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# Human Placenta Extract Therapy for Feline Hepatic Lipidosis

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**Abstract**: Feline hepatic lipidosis (HL), the most common hepatobiliary disease in cats, is characterized by the accumulation of excessive triglycerides (TGs) in more than 80% of hepatocytes. Forced oral feeding is recommended as the only therapy for this disease but the prognosis is often poor. As human placenta extract (Laennec) has been used to improve hepatic metabolism, we investigated the efficacy of this drug for the treatment of cats with HL. Ten cats diagnosed with HL in this study were treated with Laennec as a therapeutic drug. An immediate improvement in clinical symptoms was observed in all 10 cases. A statistical analysis indicated that the blood level of ALT (P = 0.029), AST (P = 0.029), ALP (P = 0.005), and T-Bil (P = 0.012) decreased significantly after treatment. All 7 hospitalized cases were eventually discharged from the hospital with a mean hospitalization duration of 4.8 days. The results of this study suggest that Laennec preparation has potential as an effective medicine for feline HL.
**Key words**: Feline Hepatic Lipidosis, Human Placenta, Laennec, ALT, ALP

## Ⅰ Introduction

Feline hepatic lipidosis (HL) is characterized by severe triglyceride accumulation in hepatocytes, causing cholestasis and liver disorders[1-4]. The triglyceride content in the liver tissue of cats with lipidosis averaged 43% compared to 10% in that of healthy cats[1-4]. HL is a secondary disorder concurrent with other essential diseases; more than 90% of lipidosis cases also had small intestinal diseases, pancreatitis, neoplasia, kidney disease, or diabetes mellitus[1-5]. Therefore, it is very important to treat the underlying disease. Refeeding syndrome is characterized by severe hypophosphatemia that occurs in patients given enteral or parenteral nutrition[6]. Therefore, even if treatment of the underlying disease was successful and only HL remained, the treatment period may be extended over the long-term. According to a specific report, in the absence of a diagnosis of a fatal underlying condition, recovery rates of 80%–88% could be expected if enteral feeding was initiated early in the course of the disease and sustained until voluntary intake resumed. However, cats might need tube feeding for several (3–6) weeks, requiring the owner to be an active participant in the recovery of their pet[7, 8]. Forced oral feeding is of limited benefit in cats that have been anorectic for prolonged periods and can be stressful when performed as long-term hospital care. At present, there are no reports on an effective medical therapy for fatty degeneration of hepatocytes. Human placenta extract (Laennec; JAPAN BIO PRODUCTS Co., Ltd., Tokyo, Japan) contains HGF, a hepatocyte growth factor that has a number of positive effects: (1) promoting liver regeneration, (2) promoting hepatocyte DNA synthesis, (3) preventing liver damage, (4) anti-fat liver, and (5) preventing liver fibroplasia[9-11]. In

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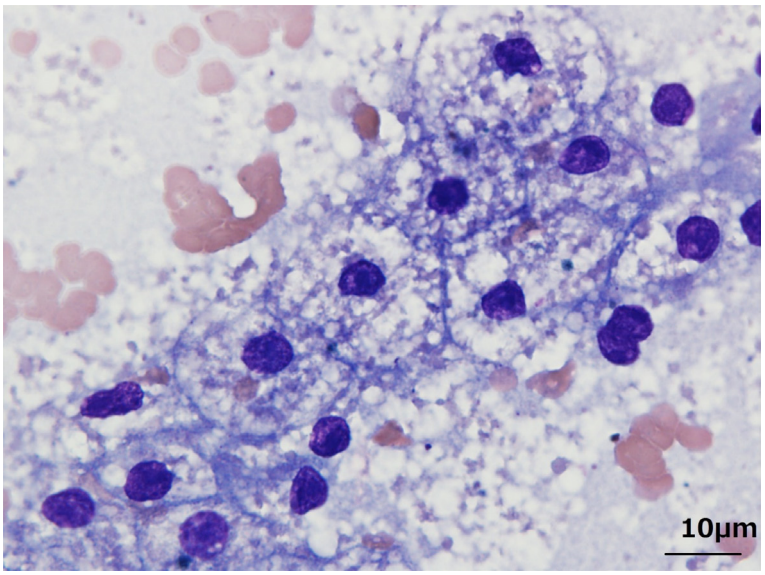


Fig. 1 Cytology findings of hepatic lipidosis.
Hepatocytes showed intense vacuolation. The sample was collected by fine needle biopsy (Case 2; Wright's Giemsa Staining, × 1,000).

this report, Laennec was administrated to 10 cats with HL as an adjunct therapy during the treatment of an underlying disease with conventional nourishment therapy.

## II Materials and Methods

A diagnosis of HL was based on a compatible history, clinical symptoms, biochemical and sonographic findings, plus the cytology of a fine needle liver biopsy[5, 12–18]. The findings of cytological examination showed a vacuolation in the majority of sampled hepatocytes in all 10 cats (Fig. 1). In addition, all 10 cats had underlying diseases; acute pancreatitis (Case 1), inflammatory bowel disease (IBD) (Cases 2 and 10), immune-mediated neutropenia and thrombocytopenia (IMNP/IMTP) (Case 3), diabetic ketoacidosis/acute pancreatitis (Cases 4 and 7), gastrointestinal low-grade lymphoma (Case 5), IBD/acute pancreatitis (Case 6), feline infectious peritonitis (FIP) dry type (Case 8), and FIP wet type (Case 9). These 10 cases were diagnosed with HL at a private veterinary hospital (AKIYOSHI ANIMAL CLINIC [author's] between April 2013 and March 2015.

On the day that HL was diagnosed, Laennec (2 ml/head; Laennec contains a water-soluble agent of the human placenta extract resolvent 112 mg out of 2 ml of 1 ampoule) was subcutaneously administered to all cats undergoing forced oral feeding and treatment for the underlying disease. All procedures in this study were performed in accordance with the Animal Protection Guidelines of Azabu University (authorization no. 1707019). This study, using client-owned cats, demonstrates a best practice of veterinary therapy and involves informed client consent.

For the seven cases of hospitalized cats, Laennec was administered every day until discharge. For the remaining three ambulatory cases, Laennec was injected from days 3 to 7. Physical examination, the time taken to recover appetite, and a complete blood workup and blood chemistry that included alanine transaminase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), γ-glutamyl transferase (GGT), total bilirubin (T-Bil), total cholesterol (T-cho), total protein (TP), albumin (Alb), blood urea nitrogen (BUN), creatinine (Cre), calcium (Ca), phosphorus (P), glucose (Glu), triglycerides (TG), and electrolytes (Na, K, Cl) were investigated. Table 1 summarizes the information on the concurrent disease, treatment, prognosis and number of Laennec injection in the 10 cats.

Table 1 Information relative to the concurent disease, treatment, prognosis and number of Laennec injection in 10 cats

| | Case1 | Case2 | Case3 | Case4 | Case5 |
|---|---|---|---|---|---|
| Signalment | Age:8y<br>NM<br>Norwegian forest cat | Age:17y<br>NM<br>Domestic short hair | Age:2y<br>NM<br>Scottish fold | Age:10y<br>NM<br>Domestic short hair | Age:15y<br>NM<br>Domestic short hair |
| History | Anorexia (4days),<br>Vomiting, (firsr opinion)<br>Ran a fever | Anorexia (5days), Vomitting | Anorexia (5days), Vomitting | Anorexia (7days)<br>Weight loss (2months) | Anorexia (7days), Vomitting |
| Abnormalities on the CBC | Normal | Normal | Normal | Normal | Normal |
| Abnormalities on the initial biochemical panels | ALT:524U/l,<br>AST:410U/l,ALP:310U/l<br>fPLI:50μg/l<,TBA147.1μmol/l | ALT:907U/l, AST:401U/l<br>ALP:932U/l, T-Bil:4.9mg/dl | ALT:1318U/l, AST:229U/l<br>ALP:657U/l | ALT:428U/l, AST:279U/l,<br>Glu:549mg/dl, Na:137mmol/l,<br>Cl:97mmol/l, fPLI kit:Positive,<br>FIV:Positive | ALT:1132U/l, ALP:215U/l |
| Concurrent disease | Acute pancreatitis | Inflammatory bowel disease | IMNP<br>IMTP | Diabetic ketoacidosis<br>Acute pancreatitis | Gastrointestinal low-grade lymphoma |
| Treatment | Enrofloxacin 5mg/kg/sid sc,<br>Maropitant 2mg/kg/sid sc,<br>Ursodeoxycholic 10mg/kg/bid po,<br>Metronidazole 10mg/kg/bid po,<br>Meloxicam 0.2mg/kg/sid sc | Enrofloxacin 5mg/kg/sid sc,<br>Prednisolone 0.5mg/kg/sid sc<br>Maropitant 2mg/kg/sid sc | Enrofloxacin 7mg/kg/sid sc,<br>cyclosporine 7mg/kg sc,<br>Prednisolone 2mg/kg/sid sc,<br>Ursodeoxycholic acid<br>10mg/kg/bid po | Imipenem-cilastatin 5mg/kg/tid iv,<br>Maropitant 2mg/kg/sid sc<br>Regular insulin iv (0.05U/kg–0.1/kg/hr) | Maropitant 2mg/kg/sid sc,<br>Ursodeoxycholic acid<br>10mg/kg/bid po, Mosapride<br>0.5mg/kg/bid po |
| Hospitalization management | NA | 7days | NA | 5days | NA |
| Number of Laennec injection | 1A SC, 1day | 1A SC, 7days | 1A SC, 3days | 1A SC, 5days | 1A SC, 3days |
| Prognosis | Survival >1years | Survival>1years | Survival>1years | Survival>6months | Survival>1years |
| | Case6 | Case7 | Case8 | Case9 | Case10 |
| Signalment | Age:9y<br>NM<br>Domestic short hair | Age:8y<br>NM<br>Domestic short hair | Age;16y<br>NM<br>Domestic short hair | Age;2y<br>NM<br>Domestic short hair | Age;14y<br>NM<br>Domestic short hair |
| History | Anorexia (4days), Vomitting | Anorexia (2days), PU/PD(+) | Anorexia (3days)<br>Weight loss (2months) | Anorexia (4days) | Weight loss |
| Abnormalities on the CBC | Normal | WBC: 23400/μl,<br>Neu: 14800/μl | WBC: 32800/μl<br>Neu: 18900/μl, Mono: 11100/μl | WBC:27500/μl<br>Neu: 21800/μl, Mono:2200/μl | Normal |
| Abnormalities on the initial | ALT: 266U/l, AST: 512U/l, ALP: 83U/l, Alb:2.2g/dl,<br>T-BiL: 1.3mg/dl, fPLI kit:Positive | ALT: 352U/l, AST: 493U/l,<br>ALP:208U/l, Glu:584mg/dl,<br>T-BiL 1.5mg/dl, f-PLI kit:Positive | ALT: 477U/l, AST: 239U/l<br>ALP: 180U/l, T-BiL: 0.8mg/dl<br>TP:10.8g/dl, BUN:39.1mg/dl | ALT: 269U/l, AST: 639U/l<br>ALP: 121U/l, T-BiL: 3.9mg/dl | ALT: 372U/l, AST: 136U/l<br>ALP: 809U/l,<br>T-bil:2.1mg/dl, Alb 2.0g/dl |
| Concurrent disease | Acute pancreatitis<br>Inflammatory bowel disease | Diabetic ketoacidosis<br>Acute pancreatitis | FIP/dry type | FIP/wet tipe | Inflammatory bowel disease |
| Treatment | Imipenem-cilastatin 5mg/kg/tid iv,<br>Maropitant 2mg/kg/SID sc,<br>Predonizolone 1mg/kg/sid sc | Imipenem-cilastatin 5mg/kg/tid iv,<br>Maropitant 2mg/kg/sid sc,<br>Regular insulin iv (0.05mg–0.1mg/kg/hr) | Imipenem-cilastatin 5mg/kg/tid iv,<br>Predonizolone 2mg/kg/sid sc,<br>IFN 1MU/kg/sid sc | Imipenem-cilastatin 5mg/kg/tid iv,<br>Predonizolone 2mg/kg/sid sc,<br>IFN 1MU/kg/sid sc | Enrofloxacin 5mg/kg/sid sc,<br>Maropitant 2mg/kg/sid sc,<br>Prednisolone 1mg/ks/sid sc |
| Hospitalization management | 3days | 3days | 4days | 5days | 7days |
| Number of Laennec injection | 1A SC, 3days | 1A SC, 3days | 1A SC, 4days | 1A SC, 5days | 1A SC, 7days |
| Prognosis | Survival > 1years | Survival > 6months | Survival: 2.5Months (FIP cause) | Survival: 1.5months (FIP cause) | Survival>6months |

Reference range: WBC: 4800–17000/μl; Neu: 2500–12500/μl; Mono: 50–1500/μl; ALT: 20–84U/l; AST: 12–45U/l; ALP: 26–160U/l; T-Bil: 0–0.7mg/dl; TP: 5.4–7.8g/dl; Alb: 2.5–3.9g/dl; BUN: 12–32mg/dl; Na: 145–156mmol/L; Cl: 111–125mmol/L; NM = neutered male; PU/PD = polyuria and polydipsia; CBC = complete blood count; ALT = alanine aminotransferase; AST = aspartate aminotransferase; ALP = alkaline phosphatase; fPLI = specific feline pancreas lipase; TBA = total bile acids; T-Bil = total bilirubin; Glu = glucose; Na = sodium; Cl = chlorine; FIV = feline immunodeficiency virus; Alb = albumin; WBC = white blood cells; Neu = polymorphonuclear neutrophils; Mono = monocytes; FIP = feline infectious peritonitis; IMNP = immune-mediated neutropenia; IMTP = immune-mediated thrombocytopenia; NA = not applicable: 1A = 1 ampoule.

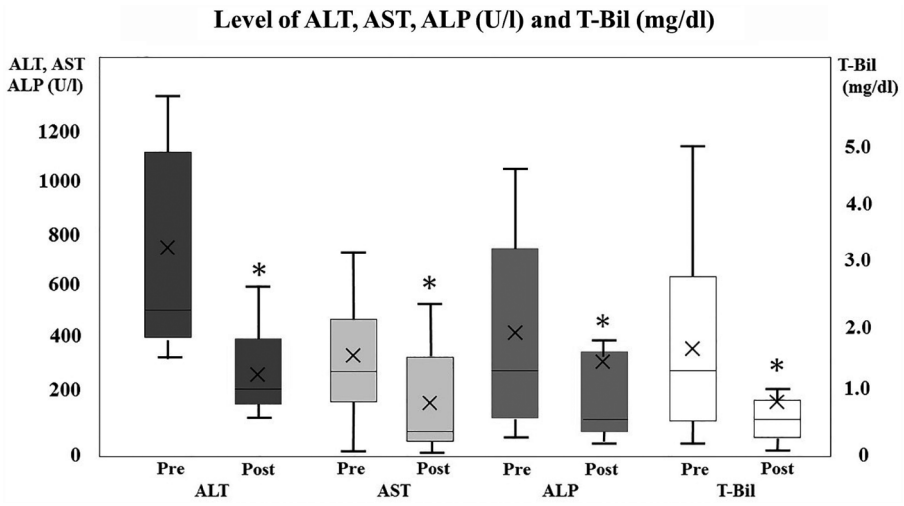


Fig. 2 Changes in blood chemistry.

The boxplot of ALT (dark gray), AST (light gray), ALP (middle gray) and T-Bil (white) levels of 10 cases, pre- and post- Laennec injection. The cross shows the mean and the bar shows the median. The values of ALT, AST, ALP, T-Bil were statistically compared between pre-treatment (pre) and post-treatment (post) values. The asterisk shows a significant difference (P< 0.05) in statistics.

Table 2 The transition of blood test results of 10 cases.

Pre shows the value of the blood test before Laennec injection and Post shows the value of the blood test at the time of completion of Laennec injection. NA means that Laennec injection was administrated by going to hospital for tratment

| | ALT Pre | ALT Post | AST Pre | AST Post | ALP Pre | ALP Post | T-Bil Pre | T-Bil Post | Hospitalization |
|---|---|---|---|---|---|---|---|---|---|
| Case 1 | 524 U/l | 425 U/l | 410 U/l | 39 U/l | 319 U/l | 72 U/l | 0.8 mg/dl | 0.3 mg/dl | NA |
| Case 2 | 907 U/l | 545 U/l | 401 U/l | 258 U/l | 932 U/l | 1159 U/l | 4.9 mg/dl | 1.1 mg/dl | 7days |
| Case 3 | 1318 U/l | 159 U/l | 229 U/l | 32 U/l | 657 U/l | 253 U/l | 0.6 mg/dl | 0.4 mg/dl | NA |
| Case 4 | 428 U/l | 142 U/l | 279 U/l | 73 U/l | 112 U/l | 80 U/l | 0.5 mg/dl | 0.3 mg/dl | 5days |
| Case 5 | 1132 U/l | 95 U/l | NA | NA | 215 U/l | 172 U/l | 0.09 mg/dl | 0.1 mg/dl | NA |
| Case6 | 266 U/l | 123 U/l | 512 U/l | 41 U/l | 83 U/l | 68 U/l | 1.3 mg/dl | 0.6 mg/dl | 3days |
| Case7 | 352 U/l | 273 U/l | 208 U/l | 275 U/l | 49 U/l | 90 U/l | 1.5 mg/dl | 0.6 mg/dl | 3days |
| Case8 | 477 U/l | 176 U/l | 239 U/l | 64 U/l | 180 U/l | 58 U/l | 0.8 mg/dl | 0.2 mg/dl | 4days |
| Case9 | 269 U/l | 141 U/l | 639 U/l | 514 U/l | 121 U/l | 90 U/l | 3.9 mg/dl | 3.3 mg/dl | 5days |
| Case10 | 372 U/l | 132 U/l | 136 U/l | 129 U/l | 809 U/l | 350 U/l | 2.2 mg/dl | 0.6 mg/dl | 7days |

Reference range: ALT: 20–84U/l, AST: 12–45U/l, ALP: 26–160U/l, T-Bil: 0–0.7mg/dl.

## III Results and Discussion

The median pre-treatment ALT, AST, ALP, and T-Bil values were 453 U/l, 279 U/l, 198 U/l, and 1.1 mg/dl, respectively. The median post-treatment ALT, AST, ALP, and T-Bil values were 151 U/l, 73 U/l, 90 U/l, and 0.5 mg/dl, respectively. (Fig. 2) The degree of decreased liver enzyme levels (ALT, AST, and ALP) and T-Bil varied, but a prompt improvement was confirmed by an alleviation of symptoms in all cases. Table 2 shows the transition of blood test results of 10 cases. A statistical analysis included a Wilcoxon signed-rank test using EXCEL (Microsoft, Washington, U.S.A.) and STATMATE (Nihon 3B Scientific, Nigata, Japan). Statistical analysis indicated that ALT ($P = 0.029$), AST ($P = 0.029$), ALP ($P = 0.005$), and T-Bil ($P = 0.012$) decreased significantly after treatment (P value<0.05). All cases were eventually discharged with a mean hospitalization duration of 4.8 days.

This report demonstrated the therapeutic potential of a Laennec preparation as an effective medicine to treat Feline HL. A decrease in ALT levels was seen in all cases and the mean time taken for appetite improvement was 4.8 days,

which was dramatically shorter than that presented in other reports[7, 8]. Furthermore, the ratio of decreased liver enzyme (ALT, AST, ALP) went along with hospitalization days for time to appetite improvement and the average hospitalization days of the non-administrated group was 12.0 days in our hospital. (Akiyoshi, M. and Hisasue, M., unpublished results) A particularly remarkable effect was seen in Case 1. This cat had acute pancreatitis and developed HL several times in the past, always necessitating hospitalization for 10 to 14 days. However, the symptoms and hepatic enzyme abnormalities of HL in this cat improved following a single injection of Laennec with conventional treatment and hospitalization management, and forced feeding was not required. HL and pancreatitis recurred 5 times over the following 2 years, and each time the cat was given Laennec recovery was evident by day 3. Furthermore, in HL cases with IBD or acute pancreatitis (Cases 2, 4, 6, 7, and 10), the length of hospitalization was less than 1 week, and it was suggested that the Laennec injection was more effective than the previously reported treatment in which tube feeding was necessary for 3–6 weeks[7,8]. In this study, the therapeutic dose of Laennec was extrapolated with reference to the therapeutic dose in humans[19]. The determination of a concrete therapeutic dose will be necessary in the future.

In this report, no side effects of a Laennec injection were seen in all cases. The maximum number of Laennec injections given to an individual cat in this study was seven (Cases 2 and 10), and no abnormalities were detected by physical examination. In human clinical trials, side effects were reported in 3.7% of 237 patients. The main side effects were pain at the injection site (2.56%), hypersensitivity (0.37%), and induration of the injection site (0.37%)[19]. Laennec was extracted from human placental tissue and is an exogenous material for cats. An increased risk of hypersensitivity and anaphylaxis was thus presumed to occur in cats compared to human patients. As such, careful follow up of the treated cats is required.

There were three problems in this study. The first problem was variability in the underlying disease of the 10 cases. The second problem was that there was no comparison with a control group that was not given Laennec. The third problem was that a definitive diagnosis of HL was not completed. We were unable to exclude other hepatobiliary diseases such as inflammatory, neoplastic, and blood vessel dysplasia since open wedge liver biopsies were not conducted. Therefore, it will be necessary to solve these problems in the future to prove the effect of a Laennec injection treatment for feline HL. It was suggested that enforcing an evaluation by unifying the underlying diseases, comparing a Laennec injection group with a non-administered group, and a histopathological examination of the liver will be necessary for further confirmation of the efficacy of Laennec.

In this study, clinical symptoms and liver enzyme levels in all cats with HL recovered following a treatment of Laennec injections with a mean hospitalization duration of only 4.8 days. A Laennec injection will be expected to reduce the cost and term of hospitalization and may contribute to improved feline HL outcomes. Further investigations that include a prospective study are necessary to determine the efficacy of Laennec.

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