



Clinical, haemato-biochemical, ultrasonographic and radiological findings in septic effusions in cats caused by *Klebsiella* spp.

Varghese Raina¹, K. Vinodkumar¹, P.V. Tresamol¹, K. Justin Davis¹,
 Ancy Thankachan¹ and P.M. Priya²

¹Department of Veterinary Epidemiology and Preventive Medicine, College of Veterinary and Animal Sciences, Mannuthy, Thrissur- 680 65, ²Department of Veterinary Microbiology, College of Veterinary and Animal Sciences Mannuthy, Thrissur-680 65, Kerala Veterinary and Animal Sciences University, Pookode, Wayanad, Kerala

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Abstract

Feline patients presenting with body cavity effusions are frequently encountered in veterinary clinical practice. Klebsiella spp. were isolated and identified from the septic effusions of three out of 23 cats with body cavity effusions. The morphological and biochemical identification was further confirmed through genus-specific PCR. Sequencing of the PCR products revealed the organism to be Klebsiella pneumoniae. Antibiotic susceptibility profiling of the organism indicated maximum susceptibility to doxycycline and enrofloxacin, while amoxicillin-clavulanate was the least effective. Haematological analysis revealed elevated total leukocyte count, with a marked increase in both the absolute neutrophil count and neutrophil percentage in all three cases of septic effusion. Thrombocytopenia was evident, while total erythrocyte count, haemoglobin, and haematocrit levels remained relatively unchanged. Biochemical analysis revealed significant increase in AST (3/3), increase in BUN (3/3), increased ALP (1/3) and elevated TP (2/3), with hypoalbuminemia in one case. The total nucleated cell count (TNCC) in all samples exceeded 100,000 cells/ μ L, indicating a robust inflammatory response characteristic of septic effusions.

Keywords: *Klebsiella* spp., amoxicillin-clavulanate, septic effusions

Septic peritonitis (SP) and pyothorax is a critical condition in cats that demands immediate medical attention to prevent life-threatening complications. Septic effusion diagnosis is confirmed by detecting intracellular bacteria in the peritoneal fluid, obtaining a positive culture result, or by identifying a ruptured or perforated organ (Scotti *et al.*, 2019). The condition arises from bacterial leakage into the peritoneum, typically from the gastrointestinal tract (Culp *et al.*, 2009) and hence Gram-negative organisms like *E. coli* and *Klebsiella* predominate.

Klebsiella spp. ranks as the second most common member of the Enterobacteriaceae family and is frequently found on the mucosal surfaces of mammals. It also exists in environmental reservoirs such as water, food, and soil. *Klebsiella pneumoniae* is a major cause of pneumonia, wound infections, urinary tract infections, sepsis, and meningitis in neonates, the elderly, and immunocompromised individuals. β -lactam antibiotics are among the most widely prescribed antimicrobials for treating infections caused by Enterobacteriaceae, including *Klebsiella* spp. (Rubin and Pitout, 2014).

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*Corresponding author: rainavarghese123@gmail.com, Ph. 9497394139

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However, the overuse of these antibiotics has led to the rise of *Klebsiella* strains that produce broad-spectrum β -lactamases, contributing to antimicrobial resistance. Moreover, the spread of antibiotic-resistant bacteria from companion animals to humans poses a significant public health concern.

This paper discusses the *Klebsiella* spp. associated with feline septic effusions, as well as their susceptibility to antibiotics typically employed in treatment protocols. Additionally, it explores the haematological and biochemical changes observed in affected cats, providing insights into the systemic impact of these conditions and the effectiveness of therapeutic interventions.

Materials and methods

Study samples

The present study was a part of work carried out in the Department of Veterinary Epidemiology and Preventive Medicine, College of Veterinary and Animal Sciences, Mannuthy, during the period from June 2023 to July 2024. Twenty-three cats presented to the Teaching Veterinary Clinical Complex (TVCC), Mannuthy and University Veterinary Hospital (UVH), Kozhikode with pleural effusion and ascites were screened, and cats that had septic effusions were selected for the present study. The presence of fluid was confirmed based on either ultrasonographic or radiological evaluations. Ultrasonography was performed using Esaote MylabX8eXP ultrasound machine, using (4-15 MHz) and phases array (1-5 Hz) transducers. Radiographic examination of the patients were carried out either in dorso-ventral or right lateral views. Siemens X ray machine (200mA) supported with Carestream Image suite Vita CR was used for radiography.

Ten millilitres of effusion were collected from cats with pleural effusion through thoracocentesis done on sternal recumbency at the seventh intercostal space, by draining the pleural fluid through a 10ml syringe with a 20G butterfly needle. Ten millilitres of peritoneal fluid were collected by abdominocentesis in a 10mL syringe by inserting a 20G butterfly needle 1-2 cm caudal to the umbilicus on the midline under aseptic precautions. Two millilitres each of both fluids were transferred to a sterile collection vial and subjected to isolation of bacteria using standard microbial culture techniques. Three millilitres of blood were collected from the medial cephalic or saphenous vein of each cat using 23-24 G scalp vein into 5 mL disposable syringe. One millilitre was transferred into an EDTA coated vial and two millilitres were transferred into another vial coated with clot activators. Ultrasound and X-ray findings of septic peritonitis case were recorded.

Isolation and identification of bacteria

Bacterial isolation from the effusion sample was performed by directly streaking the sample onto brain

heart infusion agar (BHIA; M211) or blood agar plates. These plates were incubated at 37°C for 24 hours, with colony growth assessed between 12- and 24-hours post-incubation. Identification of the bacterial isolates was conducted based on their morphological and cultural characteristics, as well as biochemical tests, following the methods outlined by Barrow and Feltham (1993) and Quinn *et al.* (2013).

Genotypic characterisation

All the clinical isolates were subjected to Gram's staining and all isolates turned to be Gram-negative and were subjected to biochemical tests. Biochemical tests revealed the presumptive isolate as *Klebsiella* spp. and isolates were subjected to genotypic characterisation and molecular confirmation by amplicon of the *gyrA* gene of *Klebsiella*. The DNA extraction from three bacterial isolates was done by the snap-chill method or heat lysis method and the extracted DNA was subjected to PCR. Primers targeting the *gyrA* gene of *Klebsiella*. Forward primer: 5'-CGCGTACTATACGCCATGAACGTA-3' and Reverse primer: 3'-ACCGTTGATCACTTCGGTCAGG-5' were used to generate an amplicon of approximately 441 bp, as described by Brisse and Verhoef (2001). Amplification was performed using a thermal cycler (Bio Rad, USA) under the following conditions: an initial denaturation at 94°C for 5 minutes, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 55°C for 45 seconds, and extension at 72°C for 45 seconds. A final extension was carried out at 72°C for 10 minutes.

Disc diffusion assay for antimicrobial profiling

Phenotypic antimicrobial susceptibility testing was performed on three bacterial isolates using *in vitro* disc diffusion assays. The susceptibility was evaluated by measuring the diameter of the inhibition zones around the antibiotic discs. Discs containing fixed concentrations of five antimicrobial agents *viz.* amoxicillin-clavulanate, ceftriaxone, doxycycline, enrofloxacin, and gentamicin were used for this analysis.

Haemato-biochemical analysis

A complete blood count, including the following parameters: total leucocyte count (TLC), total erythrocyte count (TEC), haemoglobin (Hb), thrombocyte count (PLT), haematocrit (per-cent), volume of packed red cells (VPRC), absolute neutrophil count (Neu#), lymphocyte (Lym#), absolute monocyte count (Mon#) and absolute eosinophil count (Eos#) in blood, was done for each sample using an Automatic Haematology Analyzer (Orphe Mythic 5 Vet PRO).

Sera samples from cats with pleural effusion and peritoneal effusion were separated and stored in sterile two mL microcentrifuge tubes at -20°C. Assays were performed in a fully automated analyser (SELECTRA Pro S Lite,

Netherland) according to manufacturer's instructions.

Cytological analysis and Total Nucleated Cell Count (TNCC)

The pleural/peritoneal fluid sample was centrifuged at 3000 rpm for 5 minutes and a smear was prepared with sediment, stained using Field's stain and cytological evaluation was done under oil immersion objective. TNCC was calculated using Neubauer's haemocytometer using following formula:

$$\text{Total nucleated cell count} = \frac{\text{cells counted} \times \text{dilution factor (20)}}{\text{area counted (mm}^2\text{)} \times \text{depth (0.1)}} \text{ (cells/mm}^2\text{ or cells/}\mu\text{L)}$$

Results and discussion

The present study involved three male cats: one non-descript cat, aged one year, presented with clinical signs of sternal positioning with abducted elbows, dyspnoea suggestive of pleural effusion; a two-year-old Persian cat; and another non-descript cat, aged one year, both having distended abdomen and diagnosed with septic peritonitis. All three cats had septic effusions. Pyrexia was observed in only one cat with septic peritonitis, while the other two cats exhibited subnormal body temperatures. No haemoparasites or bacteria could be detected on blood smear examination in all three cats. Among the 23 cats with body cavity effusions, bacterial growth was identified in only three cases mentioned above, while Feline Coronavirus (FCoV) was detected in the effusion samples of eight other cats.

Radiography and ultrasonography

Radiographic imaging demonstrated diffuse abdominal effusion, characterized by a "ground glass" appearance, supporting the presence of significant fluid accumulation in the abdominal cavity (Fig.9)

Ultrasonographic examination revealed the presence of homogeneously echogenic peritoneal fluid (Fig.10a., Fig.10b.) suggestive of high cellularity and consistent with septic peritoneal effusion. Additionally,

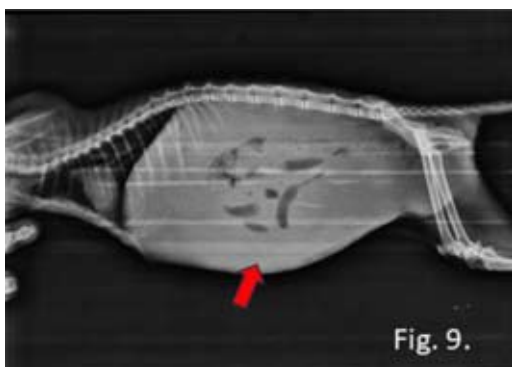


Fig. 9. Ground glass appearance of the abdomen

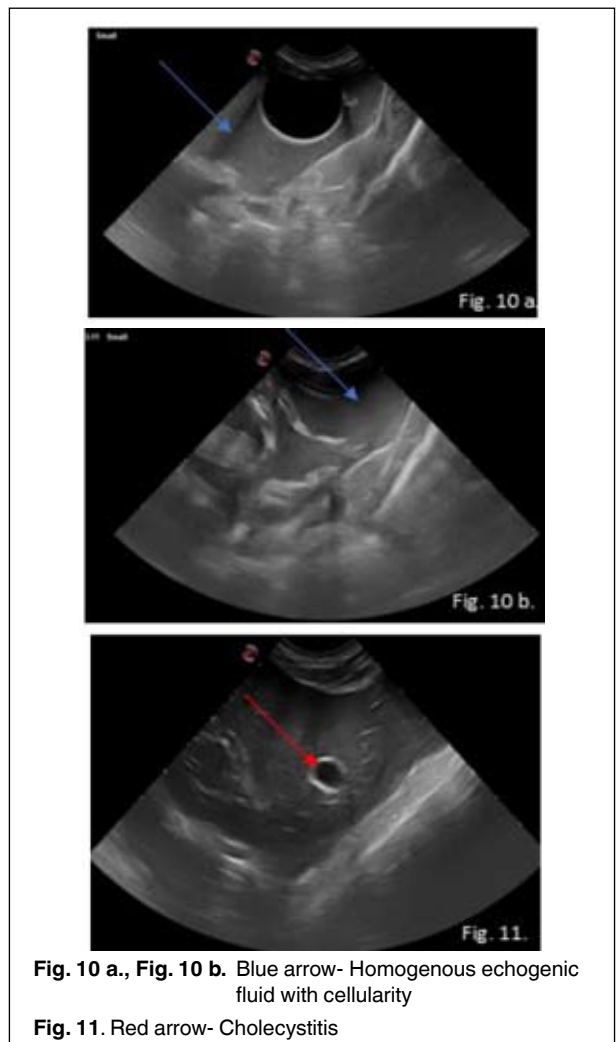


Fig. 10 a., Fig. 10 b. Blue arrow- Homogenous echogenic fluid with cellularity

Fig. 11. Red arrow- Cholecystitis

findings indicative of cholecystitis (Fig.11) and hepatomegaly with hepatic congestion were noticed (Fig. 12)

Bacterial isolation and identification

Stillion and Letendre (2015) emphasized the importance of closely observing the gross characteristics of pleural fluid, noting that septic effusions are commonly turbid or opaque and may have a foul odour. The present study also noticed cream-coloured, opaque, and malodorous effusions in all three septic effusion cases.

Klebsiella spp. was identified by culture method after initial streaking on BHIA (Fig. 3a.) or blood agar plates (Fig. 3b). Colonies on BHIA appeared as small mucoid colonies whereas large mucoid colonies were noticed on blood agar. Gram staining revealed the presence of Gram-negative organisms in three effusions in accordance with findings of Mueller *et al.* (2001) who observed that peritonitis in animals is most frequently linked to infections caused by Gram-negative bacteria. The results of biochemical tests for identification of bacterial isolates noted all three isolates were catalase, VP, and

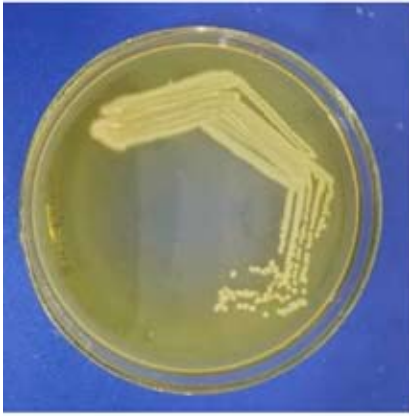


Fig. 3a. Growth of Klebsiella on BHI agar

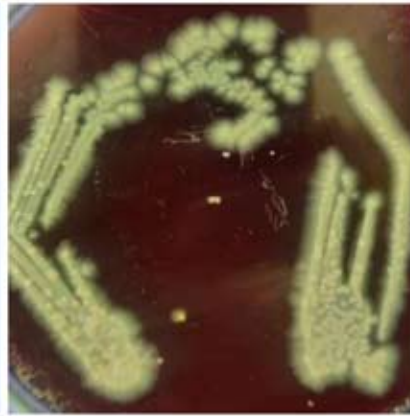


Fig. 3b. Growth of Klebsiella on blood agar



Fig. 4. Mucoid colonies of Klebsiella on Macconkey agar

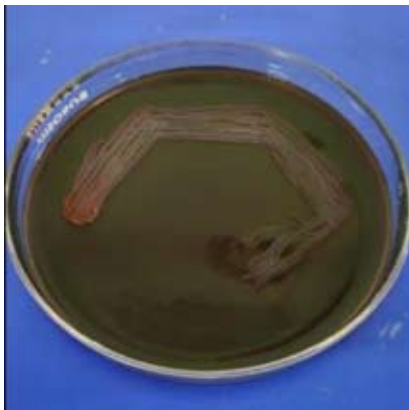


Fig. 5. Mucoid pink colonies of Klebsiella on EMB agar

citrate utilisation tests positive and negative for oxidase, indole and MR tests which indicate isolate to be *Klebsiella* spp. presumptively.

All three Gram-negative isolates were streaked on MacConkey agar producing colonies pinkish mucoid in colour (Fig 4.). Growth on EMB agar also yielded mucoid pink colonies (Fig 5.) which were suggestive as *Klebsiella* organism.

The presence of *Klebsiella* spp. in the pyothorax case of the present study is not in agreement with Odunayo (2016) who noted that feline pyothorax frequently involved mixed isolates of oropharyngeal flora, mainly comprised of *Bacteroides* spp., *Clostridium* spp., *Streptococcus* spp., *Mycoplasma* spp., and *Pasteurella* spp., whereas Gorris *et al.* (2017) observed that around 20 per cent of feline pyothorax cases were attributed to infectious agents other than oropharyngeal flora, including *Rhodococcus equi*, *Nocardia* spp, *Klebsiella* spp, *Proteus* spp, and *Pseudomonas* spp. which agrees with the present study. Scotti *et al.* (2019), reported the isolation of *Klebsiella* spp. in only one cat with secondary peritonitis in their study on prognostic indicators in 83 cats with septic peritonitis.

Genotypic identification of the isolates

Among the three tested isolates, all yielded amplicons 441 bp amplicons of the *gyrA* gene for *Klebsiella*

spp. in PCR (Fig. 6.). The biochemical characterisation results of the isolates were fully consistent with PCR for all three isolates in this study, demonstrating the reliability of biochemical identification methods.

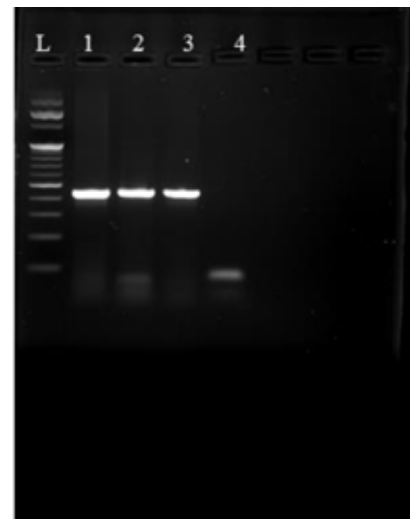


Fig. 6. Agarose gel electrophoresis of *gyrA* specific PCR of *Klebsiella*

Lane L : DNA marker 100 bp
Lane 1 and 2 : Positive samples (441 bp)
Lane 4 : Negative control
Lane 3 : Positive control

Sequencing of one sample which yielded amplicon of 441 bp of *klebsiella* spp. was done. The aligned sequence was submitted to NCBI, GenBank and accession number PQ362592 was acquired. Nucleotide Basic Local Alignment Search Tool was accessed and the isolate showed hundred per cent identity towards *Klebsiella pneumoniae* isolate SB5881 genome assembly form Paris, France (Accession number: LR792628.1). Cats develop *Klebsiella pneumoniae* infection in association with effusion if the bacteria translocate into the pleural cavity, often due to pre-existing conditions or secondary infections. *Klebsiella pneumoniae* can infect the peritoneal cavity via bacterial translocation from the gastrointestinal tract, especially in cases of intestinal compromise or

immunosuppression (Bray and Zafar, 2024). Peritoneal effusion provides a favourable environment for bacterial growth, potentially leading to peritonitis and septicemia.

Antimicrobial susceptibility testing

Results of antimicrobial testing are given in Table 3 and Fig. 7. The most sensitive antibiotics against *Klebsiella* spp. isolated from cats with body cavity effusions in this study were doxycycline and enrofloxacin, whereas amoxicillin-clavulanic acid, commonly used by veterinarians for treating bacterial pneumonia in dogs and cats, was the least effective. These results are consistent with Krupa (2020), who found that *Klebsiella* spp. isolated from mastitis also exhibited sensitivity to tetracyclines and enrofloxacin. This resistance against β -lactams was reported by Zhang *et al.* (2022) who noted *Klebsiella* isolates from cats demonstrating alarmingly high levels of multidrug resistance, with resistance rates of 82.9 percent to amoxicillin-clavulanate and 77.1 percent to trimethoprim-sulfamethoxazole and research from Bogor, Indonesia, also reported 76 percent resistance to β -lactams, further complicating treatment options (Ramadhan *et al.*, 2021).

Haemato-biochemical analysis

Haematological parameters of three septic effusion cases are given in Table 4 and biochemical parameters are given in Table 5. Haematological analysis revealed an elevated total leukocyte count, with a marked increase in both the absolute neutrophil count and neutrophil percentage in all three cases of septic effusion. Additionally, marked thrombocytopenia was observed, while total erythrocyte count, haemoglobin, and haematocrit levels remained relatively unchanged.

The observations noticed in the pyothorax case were in accordance with Barrs *et al.* (2005) who observed

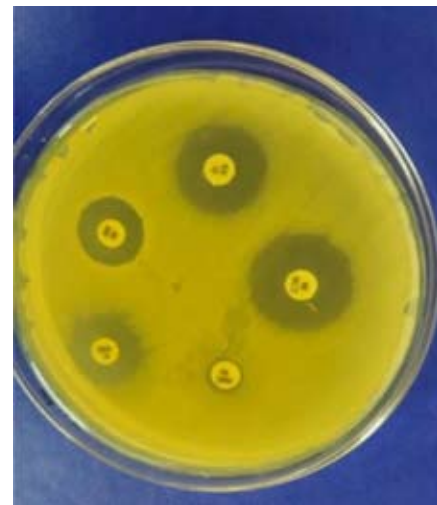


Fig.7. Phenotypic characterisation of antimicrobial resistance: Kirby- Bauer disc diffusion assay (CLSI, 2021)

Amoxicillin-clavulanate (AMC)
Gentamicin (G)
Ceftriaxone (CTR)
Doxycycline (DO)
Enrofloxacin (EX)

that consistent with pyogenic infections, neutrophilic leucocytosis with a left shift was frequently observed in pyothorax cases. One cat with septic peritonitis had a history of being bitten by another cat, while the other had no history of trauma, making it difficult to classify the cases as primary or secondary peritonitis. Haematological findings in both cases, such as neutrophilia and leucocytosis, were consistent with those reported by Culp *et al.* (2009) in secondary peritonitis in cats, although anaemia noted in their study was not observed here. Bentley *et al.* (2007) have reported severe thrombocytopenia in dogs with septic peritonitis, in agreement to this study.

Table 3. Results of antibiogram pattern of isolates from effusion

Antibiotic discs	Isolate 1		Isolate 2		Isolate 3	
	S	R	S	R	S	R
Amoxicillin- clavulanate		yes	yes			yes
Gentamicin	yes			yes	yes	
Ceftriaxone	yes		yes			res
Doxycycline	yes		yes		yes	
Enrofloxacin	yes		yes		yes	

Table 4. Haematological parameters

Sl. No.	TLC (10 ⁹ /L)	Neu# (10 ⁹ /L)	Lym# (10 ⁹ /L)	Eos# (10 ⁹ /L)	Hb (g/dL)	TEC (10 ⁶ / μ L)	HCT (%)	PLT (10 ³ / μ L)
Normal range	5.5-19.5	2.320-12.580	0.730-7.860	0.060-1.930	8.0-15.0	5.00-10.00	24.0-45.0	300-800
1.	25.3	23.14	1.06	0.27	12.4	9.5	40.3	132
2.	29.09	27.129	1.029	0.302	14.9	9.02	45.6	202
3.	24.12	20.601	2.206	0.332	13	10.04	42	139

Table 5. Biochemical parameters

Sl. No.	BUN mg/dL	Creatinine mg/dL	ALT (U/L)	ALP (U/L)	AST (U/L)	TP (g/dL)	Albumin (g/dL)	Globulin (g/dL)
Normal range	0.9-2.2	19-34	25-97	0-45	7.0-38.0	6.0-7.9	2.8-3.9	2.6-5.1
1.	0.86	52.1	35.26	17.4	66.3	8.76	4.52	4.24
2.	1.45	35.2	35.62	59.6	63.4	9.3	3.923	5.377
3.	0.8	51.5	35.8	17.2	66.4	5.6	2.8	2.8

Biochemical analysis revealed a significant increase in AST (3/3), increase in BUN (3/3), increased ALP (1/3) and elevated TP (2/3) with hypoalbuminemia in one case (Table 5). The most common abnormalities detected via serum biochemical analyses in secondary peritonitis included high aspartate transaminase activity, and high BUN concentration (Culp *et al.*, 2009). Elevated liver enzymes were also reported by Stillion and Letendre (2015) in feline pyothorax cases.

Total protein and albumin of effusion

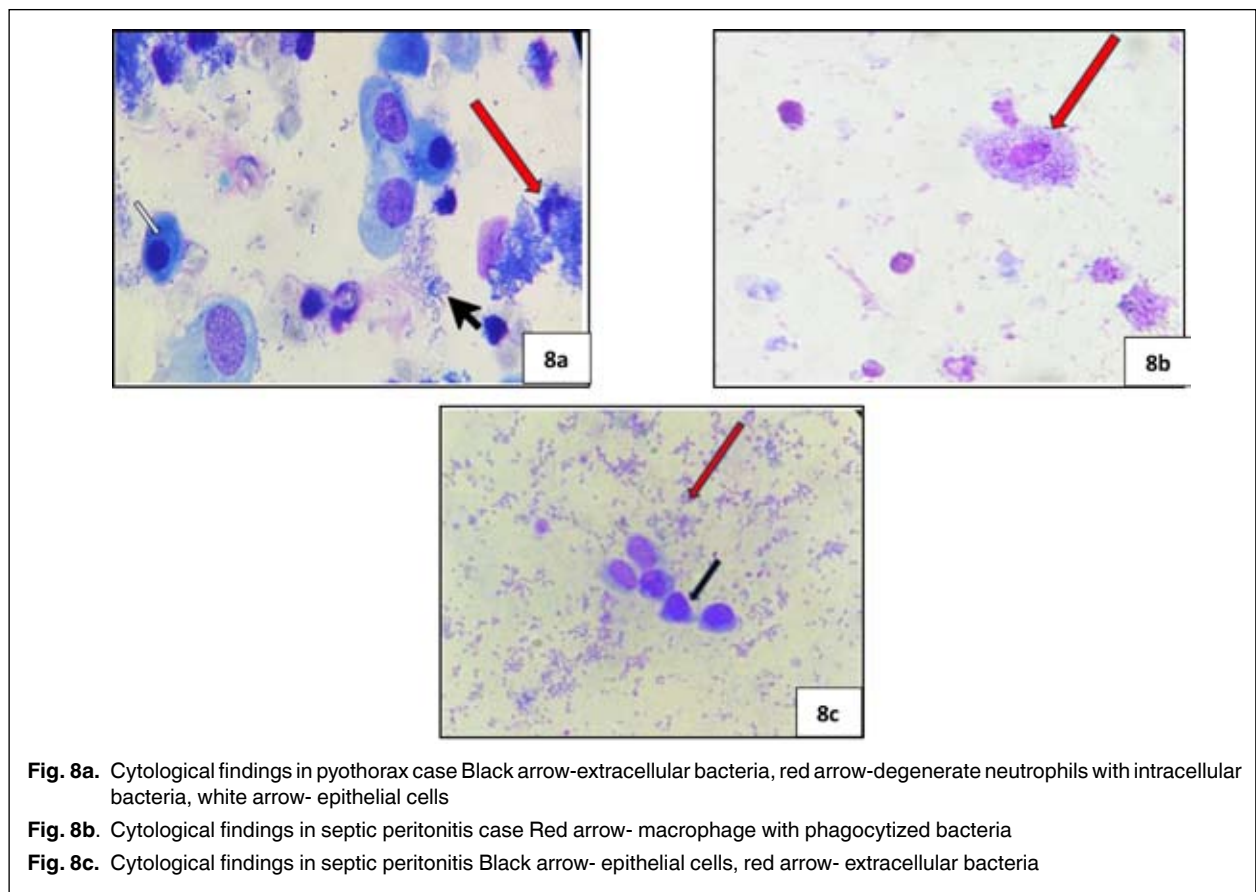
The total protein, albumin and globulin levels of septic effusions are given in Table 6. The total protein levels were found to be high with an increased globulin level in three septic effusions. DiDomenico *et al.* (2024) also noted a significant increase in the total protein of septic effusions compared to non-septic effusions which concurs with present study.

Table 6. Effusion parameters

Sl. No.	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)
1.	7.284	3.042	4.242
2.	8.347	2.837	5.51
3.	22.54	2.676	19.864

Cytological analysis and Total Nucleated Cell Count (TNCC)

Cytological evaluation of the three samples revealed abundant intracellular and extracellular bacteria, with degenerated neutrophils as the predominant cell type (Fig. 8a, 8b, 8c). The total nucleated cell count (TNCC) in all samples exceeded 100,000 cells/ μ L (Cat 1: 182,400; Cat 2: 163,200; Cat 3: 126,800) indicating a robust inflammatory response characteristic of septic effusions. The elevated TNCC, combined with the presence of bacteria and



neutrophil degeneration, strongly supports the diagnosis of severe bacterial infection in these cases. These findings are consistent with the observations of Medardo *et al.* (2024), who reported that septic fluids were marked by a high concentration of neutrophils and a lower proportion of macrophages. They also noted that cell counts frequently surpassed 13×10^9 cells/L, with most neutrophils showing signs of degeneration or toxic changes, some exhibiting bacterial phagocytosis, and occasional free bacteria visible in the background.

One case of pyothorax and septic peritoneal effusion was presented as an emergency, with the animals collapsing before treatment could be initiated, despite prior administration of antibiotic therapy (Inj. Amoxicillin Sulbactam (12.5 mg/kg, BID IV) at the previous veterinary facilities. In contrast, another case of septic peritonitis was treated with Inj. Amoxicillin Sulbactam (12.5 mg/kg, BID, IV), Inj. Pantoprazole (1 mg/kg, OD, IV), and Inj. Frusemide (2 mg/kg, BID, IV). The cat made an uneventful recovery after five days of treatment and treatment was continued orally for next 7 days. Notably, this was the only cat that demonstrated susceptibility to amoxicillin-clavulanate in antimicrobial susceptibility testing. These cases emphasize the critical need for early detection, accurate identification of the infectious agent, and tailored antimicrobial therapy to improve prognosis in septic effusions.

Conclusion

Klebsiella species were the sole organisms isolated from three septic effusions in this study and rest 20 samples showed no bacterial growth. Antibiotic susceptibility testing revealed that doxycycline and enrofloxacin were the most effective agents against these isolates. Notably, two cases demonstrated resistance to amoxicillin-clavulanate, a commonly prescribed antibiotic in veterinary practice. This finding underscores the increasing importance of antimicrobial resistance to frequently used antibiotics, emphasizing the critical need for routine antibiotic sensitivity profiling in cases of septic effusion. Such profiling ensures targeted and effective treatment, preventing the use of ineffective antibiotics and potentially reducing the emergence of resistant bacterial strains.

Conflict of interest

The authors declare that they have no conflict of interest

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