Original Article

Comparison of Sensitivities and Specificities of ELISA and Histopathology to Diagnose Feline Infectious Peritonitis

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ABSTRACT

Feline infectious peritonitis (FIP) is one of the most prevalent viral infectious diseases in cats. It presents a number of challenges for veterinarians in terms of diagnosis. The objective of this study was to compare the sensitivity and specificity of ELISA with that of histopathology. Samples were obtained from 28 cats exhibiting signs consistent with feline infectious peritonitis (FIP) at the northwest animal clinics in Tehran, Iran, between January 2013 and 2015. Of the cats examined, five were deemed healthy, 14 exhibited indications of wet FIP, and nine displayed symptoms of dry FIP. Furthermore, the sensitivities and specificities of biochemical parameters were determined. The sensitivity and specificity of the ELISA test for diagnosing effusive FIP were found to be 100%, which was identical to the results obtained from histopathology. The AST (AUC=0.708) and total bilirubin (AUC=0.74) demonstrated moderate clinical accuracy in diagnosing FIP. The optical densities (ODs) in positive cats and the negative control group exhibited no statistically significant difference between the effusive and non-effusive forms of FIP. The Youden index was employed to determine the optimal cut-off point for the ratio of ODs in positive and negative cats, which was estimated to be 3.375. In conclusion, the ELISA demonstrated high predictive values for the diagnosis of effusive FIP and has the potential for use in the serological diagnosis of feline coronavirus infection.

Keywords: ELISA Test, Feline infectious peritonitis, Effusion, Histopathology

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1. Introduction

The potential for cross-species transmission of coronaviruses represents a significant concern for both animal and human health (1, 2). Feline coronavirus (FCoV) is an enveloped single-stranded RNA and positive-stranded RNA virus belonging to the family Coronaviridae within the order Nidovirales (3). Itincludes two biotypes: feline infectious peritonitis (FIP) virus and feline enteric coronavirus (FECV). The FIP virus biotype is more virulent and has the potential to cause peritonitis or even death in wild and domestic cats (4). The diagnosis of FIP represents a significant challenge in veterinary medicine, largely due to the inability of existing diagnostic tests to differentiate between FECV and FIPV (5). As a consequence of the fact that cats with FIP are typically euthanized (6), it is of the utmost importance that FIP is accurately differentiated from other conditions. A definitive diagnosis can only be made post-mortem (7). FCoV takes control of the host body's immune system, resulting in a sequence of inflammatory responses that eventually lead to the development of pyogranulomatous lesions around the blood vessels. Ultimately, leakage from the damaged blood vessels gives rise to clinical symptoms characteristic of effusive FIP, including the accumulation of fluid in the cat's pericardium, thorax, or abdomen. In the effusive form of FIP, a cat with pleural effusion will exhibit signs of dyspnea (8, 9). Despite the existence of laboratories and manufacturers producing test kits, there is currently no single definitive test available for diagnosing FIP. The diagnostic process for FIP entails following a defined sequence of steps, as outlined in an algorithm (10, 11). A variety of serologic tests have been employed for the diagnosis of FIP, including the indirect immunofluorescent antibody test (IFA) (12), virus neutralization (13), and enzyme-linked immunosorbent assay (ELISA) (14). ELISA tests are designed to be highly sensitive, enabling the accurate detection of even low levels of FIP-related antibodies or antigens in the blood (16). This is particularly significant in instances where the disease is in its initial stages or when the clinical indications are not conclusive. This is particularly advantageous in multi-cat settings, such as catteries or shelters, where prompt identification and appropriate management can help prevent the spread of the disease (17, 18). Some authors posit that the ELISA test is the most sensitive for diagnosing FIP (15). While the ELISA test is a valuable diagnostic tool, it should be noted that it is not a definitive diagnostic method in and of itself. It is recommended that the ELISA test be used in conjunction with other clinical findings, such as a physical examination, medical history, and additional diagnostic tests, to establish a comprehensive diagnosis of FIP. A biopsy with subsequent histologic evaluation of the abnormal tissues is frequently required for diagnostic confirmation. In other words, a definitive diagnosis typically necessitates a histological examination with FCoV antigen detection, which represents the gold standard (19). The objective of this study was to evaluate the sensitivity and specificity of the enzyme-linked immunosorbent assay (ELISA) in comparison to the histopathology approach for the accurate diagnosis of feline infectious peritonitis (FIP).

2. Materials and Methods

2.1. Animals and Sampling

Samples were obtained from 28 cats (of both sexes and aged between 6 and 24 months) suspected of having FIP at the northwest animal clinics in Tehran, Iran, between January 2013 and 2015. The samples were categorized as follows: five healthy cats, 14 exhibiting signs of wet FIP, and nine displaying signs of dry FIP. Samples were obtained from 28 cats (of both sexes and aged between 6 and 24 months) suspected of having FIP at the northwest animal clinics in Tehran, Iran, between January 2013 and 2015. The samples were categorized as follows: five healthy cats, 14 exhibiting signs of wet FIP, and nine displaying signs of dry FIP.

2.2. Serum Analysis

Following a 30-minute coagulation period, the anticoagulant-free blood was properly separated into serum and supernatant for the biochemical tests. The biochemical tests were conducted using the BT 1500, a device manufactured in Italy (20).

2.3. Histopathological Assay

The reference standard for assessing the sensitivities and specificities of the tests was histopathology. In accordance with the previously described methodology by Stranieri et al. (21), cats exhibiting effusions or yellow-to-white foci or nodules in various organs, accompanied by characteristic histologic lesions, were identified as having FIP. A variety of lesion types were observed, including plasma cellular perivasculitis, plasma cell accumulations with necropurulent centers, and combinations of both. The lesion was typically characterized by a central area of necrosis encircled by an arteriole or venule, surrounded by macrophages, lymphocytes, plasma cells, and neutrophils (16, 22). In our study, the presence of histologic lesions, including pyogranulomatous inflammation and vasculitis in selected organs (liver, spleen, kidney, and heart), was deemed a reliable indicator for FIP diagnosis.

2.4. Antigen-Antibody Complex Detection in Serum

The FCoV Ab ELISA kit from Biopronix (Italy) was employed to quantify antibodies directed against FCoV in serum and abdominal fluid samples, in accordance with the instructions provided by the manufacturer. The absorbances were read at 450 nm using a microplate reader (DANA-3200, Iran). The receiver operating characteristic (ROC) curve was employed to calculate the area under the curve (AUC) of the ELISA results in comparison with histopathology as the reference standard. Furthermore, the Youden index was utilized to identify the optimal cut-off point with the highest sensitivity and specificity (23).

2.5. Statistical Analysis

The data were analyzed using the statistical software package SPSS (Version 24, SPSS Inc., USA). The statistical analyses were conducted using receiver operating

characteristic (ROC) curves with a 95% confidence interval for the area under the ROC curve (AUC) and the Youden index. A Mann-Whitney U test was employed to ascertain whether there were any statistically significant differences in the means of the cut-off points between cats affected by the dry and wet forms of FIP. A p-value of 0.05 was considered to be the level of significance.

3. Results

3.1. Biochemistry

Table 1 presents the biochemical parameters of blood serum in various groups to diagnose FIP. The mean concentration of albumin to globulin and albumin differed significantly between groups ($p<0.05$). The mean creatinine concentrations between the healthy, dry, and effusive groups were found to be significantly different ($p<0.05$).A statistically significant difference was observed between the creatinine means of the dry and effusive groups ($p<0.05$). The results of the AST activities demonstrated moderate clinical accuracy, as indicated by an area under the curve (AUC) of 0.708, which is considered to be within the acceptable range. The optimal cutoff point was determined to be 33 U/L. The sensitivity and specificity of AST at the proposed cut-off point were 80% and 57%, respectively. A significant difference was observed in mean AST activities between the groups ($p<0.05$). The clinical accuracy of total bilirubin for the diagnosis of FIP was calculated to be 0.74, with a significant difference between the groups ($p<0.05$). The optimal cutoff point was identified as 1.48 mg/dL, exhibiting a sensitivity of 56% and a specificity of 100%.

3.2. Histopathology

The histopathology results for the 13 cats with an effusive form of FIP are as follows:

3.2.1. Lesions of the Liver

The findings included subcapsular fibrinous exudate, neutrophil infiltration, and mononuclear inflammatory cells, including lymphocytes, plasma cells, and macrophages, in addition to vasculitis in the liver parenchyma. In some cases, pyogranulomatous granulomas were observed. All of the samples exhibited the hallmark lesions of FIP (Figure 1).

3.2.2. Lesions of the kidney

Figure 2a) The presence of pyogranulomatous granulomas is evident within the liver parenchyma. The arrow indicates the presence of neutrophils in close proximity to the liver vein, which is indicative of vasculitis. H&E staining (×60), b) Liver, Pyogranulomatous granulomas, H&E staining (×100), and c) Liver. The presence of subcapsular fibrinous exudate is indicated by the arrow (A), while the infiltration of neutrophils and mononuclear inflammatory cells (lymphocyte, plasma cell, and macrophage) is illustrated by the arrow (B). H&E staining (×60).

3.2.3. Lesions of the Spleen

The depletion of the Malphigian corpuscles in the white pulps and infiltration of neutrophils in the red pulp space are indicative of FIP lesions (Figure 3).

3.2.4. Lesions of the Heart

The presence of neutrophilic and mononuclear inflammatory cells among the myocytes (myocarditis) indicates the presence of FIP lesions (Figure 4). The results of the histopathological examination are summarized in Table 2.

3.3. ELISA

ELESA with histopathology results for the various groups are presented in Tables 3 and 4. The sensitivity, specificity, and positive and negative predictive value of the ELISA test in 13 cats suspected to be affected by the effusive form of FIP were calculated to be 100% in comparison to the results of the histopathological examination. The sensitivity and specificity of the ELISA test for diagnosing FIP (effusive form) were found to be equivalent to those of histopathology. The results of the descriptive statistics and the clinical accuracy of the ELISA test, as determined by ROC analysis, are presented in Table 5. The Youden index was employed to ascertain the optimal cut-off point for the ratio of ODs in positive and negative cats, resulting in a value of 3.375. At the established cutoff point, the sensitivity and specificity were both 100%. The proposed cutoff value was found to be in close proximity to the cutoff value indicated in the kit brochure (4.0). No necropsy was performed on the non-effusive group, as the majority of cats were still alive at the time of the study. A total of three cases resulted in mortality, yet no reports were provided by the owners. No significant difference was observed between OD+/OD- in the effusive and dry groups. The Rivalta test and ELISA yielded positive results for all cats affected by the effusive form. In comparison with the pathological findings, the sensitivities and specificities were found to be 100%. The receiver operating characteristic (ROC) curve, which was used to determine the optimal albumin/globulin cut-off point, as well as the areas under the ROC curve (AUC) and the Youden index, is presented in Figure 5.

Table 1. Biochemical parameters of blood serum in different groups for diagnosis of feline infectious peritonitis

Parameters	Unit	Reference range	Groups		
			Healthy	Effusive-FIP	dry-FIP
Total protein	g/dl	5.4-7.8	7.2	7.7	8.6
Albumin	g/dl	2.1-3.3	5.0	2.5	4.3
Albumin/globulin	-	0.45-1.19	2.57	0.65	1.0
Creatinine	mg/dl	0.8-1.8	1.0	0.95	1.4
Urea	mg/dl	21.4-64.2	40.1	39.71	64.1
Total bilirubin	mg/dl	0.15-0.5	1.07	3.08	17.1
BUN	mg/dl	20-30	18.58	18.56	30.1
AST	U/L	26-43	42.9	138.25	42.2
ALT	U/L	6-83	58.8	92.06	100

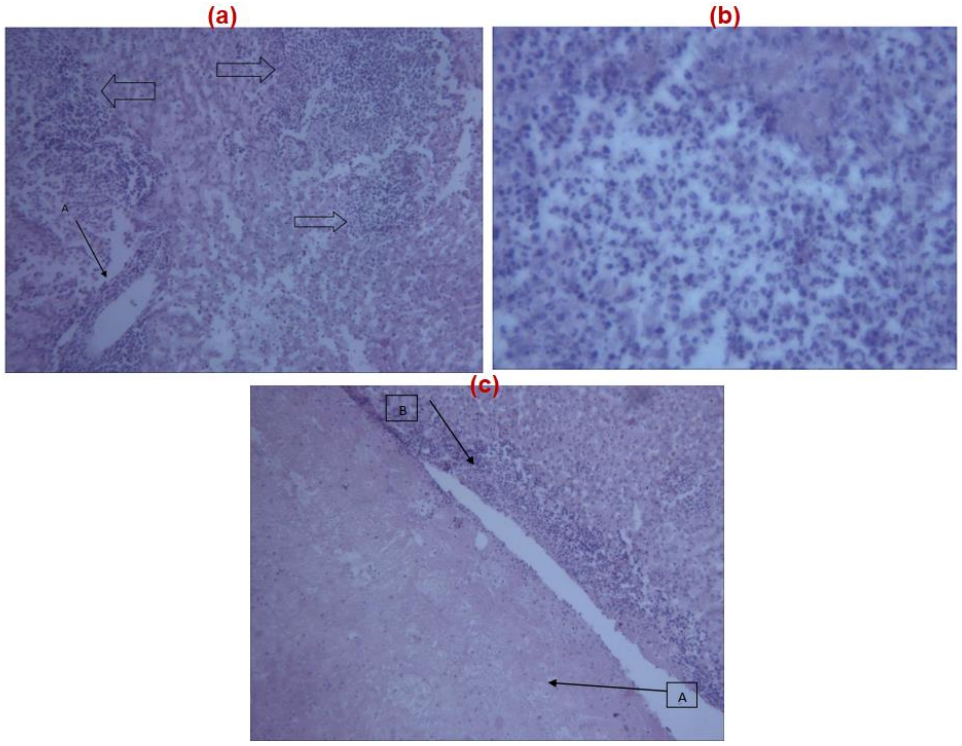


Figure 1.a) Pyogranulomatous granulomas are evident in the liver parenchyma. The arrow indicates the presence of neutrophils in proximity to the liver vein, indicative of vasculitis. H&E staining (×60), b) Liver, Pyogranulomatous granulomas, H&E staining (×100), and c) Liver. Arrow A indicates subcapsular fibrinous exudate, while arrow B illustrates the infiltration of neutrophils and mononuclear inflammatory cells (lymphocytes, plasma cells, and macrophages). H&E staining (×60).

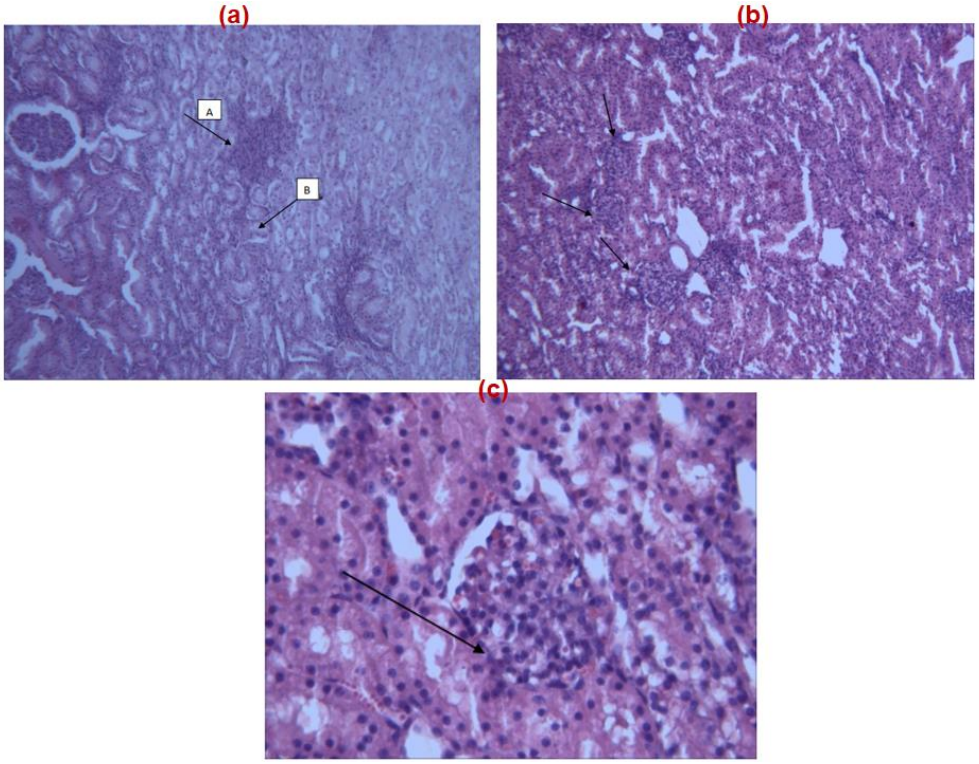


Figure 2. a) The kidney tissue displays mononuclear inflammatory cells in the interstitial space (arrow A) and hyaline casts in the tubules (arrow B), indicating nephritis, a chronic form of feline infectious peritonitis lesion. H&E staining (×60), b) The Bowman's capsule and the wall of the blood vessels in glomeruli exhibited thickening, accompanied by the proliferation of mesenchymal cells in the glomeruli and the attachment of glomeruli epithelia to the walls of Bowman's capsules (synechia), indicative of membranoproliferative glomerulonephritis (MPGN). H&E staining (×60), and c) Synechia in membranoproliferative glomerulonephritis. H&E staining (×100).

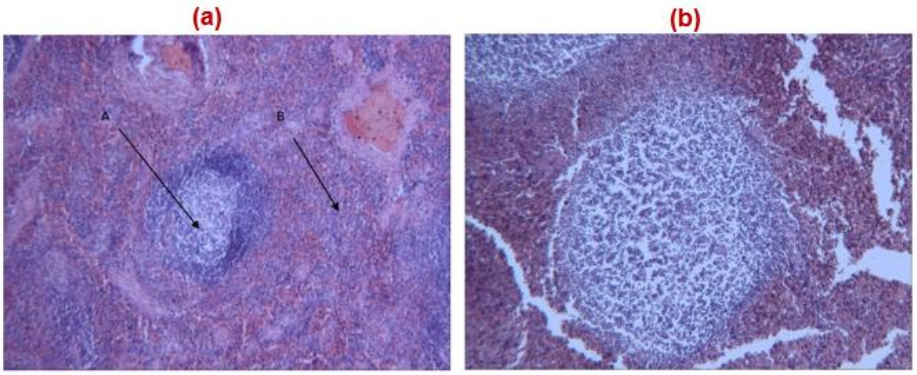


Figure. 3. a) A depletion of the Malphigian corpuscles of white pulps is accompanied by an infiltration of neutrophils in the red pulp space, which is indicative of feline infectious peritonitis lesions. H&E staining (×20) and b) depletion of the Malphigian corpuscles of white pulps. H & E staining (×60).

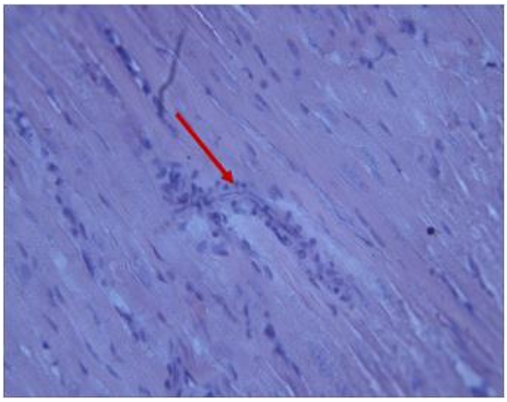


Figure 4. The presence of neutrophilic and mononuclear inflammatory cells among the myocytes (myocarditis) is indicative of feline infectious peritonitis lesions. Histological examination using the hematoxylin and eosin (H&E) staining method at a magnification of 100x.

Table 2. Histopathology results in affected cats with feline infectious peritonitis (FIP)

Cats	Kidney	Liver	Spleen	Heart
A_1	Membranoproliferative Glomerulonephritis (FIP)	Infiltration of inflammatory cells and vasculitis (FIP)	Normal	Normal
A_2	Membranoproliferative Glomerulonephritis (FIP)	Infiltration of inflammatory cells and vasculitis (Pyogranoloma) (FIP)	Lymphatic depletion (FIP)	Normal
A_3	Membranoproliferative Glomerulonephritis (FIP)	Infiltration of inflammatory cells and vasculitis (FIP)	Lymphatic depletion (FIP)	Normal
A_4	Membranoproliferative Glomerulonephritis (FIP)	Infiltration of inflammatory cells and vasculitis (FIP)	Lymphatic depletion (FIP)	Normal
A_5	Membranoproliferative Glomerulonephritis (FIP)	Infiltration of inflammatory cells and vasculitis (FIP)	Lymphatic depletion (FIP)	Normal
A_6	Membranoproliferative Glomerulonephritis With sever Synechia (FIP)	Infiltration of inflammatory cells and vasculitis (FIP)	Lymphatic depletion (FIP)	ormal
A_7	Membranoproliferative Glomerulonephritis (FIP)	Infiltration of inflammatory cells and vasculitis (FIP)	Sever lymphatic depletion in white pulp and subcapsular and Parenchymal infiltration of neutrophil (FIP)	Myocarditis (FIP)
A_8	Normal	Subcapsular and Parenchymal Pyogranolomatose Inflammation (PI)	Splenitis with lymphoid Hyperplasia (FIP)	Myocardial degeneration (vacuole in myocyte) (FIP)
A_9	Normal	Subcapsular and Parenchymal Pyogranolomatose Inflammation (PI)	Splenitis (FIP)	Normal
A_{10}	Membranoproliferative Glomerulonephritis (FIP)	Subcapsular and Parenchymal Pyogranolomatose Inflammation (PI)	Lymphatic depletion (FIP)	Normal
A_{11}	Normal	Infiltration of inflammatory cells and vasculitis (FIP)	Lymphatic depletion (FIP)	Myocarditis
A_{12}	Membranoproliferative Glomerulonephritis (FIP)	Infiltration of inflammatory cells and vasculitis (FIP)	Lymphatic depletion (FIP)	Fibrinous pericarditisis (FIP)
A_{13}	Normal	Random Hepatitis (FIP)	Lymphatic depletion (FIP)	Myocarditis (FIP)

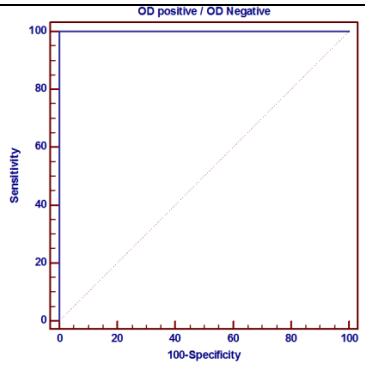
Table 3. ELISA results in different groups

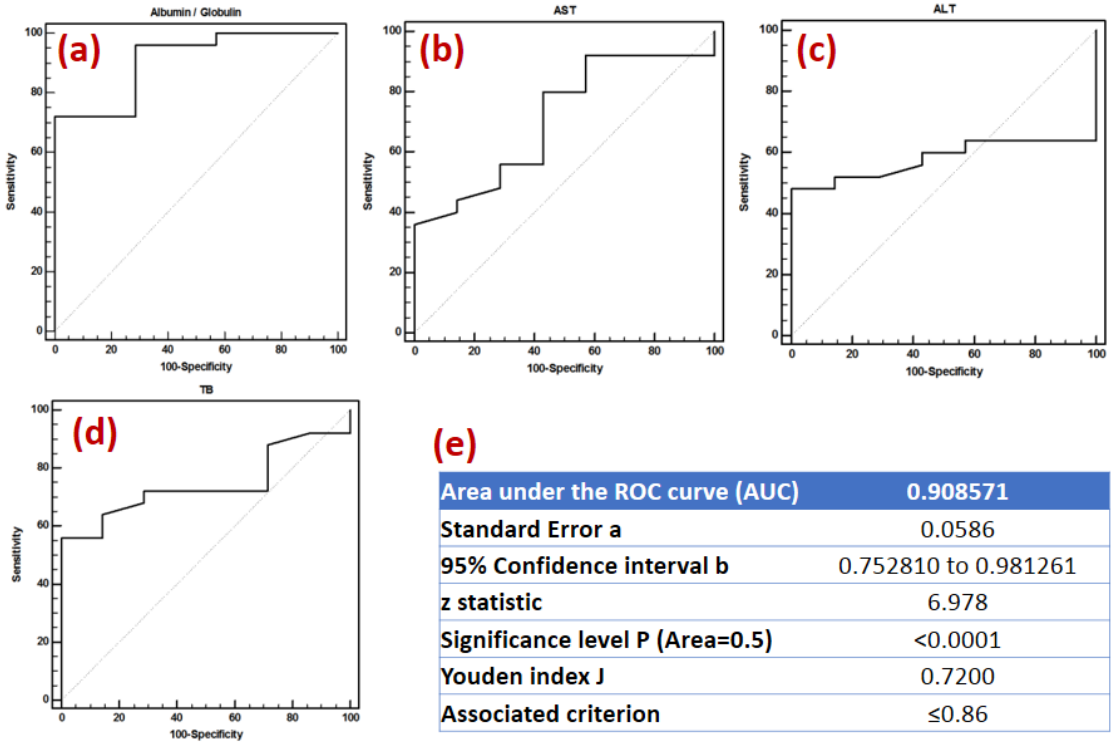
Groups	OD Serum	OD +/–	OD Effusion	OD +/–	OD Positive control	OD Negative control
Healthy						
A1	0.556				1.730	0.054
A2	0.131				1.005	0.02
A3	0.199				1.552	0.069
A4	0.139				1.424	0.059
A5	0.056				1.424	0.059
Effusive FIP						
B1	1.249	+	2.150	+	1.552	0.064
B2	2.105	+	1.823	+	1.73	0.069
B3	1.567	+	0.550	–	0.863	0.056
B4	1.875	+	1.950	+	1.73	0.069
B5	2.527	+	1.789	+	1.73	0.069
B6	0.974	–	1.153	+	0.675	0.2
B7	1.613	+	1.254	+	0.675	0.2
B8	0.993	+	1.156	+	0.863	0.056
B9	1.332	+	0.606	–	0.675	0.2
B10	0.373	+	0.286	+	0.151	0.054
B11	0.257	+	0.245	+	0.151	0.054
B12	1.416	+	1.251	+	1.005	0.007
B13	1.792	+	1.542	+	1.552	0.064
B14	2.448	+	2.122	+	1.73	0.069
Dry FIP						
C1	1.328	+			0.937	0.039
C2	0.667	+			1.552	0.064
C3	0.522	+			1.73	0.069
C4	2.315	+			1.73	0.069
C5	1.104	+			1.73	0.069
C6	2.908	+			1.73	0.069
C7	1.167	+			1.73	0.069
C8	2.098	+			1.424	0.053
C9	1.209	+			0.947	0.054

Table 4. Comparison of ELESA with histopathology results for diagnosing the effusive form of FIP

Groups	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12	B13
OD Serum	1.249 +	2.105 +	1.567 +	1.875 +	2.527 +	0.947 +	1.613 +	0.993 +	1.332 +	0.373 +	0.257 +	1.416 +	1.792 +
OD Effusion	2.150 +	1.823 +	0.550 _	1.950 +	1.789 +	1.153 +	1.254 +	1.156 +	0.606 _	0.286 +	0.245 +	1.251 +	1.542 +
Kidney	+	+	+	+	+	+	+	_	_	+	_	+	_
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	_	+	+	+	+	+	+	+	+	+	+	+	+
Heart	_	_	_	_	_	_	+	+	_	_	+	+	+

Table 5. The sensitivity, specificity, positive and negative predictive values of the ELISA test in 13 cats with effusive feline infectious peritonitis and 7 healthy cats.

	ELISA	95% Confident interval	
Sensitivity	100%	75.29-100%	
Specificity	100%	59.04-100%	
Positive Predictive Value	100%	75.29-100%	
Negative Predictive Value	100%	59.04-100%	



(e)

Area under the ROC curve (AUC)	0.908571
Standard Error a	0.0586
95% Confidence interval b	0.752810 to 0.981261
z statistic	6.978
Significance level P (Area=0.5)	<0.0001
Youden index J	0.7200
Associated criterion	≤0.86

Figure 5. The receiver operating characteristic (ROC) curves for the following variables were calculated: a) albumin/globulin cut-off point, b) aspartate aminotransferase (AST), c) alanine aminotransferase (ALT), d) total bilirubin, and e) area under the ROC curve (AUC) and Youden index.

4. Discussion

Despite the availability of numerous diagnostic tests, the diagnosis of FIP remains challenging for even the most competent veterinary clinicians. In this study, a comparison was made between cats in the control group and cats suspected to have FIP, based on their history and clinical findings. Feline infectious peritonitis (FIP) suspects were tested for anti-Feline corona virus (FCoV) antibodies using enzyme-linked immunosorbent assay (ELISA) on serum and peritoneal fluid samples. The results demonstrated that the ELISA test is a reliable and specific method for the detection of these antibodies in serum. Table 4 presents a comparison between the results of the ELISA and those of the histopathology examination in the diagnosis of the effusive form of FIP. A positive correlation was observed between the lesions in the kidney, liver, and spleen and the ELISA results. While the histopathology of lesions is highly specific for a diagnosis of FIP, it can only be performed through invasive or post-mortem procedures (24). As demonstrated in Tables 2 and 3, the diagnostic utility of the aforementioned parameters is more pronounced when evaluated in the context of the effusive form. It is crucial to assess the prevalence of feline coronavirus (FCoV) antibodies in an environment where multiple feline species coexist. Such measures are necessary for several reasons, including the prevention of FCoV-infected cats entering FCoV-free catteries. In the context of FCoV eradication programs, cats can be classified according to their shedding level for the purpose of implementing appropriate isolation measures. Furthermore, the FCoV status can be utilized to develop breeding programs. Some researchers have examined the antibody titer in serum samples and proposed a titer of 1:1600 or greater as the cut-off point (16, 25). The interpretation of serum antibody titers is a crucial aspect in the diagnosis of FIP (11). A high percentage of healthy cats exhibit antibody titers indicative of prior exposure to FCoV, reflecting the pervasive prevalence of the virus and the majority of cats that do not develop clinical disease. Accordingly, the elevated antibody titer should be interpreted with caution (15, 26). The reference standard for diagnosing effusive FIP is immunofluorescence staining of effusion cells. Some authors have asserted that the specificity of the staining is 100%, while the sensitivity is between 70% and 95% (16). In a recent study, Litster and Pogranichniy (27) found that the specificity was only 71.4%. In the absence of immunofluorescence staining, elevated AGP levels may prove useful in cases where histopathology is inconclusive (10). Among the 25 cats with FIP, 64% exhibited effusions, a finding that is comparable to the results of Lutz et al. (28), who observed effusions in 60% of their cats with effusions. However, this rate is higher than that reported by Walter and Rudolph (29), who observed effusions in 84% of their cats with FIP. As the study exclusively incorporated FIP cases confirmed through postmortem examination, it is possible that the actual prevalence in clinical practice may differ from the 64% reported. The presence of clinically apparent effusion increases the likelihood of FIP. Consequently, cases of FIP lacking observable effusion may be underrepresented in the data set. In the course of this study, cats with FIP were compared with control groups in which FIP was a serious differential diagnosis considered by the clinician based on history and clinical findings, but in which postmortem examination revealed other diseases or in which the animal survived beyond 12 months. As there is currently no effective treatment for clinically evident FIP, surviving cats were included in the control group, as they would not have survived for 12 months. Previous reports have demonstrated that an elevation in total serum protein concentration is one of the most reproducible clinicopathologic findings in cats with FIP. This finding is observed in approximately 50% of cats with effusions and 70% of cats without effusions (30). This is typified by a reduction in the albumin-to-globulin ratio, which elevates the total protein count due to the increase in globulin levels, predominantly γ-globulins (31). Similar findings have been reported by Paltrinieri et al. (32) and Paltrinieri and Gelain (33). As reported by Hartmann and Binder (16), the authors conducted a comparative analysis of various diagnostic tests. Competitive ELISA tests were employed to detect the Ag-Ab complex in cats without cavitary effusion, whereas IFA tests were utilized to detect FCoV antibodies in cats with and without cavitary effusion. The authors concluded that the detection of the Ag-Ab complex may prove a useful tool for diagnosing FIP, although they noted that the sensitivity and specificity were low in clinical cases. It is imperative to exercise caution when interpreting the results of anti-FCoV tests, as a negative titer does not necessarily exclude the possibility of FIP in cats exhibiting clinical signs. In this study, the sensitivity and specificity of a commercially available kit were determined to be 100% for the diagnosis of an effusive form of FIP. The sensitivity and specificity of the test may be subject to variation depending on the antibody cut-off titer utilized (5). The diagnostic efficacy of the ELISA test in 13 cats with effusive FIP was determined to be 100%, which was identical to the results obtained from histopathologic analysis. The cut-off point for the ELISA test was estimated to be 3.375, which was in close alignment with the proposed cut-off value outlined in the kit brochure. The optical densities (ODs) of the positive cats and the negative control group did not exhibit notable distinctions between the effusive and non-effusive forms of FIP. In conclusion, the results demonstrate that the ELISA test is an effective method for diagnosing the effusive form of FIP with high clinical accuracy. However, due to the lack of sufficient data, it was not feasible to extend these findings to the diagnosis of the dry disease form of FIP. Consequently, the evaluation of additional FIP diagnostic tests, including ELISA, may facilitate the development of a more precise logistic regression model for diagnosis.

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Authors' Contribution
Study concept and design: S.S. Analysis and interpretation of data: S.S, M.S., K.F., M.S.P. Drafting of the manuscript: S.S. Critical revision of the manuscript for important intellectual content: M.S., K.F., M.S.P. Statistical analysis: S.S. Administrative, technical and material support: S.S M.S., K.F., M.S.P. Study supervision: S.S, M.S., K.F., M.S.P.

Ethics
This survey was conducted in accordance with the National Institutes of Health's "Laboratory Care and Use Guidelines" pertaining to the use of animals in research. Approval for the research was granted by the Ethics Committee of the Islamic Azad University, Tehran Branch, Iran (10-E-IR-IAU.REC.10-B-2013). The owners of the cats have granted permission for the collection and publication of data.

Conflict of Interest
None.

Data Availability
The data that support the findings of this study are available on request from the corresponding author.

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