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Clinical , Hematological, Acute phase proteins and Radiographic changes in different respiratory affections in dogs and cats.

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ABSTRACT

Respiratory affections are important clinical problems recorded in dogs and cats affecting their health condition. This study was carried out on a total of 84 animals including 32 dogs and 52 cats of both sexes and different breeds suffering from different respiratory affections including pneumonia, aspiration pneumonia, Feline upper respiratory diseases(FURD) and Canine infectious respiratory disease(CIRD). Clinical, hematological, biochemical and radiographic changes during these affections were evaluated. In addition the most common incriminated bacteria was isolated and identified. The affected cases showed variable respiratory signs including dyspnea, nasal and ocular discharge, sneezing, cough, abnormal respiratory sound and abnormal lung sound. Hematological changes showed inflammatory leukogram represented by increasing in WBCs and neutrophil count Serum analysis showed marked increase in CRP, SAA and HP levels, with hyperproteinemia and hypoalbuminemia compared to reference value. The most common bacteria isolated from pneumonic cases were Klebsiella, E.coli, Staph., Pseudomonas, Pasteurella, Proteus and Serratia. Radiographic examinations revealed abnormal radiographic patterns associated with the different affections. The present study concluded that clinical, hematological, biochemical combined with chest radiographic findings are essential for precise diagnosis of different respiratory affections in dogs and cats and the early diagnosis facilitate the prescription of relevant therapy and follow up procedures.

1. INTRODUCTION

Respiratory diseases in dogs and cats currently receive a great deal of attention, particularly the diagnosis and treatment. Pneumonia is one of the most common systemic diseases in dogs and cats, may be caused by primary bacterial infection or secondary to other affections such as viral infection, aspiration, or inhalation of any foreign materials, also may be resulted from immunodeficiency syndrome (Kogan et al., 2008).

Canine infectious respiratory disease (CIRD)or kennel cough is a syndrome occurs in dogs especially young puppies, manifested clinically by acute onset of cough that may be accompanied by other signs such as sneezing, nasal discharge, conjunctivitis, and ocular discharge according to causative agent. Bacterial infection may be implicated as a primary pathogen or secondary to viral infection (Mochizuki et al., 2008). FURD is a syndrome occurs in cats, manifested clinically by sneezing, nasal discharge, fever, salivation, conjunctivitis, ocular discharge, oral ulceration, and epistaxis according to the cause of infection. One or more pathogens including viral pathogen such as herps virus, calici virus and canine distemper virus or bacterial pathogen such as Bordetella bronchiseptica, Chlamydia spp. and mycoplasma spp. or mixed infection are occurred (Quimby and Lappin., 2009).

The acute phase proteins (APPs) are blood proteins that can be used to assess the innate immune system's systemic response to infection and inflammation. The major APPs that respond to inflammatory stimuli are CRP and SAA in dogs, SAA in cats while the moderate APPs are HP and α1 acid glycoprotein (AGP) in dogs and cats (Eckersall and Bell., 2010).

This study was aimed to assess the clinical features, hematological, acute phase proteins, and radiographic changes in case of different respiratory affections in dogs and cats, in addition to isolation and identification of the most common bacterial agents that may be included in case of pneumonia.

2. MATERIAL AND METHODS

Ethical approval:

The study was done after the approval of the Ethics committee of Benha University with the approval number (BUFVM15-03-22). All samples were collected after owner consents.

2.1. Animals:

This study was applied on total of 84 animals including 32 dogs and 52 cats belonged to pet animal veterinary clinic of faculty of veterinary medicine, Benha University and private pet animal clinics located in Cairo governorate, Egypt during

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the period from December2020 to February2022. These cases suffered from different respiratory affections. The diagnostic evaluation included history, physical examination (84/84), hematology (25/84), serum biochemistry (25/84), thoracic radiographs (38/84) bacterial isolation and identification (24/84). Dogs and cats suspected to be suffering from respiratory disease were visually examined for the presence of nasal or ocular discharge, dyspnea, cough, depression and inappetence. Using the clinical respiratory scores (Maboni et al., 2019) summarized in Table (1). The affected animals were classified into score1 or score2.

Table 1 Clinical respiratory scores in dogs and cats.

Clinical score	Clinical signs
0 (asymptomatic)	No history of respiratory disease
1 (mild)	Cough or sneeze or nasal discharge
2 (moderate/severe)	Cough or sneeze or nasal discharge, in addition to one of the following signs: Fever or lethargy/depression or inappetence or pneumonia

2.2. Samples:
Two sets of blood samples were collected from cephalic vein. The first blood sample was collected on a labeled test tube with anticoagulant (potassium salt of EDTA) for determination of hematological parameters. The second blood sample was collected without anticoagulant, clotted at room temperature for 20 min, centrifuged at 3000 rpm for 10 min, and then the clear non-hemolyzed serum samples were separated and stored at −20°C until subsequent biochemical analysis.

2.3. Hematological examination:
Hematological parameters were determined by hematological analyzer (Model No.93-91098-00-GF) as previously described by Feldman et al. (2000). Results were compared with normal reference values according to Latimer (2011).

2.4. Bacteriological examination:
Respiratory samples were collected from cases suffered from pneumonia (12 dogs and 12 cats) for bacterial culture. Sampling methods included were tracheal wash (2/24), sputum samples (8/24) and nasal swabs from nasal discharge (14/24). The respiratory samples swabs were inoculated separately in nutrient broth for activation of the microorganism at 37 °C for 24 hours. A sterile lop full of the broth with activated microorganism was directly sub-cultured on blood agar, paired barker media, Eosin methylene blue media and MacConkey's agar. Plate readings occurred at 24 hours. and 48 hours. The isolates recovered were sub-cultured and further identified using colony morphology, Gram stain and biochemical tests (Alton et al., 1996). The identification of suspected bacteria colonies was achieved by observation of colonial morphology under microscopy and the use of some biochemical tests: hemolysis, motility, indole formation, glucose, lactose, methyl red, Voges Proskauer and citrate utilization. Assay for biochemical properties of the bacteria isolates was conducted according to McFadden (2000).

2.5. Protein profile analysis:
Serum total proteins were determined spectrophoto-metrically according to the method described by Pagana and Pagana (2017). Serum albumin was determined calorimetrically by using the dye-binding technique with bromocresol green according to the method described by Fischbach and Dunning (2009). Results were compared with normal reference values according to Latimer (2011). Serum CRP was analyzed using ELISA Kit (Eucardio laboratory, Inc., Encinitas, and CA., USA), previously validated for use in dogs according to Ibraimi et al. (2013). SAA were measured with a commercially available ELISA kit according to method described by Alsemgeest et al. (1995). Serum Hp concentrations were determined by ELISA kit according to method described by Idoate et al. (2015). Results were compared with normal reference values according to Ceron (2005).

2.6. Radiographic examination:
Lateral and ventro-dorsal views of the chest are standard, both right and left lateral views are required to gain maximum information, radiographic evaluation of the lung fields is enhanced by exposing the radiograph at maximum inspiration if possible. According to Kealy et al. (2010).

3. RESULTS

3.1. Clinical findings:
Pneumonic cases were admitted with a history of vaccination, anorexia, dullness, cough, nasal discharge, and some cases with previous illness. Cases of aspiration pneumonia were admitted with a history of accidental fluid inspiration may be water or drugs, vomiting, dyspnea, nasal discharge, coughing with digestive disorders and death within few days in some cases. Cases of FURD were admitted with a history of housing with other infected unvaccinated cats with signs of anorexia, inappetence, salivation, nasal and conjunctival discharge, sneezing and epistaxis, these affected cases were not vaccinated. All cases of CIRD were admitted with a history of housing with other infected dogs, hacking paroxysmal cough, serous nasal discharge, dullness, inappetence and ocular discharge. Clinical findings are represented in Table (2).

3.2. Hematological findings:
The results of CBC analysis are represented in Table (3). The mean value of RBCs count, Hb content and PCV% were within normal reference value in cases affected with aspiration pneumonia, CIRD and FURI but lower than reference value in case of dogs and cats affected with pneumonia. The mean value of WBCs counts, and neutrophils were higher than normal reference value in case of pneumonia, aspiration pneumonia, CIRD and FURI. The mean value of lymphocyte was lower than normal reference value in case of pneumonia, FURI and CIRD but within normal level in case of aspiration pneumonia.

3.3. Bacterial isolation and identification:
The result of bacteriological isolation revealed that the isolated bacterial pathogens in pneumonic cases were Klebsiella spp., E.coli spp., Staph. Spp., Pseudomonas spp., Pasteurella spp., Proteus and Serratia spp..

3.4. Protein profile analysis findings:
The mean value of SAA and HP were higher than normal reference value in case of pneumonia, aspiration pneumonia and FURD but within the normal level in case of CIRD. The mean value of CRP was higher than normal reference value in all cases, especially in pneumonic cases was 6 times more than reference value. Pneumonic cases showed increase in total protein and globulin, decrease in globulin and A/G ratio. Cases of aspiration pneumonia showed increase in total protein and decrease in albumin, but globulin and A/G ratio were within the normal level. Cases of FURD and CIRD showed a slight increase in total protein and globulin while showing decrease in albumin and A/G ratio compared to normal reference value. All data are represented in Table (4).

Table 2 Result of clinical examination of different respiratory affections in dogs and cats.

Disease	Affected animals		Clinical parameters			Clinical score	
	Dogs	cats	Temp.	Pulse rate	Respiratory rate	Score 1	Score 2
Pneumonia (28.6 %)	(12dogs) 3German shepherd 3Golden Retriever 4 Rottweiler 2 Yorkshire Age: 9 m-4 y	(12 cats) 4 Baladi 5 Persian 3 Siami Age :1.5-3 y.	39.3± .67 in dogs 40.2±.5 in cats	129±20in dogs 135±10 in cats	57±16 in dogs 62±20 in cats	_ Other findings: congested or pale or cyanosed mucous membrane, lung crackles and wheezes, tachycardia, and abdominal respiration.	12 dogs 12 cats
Aspiration pneumonia (9.5 %)	(3 dogs) 1 Mixed breed 1 Griffon 1 Golden Age: 9 m -3 y.	(5 cats) 3 Persian 2 Himalayan Age: 1-3 y.	39.1 ± .5 in dogs 39.9± .4 in cats	120 ±5 in dogs 130±7 in cats	48±5 in dogs 54±7 in cats	_ Other findings: dyspnea, abnormal lung sound (moist rales or crackles), megaesophagus in some cases, abdominal respiration, and mouth breathing	3 dogs 5 cats
CIRD (20.2 %)	(17 dogs) 6 German shepherd 4 Golden Retriever 1 Labrador Retriever 3 Rottweiler 1 Begal 1 Griffon 1 Cocker spaniel Age :10m – 2y.		39.2 ±.14	100±7	40 ±5	12 dogs Other findings: harsh sound of trachea, recovery occur within 3-7 days.	5 dogs
FURD (41.6 %)	(35 cats) 11 Baladi cats 5 Siami 19 Persian Age :2m -1.5 y.		40.3±.4	130±10	58±10	10 cats Other findings: ulceration in oral cavity, petechial hemorrhage in gum and hard palate, corneal ulcer, abnormal respiration, severe conjunctivitis, and lacrimation.	25 cats

Table 3 Hematological changes in different respiratory affections in dogs and cats.

Hematological parameters	Pneumonia (n=7 dogs)	Aspiration Pneumonia (n=3 dogs)	FURD (n=6 cats)	CIRD (n=9 dogs)	Reference value	
					dogs	cats
RBCs (10^6/µl)	4.30±0.92	7.40±0.25	7.07±1.21	5.90±1.40	5.7-8.5	6.9-10.1
Hb (g/dl)	9.80±0.71	14.6±0.51	12.50±1.25	13.60±1.13	14.1-20.1	10.9-15.7
PCV%	32.6±3.1	47.8±3.18	40.90±8.34	36.20±8.35	41-58	31-48
WBCs (10^3/mm^3)	26.10±3.30	22.8±2.63	20.20±5.23	19.40±5.42	5.7-14.2	5.1-16.2
Neutrophil %	87.8±4.62	85.6±2.92	81.20±9.26	83.20±7.35	43-80	25-77
Lymphocyte %	5.90 ±1.94	27.30±1.43	8.10±1.72	8.30±1.21	14-45	14-61
Eosinophil %	0.47±0.30	0.52±1.21	0.60 ±0.42	0.40±0.34	1-18	2-23
Monocyte%	12.400±2.91	10.6±1.90	5.90± 2.60	8.40±0.61	2-9	1-5

Table 4 Changes in acute phase proteins and serum proteins concentrations in the different affected cases.

	Pneumonia (n=7dogs)	Aspiration pneumonia (n-3dogs)	FURD (n=6cats)	CIRD (n=9dogs)	Reference value	
					dogs	cats
CRP (mg/l)	30.00 ±7.62	17.93±2.82	7.35±1.07	12.29±0.34	< 5	0-.03
HP (mg/dl)	76.24 ±17.38	83.90±9.64	54.86±10.30	11.67±0.33	0 – 30	3.84 – 40
SAA (mg/l)	3.74±1.34	4.31±0.72	4.78 ±1.25	1.97±0.09	0 - 2.19	-
Total protein (g/dl)	9.52±.92	8.76±0.26	8.46±0.94	7.55±0.47	5.5 – 7.2	6.6 - 8.4
Albumin (g/dl)	2.01±0.49	2.42±0.09	2.31±0.19	3.00±0.02	3.2 - 4.1	3.2 – 4.3
Globulin (g/dl)	6.13±0.59	3.32±0.19	4.92±0.95	4.29±0.46	1.9 – 3.7	2.9 – 4.7
A/G ratio	0.52 ±0.06	0.72±0.10	0.73±0.19	0.72±0.08	.9 – 1.9	.8 – 1.5

3.5. Radiographic findings:

The radiographic views of cases suffering from pneumonia and aspiration pneumonia revealed a predominantly interstitial pattern and alveolar infiltrate Fig. (1), some cases showing lung consolidation. Some cases of aspiration pneumonia were caused by megaesophagus as demonstrated by x-ray examination Fig. (2). The radiographic views of some cases suffering from CIRD revealed pneumonia represented by bronchial pattern mainly, but some cases showed mixed pattern Fig. (3).

4. DISCUSSION

The dogs and cats affected by pneumonia showed signs of cough, nasal discharge, anorexia, lethargy, fever in some cases but other cases were with a normal temperature, also showed tachypnea, tachycardia, dyspnea, abdominal respiration, and abnormal lung sound, so all cases of pneumonia took score 2 according to respiratory scoring system, the same result was previously recorded by Radhakrishnan et al., (2007); Dear (2014). Cases of aspiration pneumonia showed normal body temperature, history of vomiting or digestive troubles, dyspnea, nasal discharge, and abnormal lung sound such as harsh sound or crackles or wheezes, most cases die within few days due to complication by secondary bacterial infection the same result was previously recorded by Tart et al. (2010). All cases of aspiration pneumonia took score 2 due to presence of nasal discharge in addition to pneumonia. Ten cats suffered from FURD showed signs of sneezing or cough or nasal discharge only so, these cases took score 1.

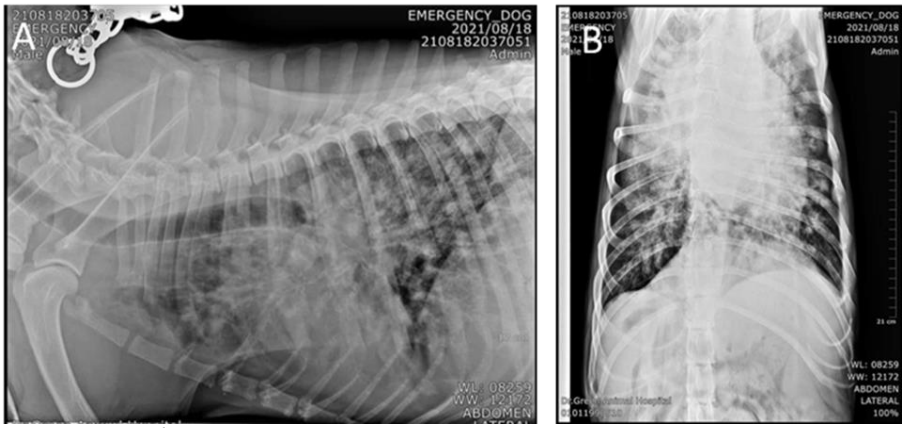

Figure 1 Lateral (A) and ventro-dorsal view (B) of chest radiography in Golden retriever dog showing interstitial pattern to patchy alveolar pattern with mild degree of pleural effusion in a dog lung suffering from pneumonia.

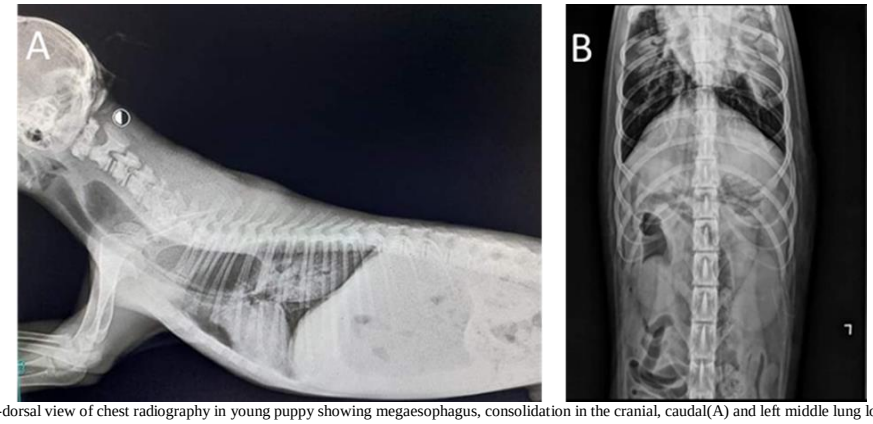

Figure 2 lateral and ventro-dorsal view of chest radiography in young puppy showing megaesophagus, consolidation in the cranial, caudal(A) and left middle lung lobes(B) in a dog suffering from aspiration pneumonia.

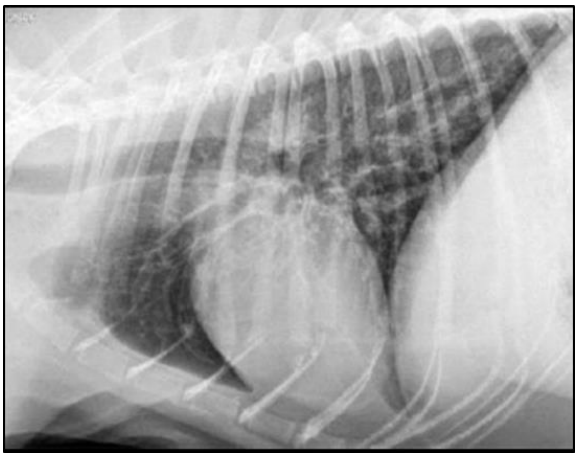

Figure 3 lateral view of chest radiography showing bronchial and alveolar pattern in the cranial and caudal lung lobes in Rottweiler dog suffering from CIRD.

Other 25 cats showed signs of fever, anorexia, sneezing, nasal discharge, ocular discharge, salivation, oral ulceration and epistaxis in some cases, these cases took score 2, the same results were previously recorded by Lappin et al. (2017). Dogs affected by CIRD(n=12) showed signs of acute onset of cough, sneezing, serous ocular and nasal discharge, normal body temperature and normal lung sound, these cases took score 1, recovery occur within 3 to 7 days. Some cases(n=5) showed signs of fever, mucopurulent nasal discharge and abnormal laryngeal and tracheal sound, these cases took score2, the same result was previously recorded by Reagan and Sykes (2020). Occurrence of dyspnea in some cases may be attributed to hypoxia that resulted from sever inflammation in the bronchioles and alveoli, which interfere with gas exchange and respiration. Nasal discharge was observed may be due to inflammatory changes in the nasal mucous membrane. Heart rate and respiratory rate were increased in some cases to compensate hypoxia. Abnormal lung sounds such as crackles or wheezes may be due to the accumulation of exudate produced by inflammatory cells and goblet cells because of pneumonia (Ramadan, 2019). Most of CIRD and FURD cases if not treated rapidly it may be complicated by secondary bacterial infection result in occurrence of pneumonia.

The mean value of RBCs count, Hb content and PCV% were within normal reference value in cases affected with aspiration pneumonia, CIRD and FURI but lower than reference value in case of dogs and cats affected with pneumonia. That was attributed to, respiratory affections especially pneumonia (Ismael et al., 2017). The mean values of WBCs count, and neutrophils count were higher than

normal reference value in all affections. The mean value of lymphocyte was lower than normal reference value in case of pneumonia, FURI and CIRD but within normal level in case of aspiration pneumonia. The same results were previously described by Kogan et al. (2008) and Reagan and Sykes (2020). The increase of WBCs, mainly neutrophils and monocytes are a frequent finding in many diseases because of acute inflammatory response Kogan et al. (2008). The bacteriological examination of the cultured swab collected from the pneumonic cases revealed that, the bacterial pathogens isolated in case of pneumonia were Klebsiella, E. coli, Pasteurella, Pseudomonas, Staphylococcus spp., Proteus and Serratia. The same result was previously recorded by Rheinwald et al.,(2015)and Viitanen.,(2017)..

The major positive APPs, such as CRP and SAA in dogs, have low physiological levels, but rise rapidly within hours after inflammatory stimulus and normalize quickly when inflammatory stimulus ceases (Eckersall and Bell, 2010). Due to these properties, the major positive APPs have received the most attention as inflammatory biomarkers than the intermediate positive APPs, such as Hp. (Ceron et al., 2005). The mean value of SAA and HP were higher than normal reference value in case of pneumonia and FURD but within the normal level in case of CIRD. The mean value of CRP was higher than normal reference value in all cases especially pneumonic cases. The same results were previously described by Cerón et al. (2005); Eckersall and Bell (2010); Viitanen., (2017). SAA is the major APPs in cats, significantly increase in the early stage of pneumonia and upper respiratory infection compared to other APPs. SAA has been clinically applied as a biomarker for occurring the disease in cats as it is the most rapidly responsive AAP in case of inflammatory and infectious conditions (Yuki et al., 2020). The pneumonic cases showed higher level of CRP, while total cases of FURD and CIRD showed slight and moderate increase in CRP level respectively. HP is the moderate APP in dogs and cats was moderately increased in case of infection and inflammation (Eckersall and Bell 2010) Pneumonic cases showed increase in total protein and globulin, decrease in albumin and A/G ratio compared to normal reference value. The same results were previously recorded by Cerón et al. (2005) and Kogan et al. (2008). Cases of FURD and CIRD showed a slight increase in total protein and globulin while showing decrease in albumin and A/G ratio. The same results were previously described by Trumel et al. (2019) and Hong et al. (2021). The changes in protein profile during acute phase response were caused by increasing in synthesis of positive acute phase proteins, complement proteins, and immunoglobulins, so hyperproteinemia is usually associated with infection and inflammation. Albumin is a negative acute phase protein that decreased during inflammation because about 30–40% of hepatic protein anabolic capacity is utilized for the creation of positive acute phase proteins during the acute phase response, resulting in a reduction in other proteins (Ramadan et al., 2019). Hypoalbuminemia could be suggestive of lung inflammation and vasculitis that resulted in leakage of albumin into the alveolar space (Kogan et al., 2008).

The radiographic views of cases suffering from pneumonia and aspiration pneumonia revealed a predominantly interstitial pattern and alveolar infiltrate, some cases showing lung consolidation, the same results were previously recorded by Dear (2014) and Levy et al. (2019). The radiographic views of dogs' chest suffering from kennel cough revealed a predominantly bronchial pattern, but some cases showed mixed pattern, the same result was previously recorded by Vindenes et al. (2015). Lung patterns are simply the radiographic appearance of disease in the lung. Common patterns include bronchial pattern, interstitial pattern, and alveolar pattern. Bronchial pattern represented by diffuse thickening of the air way lines and rings throughout the pulmonary tissue was observed in case of bronchitis and kennel cough. An unstructured interstitial pattern represented by increasing the soft tissue opacity that partially obscure the blood vessels margin was observed in case of pulmonary edema and pneumonia. A structured interstitial pattern represented by ovoid or rounded shaped nodules of soft tissue distributed all over the lung tissue was observed in case of fungal pneumonia. Alveolar pattern is more severe than interstitial pattern, it represented by an area of increased soft tissue opacity in the lung tissues that completely obscure pulmonary blood vessels, it was observed in the same cases as interstitial pattern (Thrall and Robertson, 2015).

5. CONCLUSION

The present study concluded that clinical, hematological, biochemical combined with chest radiographic findings are essential for precise diagnosis of different respiratory affections in dogs and cats and the early diagnosis facilitate the prescription of relevant therapy and follow up procedures.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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