

## EPIDEMIOLOGICAL, CLINICO-DIAGNOSTIC AND THERAPEUTIC ASPECTS OF BOVINE RESPIRATORY DISEASE IN INDIAN BUFFALOES

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### ABSTRACT

Bovine Respiratory Disease (BRD) is major health problem occurring worldwide in bovines, responsible for high morbidity and mortality in calves and heavy economic losses in terms of production losses in milch animals and cost of veterinary treatment. Hence the present study was planned to investigate epidemiological, clinico-diagnostic and therapeutic aspects of BRD in Indian buffaloes. A total of 805 buffaloes reported to TVCC, COVAS, Udgir during the study period were screened for Bovine respiratory disease. On the basis of history of varying degree of nasal discharge, coughing, dyspnoea and abnormal lung sounds, 37 buffaloes were found positive for BRD, indicating 4.60% overall prevalence. The haemoanalysis showed significant ( $P < 0.01$ ) leukocytosis associated with neutrophilia. The radiographic examination of thorax revealed variable degree of congestion and diffuse consolidation of lungs. The bacteria isolated from nasal swabs were *Staphylococcus* sp., *Streptococcus* sp., *E. coli*, *Corynebacterium* sp., *Klebsiella* sp., *Manheimia haemolytica*,

*Brevundimonas* sp. and *Pseudomonas* sp. Antibiogram of pure culture organisms isolated from BRD affected buffaloes revealed highest sensitivity for gentamicin (83.33%), followed by enrofloxacin (58.33%), ceftiofur sodium (50.00%), moxifloxacin (33.33%). Thirty-one BRD affected buffaloes were randomly distributed in four groups and treated with gentamicin 4 mg/kg, enrofloxacin 5 mg/kg, moxifloxacin 5 mg/kg and ceftiofur 1.6 mg/kg respectively. All treated buffaloes from different groups clinically cured within 5 to 15 days. Based on results of current therapeutic trial, gentamicin proved most effective and economic in treatment of BRD in buffaloes.

**Keywords:** *Bubalus bubalis*, buffaloes, BRD, radiology, antibiogram, cultural isolation, gentamicin

### INTRODUCTION

Diseases affecting bovine respiratory system have been grouped under category known as Bovine Respiratory Disease (BRD). BRD is

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multifactorial in nature and is caused by interaction of infectious agents, environmental factors and managemental practices. The infectious agents are bacteria or viruses, or viruses complicated with bacteria (Allan *et al.*, 1991). The bacteria those play important role in Bovine respiratory disease include *Mannheimia haemolytica*, *Pasteurella multocida* and *Histophilus somni* (Amir *et al.*, 2013). The viral agents commonly associated with respiratory tract diseases in bovines are Bovine herpes virus type-1 (IBR), Bovine respiratory syncytial virus (BRSV), Bovine viral diarrhoea virus (BVDV), Bovine parainfluenza virus type 3 (PI-3) and Bovine respiratory coronavirus. BRD is caused by a combination of infectious agents and predisposing factors such as poor nutrition, early weaning, dehydration, high or low temperature, poor ventilation, little rest, and transportation (Jared *et al.*, 2010). BRD is major health problem occurring worldwide in cattle, responsible for high morbidity and mortality in calves and heavy economic losses in terms of production losses in milch animals and cost of veterinary treatment (Van der *et al.*, 2001). Abundant literature is available on bovine respiratory disease in cattle especially calves (Van der *et al.*, 2000; Singh and Singh, 2009; Amir *et al.*, 2013), but literature on this topic in buffaloes is very scarce. Therefore, the present study was planned to study epidemiological, clinico-diagnostic and therapeutic aspects of BRD in buffaloes.

## MATERIALS AND METHODS

### Epidemiology

The present study was carried on 37 clinical cases of Bovine Respiratory Disease (BRD) affected buffaloes admitted to the

Veterinary Clinical Complex, COVAS, Udgir, Dist. Latur, from Marathwada region. The information pertaining to age, sex, physiological status, milk yield, month and season of illness was collected for computation of prevalence.

### History and clinical examination

The history pertaining to appetite, water intake, rumination, defecation, faecal consistency, and urination and clinical findings in respect of body temperature, heart rate, respiration, ruminal motility, color of mucus membrane, nasal discharge, coughing, chest pain, body condition, severity of weakness, posture and lung auscultation were recorded in all BRD affected buffaloes.

### Hematology

For hematological studies, 5 ml of blood was collected in sterile EDTA vials from jugular vein of six healthy and 24 BRD affected buffaloes on 0 and 5<sup>th</sup> day of trial and processed for complete blood cell count on fully automated hematology cell counter (Model: Abacus Junior Vet, Diatron GMBH, Austria).

### Radiographic examination

Buffaloes which were suspected for BRD or chronically ill and not responding to antibiotic treatment were subjected to radiographic examination of reticulo-thorax by using 1000 mA, 150 kvp computed radiographic system (Konica Minota Regius 210 Dry Pro 873).

### Culture, isolation and identification of bacteria from nasal swabs

Nasal swabs collected from BRD affected buffaloes were subjected to bacteriological examination. The isolation and identification of the bacteria was done by using MALDI-TOF mass

spectrometry (Matrix Assisted Laser Desorption Ionization Time of Flight).

### ***In-vitro* Antibiotic Sensitivity Test (AST)**

Antibiotic sensitivity test (AST) was conducted against the bacteria isolated in pure culture using standard antibiotic discs as per the disc diffusion method described by Cruickshank *et al.* (1975).

### **Treatment**

Experimental design for evaluation of efficacy of Gentamicin, Enrofloxacin, Moxifloxacin and Ceftiofur sodium therapy in BRD affected buffaloes is as given in Table 1.

The supportive treatment in all four groups comprised of meloxicam 0.5 mg/kg IM, chlorpheniramine maleate 0.25 mg/kg IM daily and multivitamin 1 ml/ 20 kg IM once daily for 5 to 7 days. In severe cases of respiratory distress deriphylline (Etophylline 84.7 mg + Theophylline 25.3 mg/ml) 3 to 4 mg/ kg bw, IV, BID and dexamethasone 0.04 to 0.1 mg/ kg bw, IM/ IV daily or in case of pregnant animals isoflupredone acetate 0.02 mg/kg, IM once daily for 3 days was given. In anorectic animals, Ringers lactate solution was given 1 to 2 lit by slow IV route.

Efficacy of each drug was evaluated on the basis of survival rate, recovery period, improvement in clinical signs, haematological values, radiological changes and results of antibiotic sensitivity test.

### **Statistical analysis**

Statistical analysis was carried out as per the methods described by Snedecor and Cochran (1994). The Chi-square ( $X^2$ ) value was calculated to determine the significance of an association between diseases and hypothesized causal factor.

## **RESULTS AND DISCUSSIONS**

### **Epidemiology**

Out of 805 clinical cases of buffaloes screened, 37 buffaloes were clinically and radiologically positive for BRD indicating an overall prevalence of 4.60%. Data on this aspect for buffaloes is not traceable for comparison. However, high prevalence rate of bovine respiratory disease ranging from 15 to 45% in feedlot cattle has earlier been reported by Kelly and Janzen (1986). Breed-wise higher prevalence of BRD in Murrah buffaloes (9.84%) as compared to local breeds of Maharashtra *viz.* Marathwadi (4.36%) and Pandharpuri (3.63%) breeds observed may be attributed to stress of high milk yield and poor adaptability of this breed to local climate. Age-wise prevalence of clinical cases of BRD in buffaloes showed higher prevalence in young (1 to 4 years) buffaloes (4.05%) than adults (4.79%). Few workers have reported occurrence of pneumonia or BRD in adult cattle and buffaloes (Robin, 1974; Makhdoomi *et al.*, 2013). The disease occurred throughout the year, but highest prevalence was recorded in monsoon season (5.74%) followed by summer (4.11%) and winter (4.09%). Earlier some workers have reported higher incidence of BRD in cattle in the fall (Lonergan *et al.*, 2001), and winter season (Makhdoomi *et al.*, 2013). The higher prevalence of BRD observed in monsoon in the present study might be attributed to exposure of animals to frequent changes in weather, exposure to rain, poor hygiene, and calving stress during this period.

### **Clinical signs**

The duration of illness in BRD affected buffaloes varied from 3 to 30 days. Non-significant changes in the rectal temperature, highly significant

increase ( $P<0.01$ ) in pulse rate, significant increase ( $P<0.05$ ) in respiration rate and highly significant decrease ( $P<0.01$ ) in the ruminal motility was seen in affected buffaloes in comparison to healthy animals (Table 2). In affected buffaloes the rectal temperature was found to be elevated in 37.84% cases, normal in 59.46% cases and subnormal in 2.70% cases. Mild to severe tachycardia ( $>60$ /minute) was noticed in 54.05% cases. The rapid respiration with mild dyspnoea was observed in 64.86% buffaloes whereas moderate to severe dyspnoea was noticed in 16.22% affected buffaloes.

The appetite, water intake and rumination were invariably reduced to absent in all affected buffaloes. In general mucus membranes were slight to moderately congested, whereas in some cases they were severely congested or cyanotic. The degree of weakness, dullness and depression increased as the disease progressed. The body condition was thin to emaciated and the skin and hair coat was dry, rough, and lusterless in sub-acute to chronic cases of bovine respiratory disease. Faeces were scanty and usually firm in consistency. Milk yield was drastically reduced in affected lactating buffaloes.

The nasal discharge was serous in early cases where as muco-purulent to purulent in later stage of disease. Coughing was harsh and dry in the initial stages and later on it became low and moist. But it is surprising to note absence of coughing in 56.76% cases of buffaloes. The signs suggestive of chest pain such as stiff gait, abducted elbows, difficulty while sitting, prolonged standing and arched back were observed in almost 50% cases. Auscultation of the lungs revealed variety of abnormal sounds. In the early stage of BVD the high-pitched breath sounds were noticed especially over antero-ventral aspects of the lungs. As the disease progressed moist rales were prominent. In

advanced cases of lung consolidation, the breath sounds were absent and hearts sounds were audible in lung area.

The presence of coughing, nasal discharge, difficult respiration and adventitious lung sounds were the result of inflammation of the respiratory tract along with secretion and exudates in air ways (Radostits *et al.*, 2010). More or less similar symptoms were earlier reported in cattle and buffaloes suffering from pneumonia by Sen and Albay (2003); Isabelle *et al.* (2008); Singh and Singh (2009); Amir *et al.* (2013).

### Hematology

TEC and PCV values were significantly ( $P<0.05$ ) lower whereas MCV was significantly ( $P<0.01$ ) higher in BRD affected buffaloes as compared to healthy animals (Table 2), indicating macrocytic normochromic anaemia which may be attributed to malnutrition owing to prolonged anorexia in chronic cases. In present study highly significant ( $P<0.01$ ) increase in TLC count, significant ( $P<0.05$ ) increase in neutrophil count with significant decrease ( $P<0.05$ ) in lymphocyte count was noticed in affected buffaloes, indicative of leukocytosis associated with neutrophilia. In affected buffaloes, TLC varied between  $3.56$  to  $45.26 \times 10^3/\mu\text{l}$ . On detail analysis of TLC data, leucopenia ( $<5 \times 10^3/\mu\text{l}$ ) was observed in 9.09% cases, normal leucocyte count ( $5-10 \times 10^3/\mu\text{l}$ ) in 30.30% cases, mild leukocytosis ( $10-15 \times 10^3/\mu\text{l}$ ) in 48.48% cases, moderate leukocytosis ( $15-20 \times 10^3/\mu\text{l}$ ) in 6.06% cases and severe leukocytosis ( $>20 \times 10^3/\mu\text{l}$ ) in 6.06% cases. The present findings of leukocytosis associated with neutrophilia are in conformity with the observations of Isabelle *et al.* (2008); Tripathi *et al.* (2012). Leukocytosis might have occurred due to normal reaction of body defense mechanism against bacterial infections

and anoxic stress.

### Radiological examination

Radiographic examination of thorax revealed variable degree of congestion in lungs, lack of minimum alveolar air-filled dark appearance of the lung fields, focal, regional, multifocal or diffuse white patches suggestive of consolidation of lungs (Figure 1), light to bright gray calcification or fibrosed nodules, clear effusion, loss of integrity among the structures, no distinction among the organs, enlargement of mediastinal lymph nodes, adhesions and over distension of diaphragm. Similar to present study other workers have used thoracic radiography for diagnosis of lung diseases in cattle and buffaloes (Robin, 1974; Verschooten *et al.*, 1974; Finley and Divers, 1997). It is interesting to note that eight buffaloes showing progressive loss of appetite and loss of body weight with normal body temperature and absence of coughing were found positive for pneumonia by radiological examination.

### Bacteriological examination

Nasal swab samples from clinical cases of BRD in buffaloes were processed for isolation and cultural examination of bacteria. The isolated species of bacteria were identified as *Staphylococcus* sp., *Streptococcus* sp., *E. coli*, *Corynebacterium* sp., *Klebsiella* sp., *Manheimia haemolytica*, *Brevundimonas* sp. and *Pseudomonas* sp. by using MALDI-TOF mass spectrometry. More or less similar findings were earlier reported in buffaloes by Praveen Kumar *et al.* (2015) but several workers have isolated *Pasteurella multocida* as major pathogen followed by *Manheimia haemolytica*, from nasal swabs collected from BRD affected cattle (Portis *et al.*, 2012; Kurcubic *et al.*, 2013, Joshi *et al.*, 2017)

### *In vitro* antibiotic sensitivity test

Antibiogram of pure culture organisms isolated from BRD affected buffaloes revealed highest sensitivity for gentamicin (83.33%), followed by ceftriaxone *plus* tazobactam (58.33%), enrofloxacin (58.33%), amoxicillin *plus* sulbactam (50%), chloramphenicol (50%), ceftiofur sodium (50%), ciprofloxacin (33.33%), moxifloxacin (33.33%) oxytetracycline (8.33%) and complete resistance to penicillin. Not much data on AST against isolated bacteria from BRD affected buffaloes is available. Earlier antibiogram studies revealed highest sensitivity to amoxicillin and clavulanic acid (Sen and Albay, 2003), ceftriaxone (Rashid *et al.*, 2011) and ceftiofur (Praveen Kumar *et al.*, 2015; Joshi *et al.*, 2017).

### Treatment

The present therapeutic trial was conducted on 31 clinical cases of bovine respiratory disease in buffaloes, divided into 4 Treatment groups *viz.* Group A (Gentamicin), Group B (Enrofloxacin), Group C (Moxifloxacin) and Group D (Ceftiofur sodium) comprising 8, 8, 6 and 9 buffaloes (Table 1). The attributes considered for comparison were recovery rate or survival rate, average recovery period, percent sensitivity to antibiotics and cost of treatment.

In treated animals, the survival rate in all groups was same i.e., 100% however, the overall recovery period was lowest in Group A ( $6.76 \pm 0.47$  days), followed by Group B ( $8.20 \pm 0.81$  days), Group C ( $8.38 \pm 0.47$  days) and highest in Group D ( $9.35 \pm 1.23$  days). In almost all groups the body temperature decreased to normal within 3 to 5 days. However, the pulse rate, respiration, ruminal motility, and appetite were gradually improved and became normal by 8<sup>th</sup> to 15<sup>th</sup> day, suggested reduction in inflammatory changes in lungs. Nasal

discharge became absent by 4<sup>th</sup> to 7<sup>th</sup> day and coughing gradually reduced and subsided 5<sup>th</sup> to 10<sup>th</sup> day of treatment. The hematological values especially total leucocyte and neutrophil counts substantially improved to near normal by 5<sup>th</sup> day owing to reduction in infection. Radiological examination in representative cases before and on 5<sup>th</sup> to 7<sup>th</sup> day of treatment revealed resolution of pneumonic changes in lungs and marked reduction in size of mediastinal lymph nodes (Figure 1 and 2). The percent sensitivity of antibiotic used in Group A was highest (83.33%), followed by Group B (54.21%), Group C (50.00%) and lowest in Group D (29.16%). The average cost of treatment of pneumonia with gentamicin in Group A was lowest followed by enrofloxacin in Group B, ceftiofur sodium in Group D and moxifloxacin in Group C. Based on the results of current therapeutic trial, Gentamicin proved most effective and economic in therapeutic management of bovine respiratory disease.

Antimicrobials *viz.* macrolide, (erythromycin, azithromycin), and fluoroquinolones (Enrofloxacin) are preferred for treatment of lung disease as they achieve therapeutic concentrations in diseased lung tissue after administration of conventional doses. The common causes for failure to respond favorably to treatment for bacterial pneumonia include advanced disease, drug resistant bacteria, inadequate dose of drug and presence of other lesions/disease (Radostits *et al.*, 2010). The choice of antimicrobial will depend on the tentative diagnosis, the experience with the drug in previous cases and the results of drug sensitivity test (Smith, 2009). The present findings on clinical efficacy of enrofloxacin are in agreement with Lekeux and Art (1988). In contrast to our findings Smith (2009) has suggested ceftiofur sodium 1.1 to 2.2 mg/kg IM for treatment of bronchopneumonia in beef cattle.

Supportive treatment with meloxicam was given to reduce inflammation of lungs. Multivitamins were given to rejuvenate ailing animals and also to improve their non-specific resistance. Dexamethasone was used for its anti-inflammatory effect in the treatment of acute and severe pneumonia. Ringers lactate solution was given to provide energy and combat respiratory acidosis in anorectic animals. Theophylline-etophylline combination was used as bronchodilator in severe cases of respiratory distress. In case of respiratory distress theophylline has been evaluated as a bronchodilator and it effectively decreases the respiratory distress (Radostits *et al.*, 2010).

## CONCLUSIONS

From current study it is concluded that radiological examination is very good tool not only for diagnosis but also for assessing severity of BRD in buffaloes and further monitoring the improvement during treatment. Further it is suggested that antimicrobial therapy should be chosen based on results of antibiotic sensitivity test for effective treatment of bovine respiratory disease in buffaloes.

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Table 1. Experimental design for evaluation of efficacy of various antibiotics in treatment of Bovine Respiratory Disease

Group	No. of animals	Drug	Dose and route
A	8	Gentamicin	4 mg/kg IM, daily for 5 days or till recovery
B	8	Enrofloxacin	5 mg/kg IM, daily for 5 days or till recovery
C	6	Moxifloxacin	5 mg/kg IM, daily for 5 days or till recovery
D	9	Ceftiofur sodium	1.6 mg/kg IM, daily for 5 days or till recovery

Table 2. Mean clinical and hematological values in BRD affected and healthy (Control) buffaloes.

Sr. No.	Parameter	Affected (n=37)	Healthy control (n=6)	t-value
1	Body temperature (°F)	101.8±0.24	101.6±0.18	0.24 <sup>NS</sup>
2	Pulse rate (Per minute)	60.62±1.99	49±1.13	5.72 <sup>**</sup>
3	Respiration (Per minute)	28.5±1.20	22.33±1.38	4.67 <sup>*</sup>
4	Ruminal motility (Per 5 minute)	2.72±0.22	5.5±0.34	12.16 <sup>**</sup>
5	Hb (g/dl)	10.20±0.36	10.20±0.51	0.24 <sup>NS</sup>
6	PCV (%)	34.34±1.21	38.07±0.94	2.90 <sup>*</sup>
7	TEC ( $\times 10^6 \mu\text{l}$ )	5.60±0.24	6.826±0.34	2.39 <sup>*</sup>
8	TLC ( $\times 10^3 \mu\text{l}$ )	11.62±1.36	6.12±0.31	4.10 <sup>**</sup>
9	MCV (fl)	62.36±1.32	51.67±2.03	7.79 <sup>*</sup>
10	MCH (pg)	18.39±0.42	16.65±0.56	2.88 <sup>*</sup>
11	MCHC (g/dl)	29.64±0.53	32.28±0.94	4.09 <sup>*</sup>
12	PLT ( $\times 10^3 \mu\text{l}$ )	246.70±43.24	280.00±0.13	2.02 <sup>*</sup>
13	Lymphocyte (%)	43.24±2.77	56.33±0.96	4.33 <sup>**</sup>
14	Neutrophil (%)	47.97±2.96	35.83±0.70	5.42 <sup>*</sup>
15	Monocyte (%)	3.24±0.19	1.84±0.30	2.92 <sup>*</sup>
16	Eosinophil (%)	5.52±0.66	5.84±0.79	0.32 <sup>NS</sup>

NS = Non significant; \* = Significant (P<0.05); \*\* = Highly significant (P<0.01).

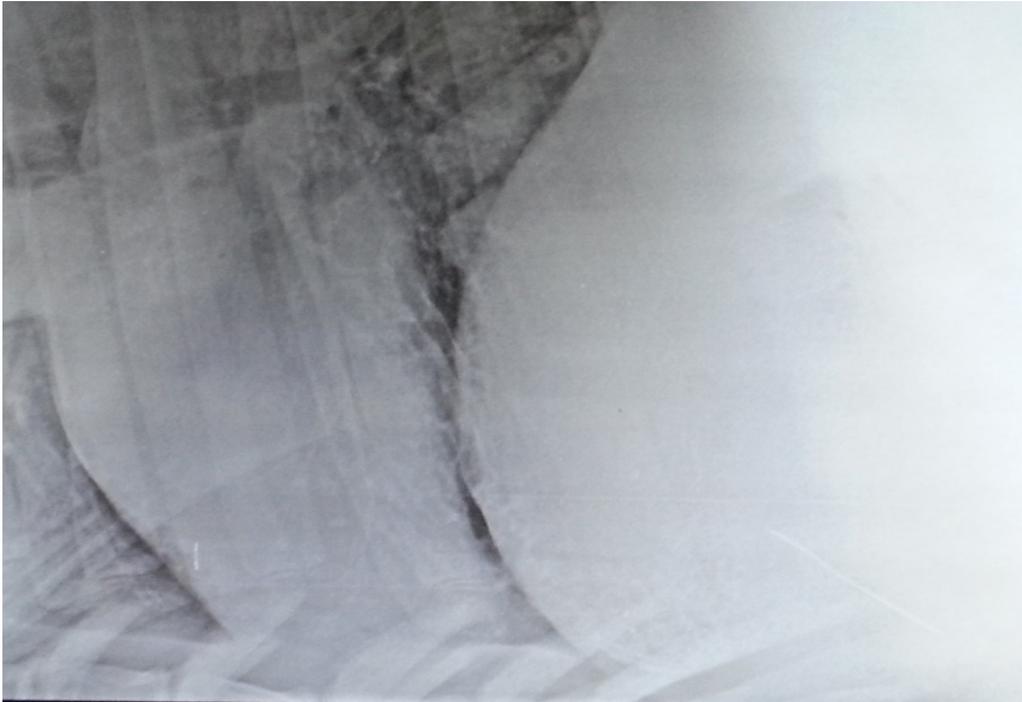


Figure 1. Severe pneumonia with consolidation and exudation in 4 years old Marathwadi buffalo before treatment with enrofloxacin.

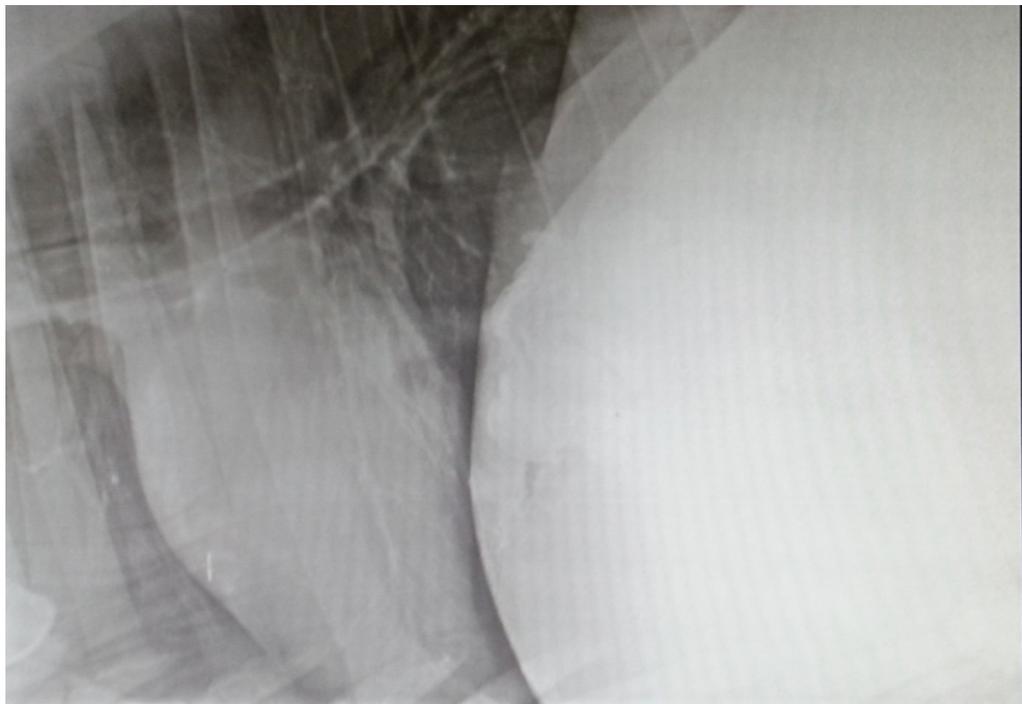


Figure 2. Disappearance of exudation and consolidation in same buffalo on 7<sup>th</sup> day of treatment with enrofloxacin.

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