2).

The lesions of the experimentally infected cats closely resembled those of the spontaneously infected ones, except that they contained not so much ascitic fluid. One cat infected experimentally had thoracic fluid (Table 3).

Histopathologic findings

In the spontaneously infected cats, fibrinous and necrotic peritonitis was found constantly in all the abdominal organs, including their serosal membranes. Many foci of necrosis and lymphocytic and neutrophilic infiltration were found on the surfaces and within the parenchyma of the liver, kidneys and lungs. In general, abdominal lymph nodes exhibited some degree of hyperplasia of reticulum cells.

The lesions seen in the experimentally infected cats were similar to those in the spontaneously infected ones. In some of the experimentally infected cats, however, phlebitis and other noticeable vascular lesions were seen in the lungs, livers, kidneys and omentum. In the omentum, arteritis and neutrophilic infiltration were also present.

Attempts to demonstrate an etiologic agent

No bacteria were demonstrated aerobically or anaerobically in the ascitic fluid from any infected cat. Some bacteria were cultured from tissues collected from dead cats several hours after death, but these were assumed to be postmortem contaminants. Attempts to isolate mycoplasmas from ascitic fluid and liver were unsuccessful. Isolation of viruses or other microorganisms was all negative in the yolk sac or chorio-allantoic membrane of embryonated eggs, mice, guinea pigs or hamsters inoculated with ascitic fluid or homogenized liver.

Tissue specimens were collected from mice and guinea pigs, stained and examined microscopically for *Toxoplasma gondii* and *Bedosonia*, but none of these microorganisms

were found. Passive hemagglutination tests for *Toxoplasma gondii* were performed with serums from mice, guinea pigs and infected cats, but the results were all negative.

DISCUSSION

The condition of the disease mentioned above was identical with that of feline infectious peritonitis which had been described since 1966 in the U.S.A. The clinical signs of that disease included fever, anorexia, weight loss and distension of the abdomen due to ascites. The disease commonly affected young cats and has been observed to affect their cage mates. The mortality was extremely high in spite of any antibiotic therapy. It is assumed from experience that the Siamese cat may have been affected more frequently than any other breed.

The disease can easily be transmitted serially to domestic young kittens with ascitic fluid or homogenized liver collected from infected cats. The infectivity of the inoculum was not altered by freezing at -20°C or treating with a broad-spectrum antibiotic. The gross lesions of feline infectious peritonitis were characterized by diffuse fibrinous peritonitis and increased ascitic fluid. Necrosis was found either on the serosal surface or within the parenchyma of the liver and other organs. In some cats affected with the experimental disease, prominent vascular lesions were found. WARD and THEILEN⁷⁾ indicated that vascular lesions, especially lesions of the vein, were present in cats affected with the experimental disease. In the present investigation it is remarkable that arteritis also occurred to cats affected with the experimental disease.

Since 1970, feline infectious peritonitis has been observed not only in the U.S.A. but also in England³⁾. Additionally, the present paper shows that the disease is a serious problem as a new fatal infectious disease of cats even in Japan.

The etiology of the disease is yet unknown. The authors have not succeeded in isolating bacteria or mycoplasmas from the affected cats. Extensive attempts have been made in vain to detect *Toxoplasma gondii* or *Bedosonia* organisms from these cats. The causative agent was resistant to broad-spectrum antibiotics and freezing. It was impossible to recover any bacteria, protozoa, mycoplasmas, or *Bedosonia* organisms from the affected cats. These results suggest that the causative agent of the disease may be a virus.

Recently, some ultrastructural and filtration studies conducted by ZOOK et al.¹⁰⁾ have shown that feline infectious peritonitis is probably caused by viruses which are presumed to be RNA viruses with the budding of viral particles in the endoplasmic reticulum. WARD^{5,8)} described that the morphogenesis of a virus in cats infected with experimental feline infectious peritonitis was similar to that of human coronavirus and mouse hepatitis virus. It has not yet been proved, however, that either of these viruses is the causative agent of the disease.

The authors are still making efforts to demonstrate a viral etiology for feline infectious peritonitis, using some primary and established cell cultures.

SUMMARY

Feline infectious peritonitis is a newly recognized disease entity which is characterized clinically fever, anorexia, depression, leukocytosis and distension of the abdomen, and pathologically by diffuse fibrinous peritonitis and focal hepatitis.

The authors described the disease which had occurred naturally to 12 cats in the Tokyo area and made attempts to transmit the disease to healthy domestic kittens. The clinical features of the cats affected with spontaneous infectious peritonitis and those

the experimental disease were fever, anorexia, weight loss, ascites and leukocytosis. The pathologic findings in the cats infected spontaneously were characterized grossly by diffuse fibrinous peritonitis, and histologically by luxuriant fibrinous exudate on the serosal surfaces and necrosis in parenchymal organs. The lesions of the experimental disease were similar to those of the natural disease, although they presented less fibrinous exudation and more extensive focal necrosis than the latter.

Isolation of any causative agent of the disease has been unsuccessful so far. It was strongly suggested that the causative agent of the disease might be a virus.

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Feline Infectious Peritonitis (FIP) に関する研究

I. わが国における自然感染例の確認と伝達試験

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FIP は 1966 年ごろから、米国の二三の研究所で観察されているネコの新しい伝染性疾患で、発熱、瘦削、腹水貯留、白血球増多症を主徴とし、著明な線維索性腹膜炎と限局性肝炎を認め、その斃死率は極めて高いといわれている。米国以外では、最近英国において、その発生が確認されているのみである。著者らは 1969 年春以来、都内を中心とした臨床家および本学家畜病院の協力を得て、12頭の自然感染例を発見するとともに、その

3 例の材料から、ネコを用いて伝達試験に成功し、疾患および病原に関する検討を行なった。

自然例の腹水と肝の混合乳剤を接種した 1~8 カ月令のネコにおいて、2~10 日の潜伏期を経て 40°C 前後に発熱し、2 万ないし 4 万/cmm の白血球増多症（一部は白血球減少症を経た後）が持続してみられた。接種後 10 日から 2 週間で斃死した例、および 5~7 週後に安楽死させた例とともに、透明無色ないし麦藁色で卵白状を呈しフィブ

リンを混じた 5~200 ml の腹水が貯留し、腹膜は濁った顆粒状を呈して、肝脾とともに表面は部分的に線維素性付着物でおおわれており、大網は著しく肥厚し、腸間膜リンパ節の著明な腫大もみられた。組織学的には線維素性ないし壊死性腹膜炎が、腹腔臓器の包膜を含め、広く認められるほかに、肝、腎、肺における壊死巣ないし細胞集簇巢

がみられる。また特徴的な静脈炎、接種例ではとくに動脈炎を示すものも認められた。

病原体については確定的でないが、細菌、*Mycoplasma*, *Toxoplasma*, *Bedosonia* のいずれもが否定され、本病はわが国においては今回はじめて発見されたネコの新しいウィルス性疾患と考えられる。

EXPLANATION OF PLATES

PLATE I

- Fig. 1. A cat affected with experimental FIP. Its abdominal cavity contained about 500 ml of ascitic fluid.
- Fig. 2. Viscera from the cat shown in Fig. 1. Fibrinous exudates are present on the liver, spleen and intestine.
- Fig. 3. Pseudomembrane and necrotic lesions of the liver. $\times 100$.

PLATE II

- Fig. 4. Focal necrosis and vascular lesions in the kidney. $\times 100$.
- Fig. 5. Necrosis in the lung. $\times 100$.
- Fig. 6. Mesenteric lymph node. Hyperplasia of reticulum cells is shown. $\times 100$.
- Fig. 7. Vascular lesion in the omentum. $\times 100$.