

**Research article****Peritoneal fluid analysis in dairy cows suffering from peritonitis**Reza Safarchi<sup>1</sup>, Aria Badiel<sup>2</sup>, Mohammad Gholi Nadalian<sup>1</sup> and Hesam Addin Seifi<sup>3</sup>

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**Abstract**

Peritonitis is an inflammatory process which involves the peritoneal cavity and its serous surfaces (the peritoneum). The purpose of this study was to analyze the peritoneal fluid in dairy cows suffering from peritonitis. In the control case study, 45 dairy cows were selected from the dairy farms in Tehran Province. Eighteen cows that showed no sign of peritonitis were considered for the control group (C) and the remaining 27 were divided into two groups: the group with peritonitis (A, n=21) and the group suffering from some miscellaneous disorders (B, n=6) in abdominal cavity (displacement of the abomasum, ascites, as well as liver and spleen abscesses). In the peritoneal fluid analysis, only one case in group (A) had traditional indicators of peritonitis and, in the remaining cows, other measures of inflammation (changes in cellular composition) were observed. Statistical analysis was performed using Chi-square for qualitative data and Kruskal-Wallis, ANOVA, and ROC curve for the quantitative ones. Finally, it was found that peritoneal fluid analysis does not have the required efficiency for the diagnosis of peritonitis in many cases and to reach the diagnosis with high accuracy and validity in bovine peritonitis, simultaneous use of laparoscopy and abdominocentesis is recommended.

**Keywords:** Laparoscopy; peritonitis; abdominocentesis; dairy Cow

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**Introduction**

Peritonitis is an inflammatory process involving the peritoneal cavity and its serosal surface, the peritoneum. Normal peritoneal fluid has a wide range of values. It should be clear; protein content should be less than 3 g/dl, although some authors have reported normal values up to 6.3 g/dl for cattle (Rosenberger, 1979). Normal bovine peritoneal fluid may contain some fibrinogen and may clot when exposed to air. Normal fluid contains fewer than 10000 cells with a

majority of macrophages. Lymphocytes, eosinophils, and desquamated mesothelial cells may also be present, but there are normally very few neutrophils (Smith and Jones, 2009). In 1982, Canadian researchers showed that if bovine peritonitis is infectious, no judgment can be made about Nucleated Cell Count (NCC). Furthermore, they found that peritoneal fluid analysis is only helpful for the diagnosis of chronic, non-infectious peritonitis and must not be relied on in advanced and infectious peritonitis due to slight changes in NCC and TP (Hirsch and Townsend, 1982). Another study in

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1985 which was conducted on cows determined that, if neutrophil level in the peritoneal fluid exceeds 40% and eosinophils level is less than 10%, then peritonitis will definitely occur (Wilson et al., 1985). The objective of this study was to investigate changes in the key diagnostic parameters of peritoneal fluid among the cows that are diagnosed to have peritonitis using laparoscopy.

Laparoscopy is a minimally invasive surgical technique using an endoscope inserted transabdominally to observe organs within the abdominal and pelvic cavities. During laparoscopy, the surgeon can inspect the viscera and peritoneal surfaces visually for evidence of disease and perform surgical procedures (Boure, 2005; Emrah Yanmaz et al., 2007). It must be noted that, to the best knowledge of the present authors, no formal report has ever been published on peritonitis diagnosis among cows using laparoscopy and its comparison with the peritoneal fluid analysis; thus, the application of this method is considered the first attempt in this field. Criteria of peritonitis diagnosis in laparoscopy include changes in the colour of peritoneal fluid, inflammation of peritoneum (peritoneal nodular surface, petechial and ecchymosis bleeding on the peritoneal and serous surfaces), yellow fibrin deposits on the peritoneal and visceral surfaces of peritoneum, and organized adhesion on peritoneal membrane surfaces in advanced and chronic cases (Luis, 2008).

## Materials and Methods

All the procedures involving the experimental use of animals were approved by Animal Ethics Committee, Islamic Azad University, Science and Research Branch, Tehran, Iran. This investigation was carried out on 45 milking Holstein cows (2.5-8 years old) from the dairy farm located around Tehran (capital city of Iran). After taking complete history according to the statements of the farm veterinarian, careful clinical examination was carried out on animals as described previously (Rosenberger, 1979).

### Collecting blood samples

Two blood samples were obtained by tail vein puncture in 10 ml vacutainer tubes: one with EDTA (as an anticoagulant) for hematologic analyses and another without anticoagulant for biochemical analyses. Samples were transferred to the laboratory on ice. Blood samples of biochemical analysis were allowed to clot and serum was extracted by centrifugation at 2000×g for 10 min within 2 h after collection. Sera were stored at -20°C for a short period until analysis.

### Haematologic and biochemical analyses

Haematological parameters including RBC count, WBC count, PCV and haematocrit value, platelet count,

and haemoglobin concentration were measured by auto-analyzer cell counter (Exigo Veterinary Hematology Analyzer, Art. no.1504195, March 2005). Differential leukocyte counts were performed on routinely prepared Giemsa-stained blood smears using the cross-sectional technique (Jain, 1993). Commercial kits were used to determine the serum concentration of fibrinogen, total protein, and albumin. Haptoglobin was measured to prevent the peroxidase activity of hemoglobin which was directly proportional to the amount of Hp. Analytical sensitivity of this test in serum has been determined as 0.0156 mg/mL for Hp by the manufacturer (Tridelta Development Plc, Wicklow, Ireland).

### Abdominocentesis

Abdominocentesis was performed at right caudo ventral abdominal wall medial to the fold of the flank, using a 3.8 cm, 18-gauge needle. A further site is the caudal to the xiphoid sternum and 4-10 cm lateral to the midline. The legs of animals were restrained. Sites were prepared aseptically and not infiltrated with any local anesthetic. The needle was pushed carefully and slowly through the abdominal wall, which twitched when the peritoneum is punctured. The fluid was allowed to flow into a 2 ml tube containing tri-potassium EDTA without the aid of a vacuum. After completing the procedure, the peritoneal fluid was centrifuged at 1500 g for 10 minutes. The supernatant was preserved at -20°C until further analysis. The sediment was used for preparing a smear by routine procedure. The peritoneal fluid smears were air dried, stained with Wrights stain and examined for cell differential count and cellular morphology. Differential cell counts and cytological findings were made especially at the end of the slide and at the edges of the slide, where abundance of the cells was found (Jain, 1993). Four categories of nucleated cells (neutrophils, lymphocytes, monocytes and other similar mononuclear cells, and eosinophils) were reported. Large mononuclear cells including macrophages, activated macrophages and mesothelial cells were all grouped together as monocytes. Commercial kits were used to determine the levels of fibrinogen (MahsaYaran Company) and total protein (Farasmad Company).

### Laparoscopy

In this study, diagnostic laparoscopy was performed on the animals in a standing position. Before preparing the surgical sites, the animal tail was tied to its hock or neck on the opposite side of the surgery. Both paralumbar fossa were clipped and prepared for aseptic surgery. The abdominal wall was infiltrated using a local anesthetic (8-10 ml of 2% lidocain solution per site) at portal sites. The laparoscopic cannula was inserted in the middle of the fossa, dorsally to the crus of the internal oblique muscle. The other

sites of penetration were located as one hand width ventral to the lumbar transverse processes and one hand width caudal to the last rib. When cannula was placed in the body wall, the trocar was replaced with a 10-mm rigid laparoscope (Fritz, Germany), which had an installed video camera and light source, and was connected to the laptop via a USB cable. A rapid examination of the surrounding intra-abdominal structures was performed to determine if trauma caused by the cannula insertion had occurred or not. Tubing connected to the insufflators was attached to the cannula, insufflation was stopped when organs were visualized, and the laparoscope could freely move in the abdomen. In patients with peritonitis, inflammatory signs were readily visible in the form of not only reddening and increased vessel injection but also varying degrees of fibrin production or adhesion between organ and body wall according to the stage of peritonitis. In some cases, peritoneal fluid was observed with changed colour and turbidity. In some patients, the strand of free fibrin in peritoneal fluid was visible. In others, heavy adhesion between organ and abdominal wall decreased further exploration of abdominal cavity. When the laparoscopic examination was completed, the endoscope was removed and the surgeon released the CO<sub>2</sub> gas/air via the open cannula. After all the gas was evacuated, the cannula was removed and the portal sites were closed. The portal site of the trocar was sprayed with tetracycline.

### Statistical analysis

Statistical analysis was carried out by SPSS16 software. All the variables for the investigation of normal data distribution were screened using the Kolmogorov-Smirnov table. In the statistical analysis, the mean and standard deviation were first obtained for all the quantitative data and the magnitude of data changes was analyzed at the confidence interval of 0.05% using ANOVA and Kuruskale Wallis, variance analysis methods. Finally, Chi-square method was utilized for analyzing the qualitative data. Also, to determine the clinical accuracy of the quantitative data, ROC curve test was used and the area under the curve was analyzed.

### Results

Eighteen cows lacked any clinical or laboratory signs of peritonitis were assigned to the control group (C). According to the laparoscopic results, 21 cows infected with peritonitis were assigned to group (A) and 6 cows suffering from other abdominal disorders were placed in group (B). In groups(C), (A), and (B), respectively, 13, 16, and 3 peritoneal fluid samples were obtained and, totally, 32 peritoneal fluid samples were taken from the 45 cows. In group (A), peritoneal

fluid analysis demonstrated that 1 out of 16 samples had the required changes for peritonitis (total number of nucleated cells was more than 10000 per  $\mu\text{L}$  and total protein was more than 3 gr/dL and the laparoscope showed heavy adhesion in 1 other sample, In contrast, no variations were observed in peritoneal parameters compared to the control group (Fig. 1). In the remaining 14 samples from which the peritoneal fluid was obtained (group A), a change was observed in the composition and ratio of peritoneal fluid cells compared to the control group (Table 1) and, in the remaining 5 cases, in which laparoscopic images proved peritonitis, no fluid could be extracted, even after frequent repetition. Changes in peritoneal fluid in the second group (B) were relatively in agreement with the variations in group (A). In the control group, no suspected variations were observed in the favor of peritonitis neither in laparoscopic nor peritoneal analyses and all the animals were healthy. Results of blood profile in all groups summarized in table 3 and 4.

### Discussion

One of the problems with which veterinarians are encountered in clinics is diagnose of peritonitis. This disease can induce a wide range of signs and make diagnosis more difficult: from a disease state to death of an animal. Sometimes, animals have symptoms such as reduced milk production, rumen contraction (frequency and amplitude) and pain in the abdominal cavity (Fecteau, 2005). These symptoms are not specific for the diagnosis of this disease and can be also observed in other disorders related to abdominal region or other systematic diseases. Because of the span spectrum of peritonitis and different situations, unlike many diseases, no pathognomonic sign has been mentioned. Among the 16 blood parameters examined in the present study, hyperfibrinogemia, hyperhaptoglobinemia, and left to the shift had significant differences from the control group. About 52% of the cows diagnosed with peritonitis had white cell counts in the normal range. However, inflammatory changes in the peritoneum can be seen by laparoscopy. Collection and evaluation of peritoneal fluid is helpful in the diagnosis and the establishment of treatment, as well as prognosis, in many gastrointestinal disorders in cattle. Abdominocentesis is considered an essential ancillary test in the approach to acute abdomen in many species (Smith and Jones, 2009). In most cases, use of peritoneal fluid analysis in cow is very difficult, because cows have very high ability in limiting infection and forming fibrin bonds; thus, although the peritoneal fluid is extracted, there may not be sufficient changes required for the diagnosis or no fluid is obtained despite the presence of peritonitis (Smith and Jones, 2009). Results of this study showed that

**Table 1: Mean ± standard deviation of peritoneal parameters in the studied groups**

Group	Nucleated cell count (μl)	Neutrophils (%)	Lymphocytes (%)	Total proteins (gm/dl)	Fibrinogen (mg/dl)	Eosinophil (%)
A	4439±707	64±14.29*	32.93±14.74*	3.07±2.18	143.29±70.97*	1.71±0.72
B	4100±367	61.33±11.37	37±13.22	2.40±1.75	111.33±12.09	1.67±2.08
C	4103±103	39.31±4.15	59.69±4.51	2.31±0.35	67.23±8.24	1±0.816

(\*:P&lt;0/01)

**Table 2: Values of peritoneal parameters and the area under the curve in group (A)**

Peritoneal fluid	Nucleated cell count	Neutrophils	Lymphocytes	Total protein	Fibrinogen	Eosinophil
(AUC)	0.30	1.00	1.00	0.71	0.97	0.74
(P value)	0.08	0.00*	0.00*	0.05	0.00*	0.03*

AUC > 0.9 = good clinical accuracy; AUC = 0.7-0.9 = Moderate clinical accuracy; AUC < 0.7 = poor clinical accuracy; \*Indicate significant differences at P<0.05

**Table 3: White blood cell and differential leukocyte count**

Group	WBC/μl		Ne/μl		Band/μl		Lymph/μl		Mono/μl		Eos/μl	
	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	Mean	SD
A	1.21×10 <sup>3</sup>	5.03×10 <sup>2</sup>	5.62×10 <sup>3</sup>	3.93×10 <sup>3</sup>	99.64*	119.49	6.26×10 <sup>3</sup>	2.54×10 <sup>3</sup>	56.86	76.12	69.07	124.04
B	11.01×10 <sup>3</sup> *	3.01×10 <sup>3</sup>	3.22×10 <sup>3</sup>	2.31×10 <sup>3</sup>	41.67	72.16	6.69×10 <sup>3</sup>	1.85×10 <sup>3</sup>	59.00	55.43	41.67	72.16
C	9.28×10 <sup>3</sup>	2.18×10 <sup>3</sup>	3.26×10 <sup>3</sup>	0.73×10 <sup>3</sup>	-	-	5.90×10 <sup>3</sup>	1.47×10 <sup>3</sup>	.	.	110.46	91.03

\*Indicate significant differences at P<0.05 compared with control group (c); S.D., standard deviation; WBC, White blood cell; Ne, Neutrophil; BN, Band neutrophil; Lym, lymphocyte; Mon; Monocyte; Eos, Eosinophil

**Table 4: Blood analysis, mean and SD in all groups**

Group	RBC×10 <sup>3</sup> /μl		Hb g/dl		Plt×10 <sup>9</sup> /μl		PCV%		Fb (mg/dl)		Tp (g/dl)		Alb (g/dl)		Hp (mg/dl)	
	mean	SD	mean	SD	mean	SD	mean	SD	Mean	SD	Mean	SD	Mean	SD	mean	SD
A	6.71	1.40	9.50	1.54	3.13	1.53	29.47	5.54	379.7*	127.98	7.29	1.102	3.6	0.33	187.9*	12.8
B	7.20	1.66	9.10*	1.83	3.27	1.33	27.83	5.63	327.0	197.61	6.66	0.30	3.56	0.56	162.3	56.87
C	6.99	0.20	10.13	0.37	2.69	0.80	30.15	0.966	293.6	27.54	7.05	0.09	3.50	0.09	2.74	1.21

\*, Indicate significant differences at P<0.05 compared with control group (c); SD ±1standard deviation; RBC, Red blood cell; Hb, Hemoglobin; Plt, Platelet; PCV, Packed cell volume; Fb, Fibrinogen; TP, Total protein; Alb, Albumin; Hp, Haptoglobin

**Table 5: Area under the curve for all quantitative data in group A**

Test Result Variable(s)	AUC	Std. Error <sup>a</sup>	Asymptotic Sig. <sup>b</sup> (P value)	Asymptotic 95% Confidence Interval	
				Lower Bound	Upper Bound
Blood Profile					
Red Blood Cell	0.52	0.13	0.80	0.26	0.79
Hemoglobin	0.45	0.12	0.69	0.20	0.70
Plateletes	0.51	0.12	0.92	0.27	0.74
Fibrinogen	0.68	0.11	0.10	0.45	0.90
Segmented Neutrophils	0.71	0.11	0.06	0.49	0.93
Band Neutrophils	0.72	0.10	0.04*	0.52	0.92
Lymphocytes	0.27	0.10	0.04	0.06	0.48
Atypical Lymphocytes	0.50	0.11	1.00	0.27	0.72
Basophiles	0.50	0.11	1.00	0.27	0.72
Monocytes	0.65	0.10	0.15	0.44	0.87
Eosinophils	0.37	0.11	0.27	0.15	0.60
Packed Cell Volume	0.53	0.13	0.75	0.26	0.80
Total Proteins	0.70	0.12	0.07	0.46	0.93
Blood Albumin	0.65	0.11	0.16	0.43	0.88
Globulins	0.57	0.12	0.52	0.32	0.81
Haptoglobins	0.89	0.07	0.00*	0.75	1.03
Peritoneal Fluid Profile					
Nucleated cell count (NCC)	0.30	0.10	0.08	0.09	0.51
Neutrophils	1.00	0.00	0.00*	1.00	1.00
Lymphocytes	1.00	0.00	0.00*	0.00	0.00
Eosinophils	0.74	0.09	0.03*	0.55	0.92
Total protein	0.71	0.11	0.05	0.48	0.93
Fibrinogen	0.97	0.02	0.00*	0.93	1.02

\*, Indicate significant differences at P<0.05 a. Under the nonparametric assumption; b. Null hypothesis: true area = 0.5; AUC > 0.9 = good clinical accuracy; AUC = 0.7-0.9 = Moderate clinical accuracy; AUC < 0.7 = poor clinical accuracy



**Fig. 1: Adhesion of abdominal viscera (grater omentum) to the right wall in the posterior of the liver**



**Fig. 2: Diffused peritonitis, fibrin deposition spots on the parietal peritoneum**



**Fig. 3: Chronic localized peritonitis, adhesion of the liver capsule to the parietal peritoneum**



**Fig. 4: Diffused peritonitis, deposition of yellow fibrin in the abdominal viscera**

variations in TP and NCC, which are the most effective traditional criteria in the peritoneal fluid, cannot always be reliable for the diagnosis of peritonitis in cows. In the present study, change in the cellular composition was more significant and important than the change in the total number of cells such that neutrophil to lymphocyte ratio was changed and increased. By investigating the area under the curve (AUC), variations of neutrophils and lymphocytes were significant as a criterion for animals' suffering from peritonitis (Table 1,2,5). By investigating various cut-point, sensitivity and specificity of neutrophils in peritonitis diagnosis was 100%. It must be noted that in the reports by other authors, the presence of eosinophil in the bovine peritoneal fluid has not been so prevalent (Oehme, 1969; Oehme and Noordsly, 1982). Findings in the present study also confirmed these results. Variations in peritoneal eosinophils in this study were significant according to the AUC ( $P=0.000$ ); however, it was not a valuable finding for making judgment about peritoneal diagnosis, because it did not have two important and desired properties (sensitivity and specificity). When this component had a high level of sensitivity at a specific cut-point, its specificity was low; with a change at the cut-point and increase in specificity, the sensitivity was reduced. The sampling method adopted by the Canadian researchers was different from the methods used in this study and other works; frequent washing of peritoneum with normal saline, might be the reason for the increased level of eosinophil in the bovine peritoneal fluid (Wilson et al., 1985). According to the findings of the present study, we found that in all normal cases as well as patients infected with peritonitis in which peritoneal fluid could be obtained, level of eosinophil was less than 10% and, in the first step, this finding was inconsistent with the results by the Canadian researchers. Furthermore, it was found that neither TP nor NCC of the peritoneum can be a sensitive index for peritoneal diagnosis in the cow species (Wilson et al., 1985). In this study, the majority of cells in peritoneal fluid extracted from the cows suffering from peritonitis with no exception were composed of neutrophils. However, in spite of the presence of peritonitis in most of them, NCC did not exceed the threshold of 10000 per  $\mu\text{l}$ , nor was the TP increase statistically significant (Table 1,2), but, laparoscopic findings indicated the presence of peritonitis (Fig. 2&3). In this study, the results showed that NCC level exceeded the threshold of 10000 only in one case of Fig. 4, while in most cases, despite the presence of peritonitis, NCC level did not increase and even decreased compared to the control group. On this basis, we showed that the judgment solely based on the cows' NCC without relying on other parameters is not helpful for peritonitis diagnosis. It must be noted that, if NCC level in a cow is low, the hypothesis of peritonitis

should not be rejected and other parameters of peritoneal fluid must be taken into account or other diagnosis methods must be utilized. In this study, the results suggested that, despite the presence of peritonitis, 33.33% of the patient, total protein were decreased, instead of increasing, and 6.66% of the patient were in the normal range despite the presence of peritonitis. The reason for such variations can be the bovine ability in limiting infection by establishing fibrin bonds which results in high proteins consumption of the peritoneal fluids (Smith and Jones, 2009). But, the important point is that, when investigating the AUC, TP variation tended to be significant ( $P = 0.058$ ) and the area under the curve was 0.714, which indicated moderate clinical correlation between this variable and presence of the disease. Therefore, according to the obtained results, it can be concluded that, in the cows with peritonitis, the high level of TP is not a requirement and many cows with peritonitis can have a normal or decreased level of proteins in their peritoneal fluid. In the cow species, the interpretation of peritoneal fluid and its value is only useful for the region in which the fluid sample is taken and the infection in other regions might be limited by the peritoneal immune system (Smith and Jones, 2009).

In the present study, in all the cases and all the cows that were diagnosed with inflammatory lesions or adhesion in the peritoneal region by laparoscopy, fibrinogen of the fluid was increased such that, by assessing various cut-point, at 84 mg/dl, its sensitivity and specificity for peritonitis diagnosis were 100 and 93% respectively. The findings in this study clearly showed variation in the colour of peritoneal fluid in the cases infected with peritonitis. This colour change was precisely observed by the laparoscope. However, it must be noted that while extracting the peritoneal fluid by the needle, if the fluid was mixed with the blood, there might be misinterpretation; thus, it is better to be interpreted along with other parameters.

In group B, in 6 cases, the laparoscopy examination diagnosed other disorders than peritonitis (liver and spleen abscess, displacement of abomasum, fibrosis of kidney capsule and ascites, or colour change of peritoneal fluid to serosanguineous colour without fibrin deposition) and peritoneal fluids were extracted only from 3 case, none of which had the traditional peritonitis indicator. Nevertheless, the number of neutrophils was increased with respect to the mononuclear cells (lymphocytes). To explain this point, it must be pointed out that, if we admit that the peritonitis is an active process and spectrum which begins from an acute condition and some other patients may experience chronic phase of the disease and also accept that attack of neutrophils along with increasing their numbers followed by the chemotaxis process during the initial stages of (acute) peritonitis in the

peritoneal fluid is a measure of inflammation (Fecteau, 2005) and the main definition of peritonitis is inflammatory reaction in the peritoneum, thus under this condition (beginning of the disease process), the only laparoscopic finding will be peritoneal hyperemia, rather than fibrin deposition or adhesion. Peritoneal fluid analysis at the beginning of acute peritonitis might be a more valuable finding than laparoscopy (under such conditions, even if there are no traditional peritoneal indicators, there are at least some changes in the cellular composition). Our finding was completely inconsistent with the results by the Canadian researchers who concluded that peritoneal fluid analysis is very helpful in the diagnosis of bovine chronic, non-septic peritonitis (Hirsch and Townsend, 1982). In the present work, obtaining peritoneal fluid in the animals suffering from advanced lesions or chronic peritonitis was very difficult and sometimes impossible.

### Conclusion

Inability of peritoneal fluid in terms of expressing the expansion of lesions is one of the most important weaknesses of this method, which is exactly the strength of laparoscopy compared to the peritoneal fluid. Accordingly, laparoscopy is able to cover the weaknesses of peritoneal fluid and, in chronic cases of peritonitis and expansion of the lesions, it has stronger and better performance than the peritoneal fluid. Similarly, regarding the laparoscopy, at the beginning of the peritonitis process, no significant finding may be observed in peritoneal exploration, except peritoneal hyperemia. The conclusion is that every method has its own advantages and disadvantages and must be selected by veterinarians considering the specific conditions. Finally, to reach the diagnosis with high accuracy and validity in bovine peritonitis, simultaneous use of laparoscopy and abdominocentesis is recommended.

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