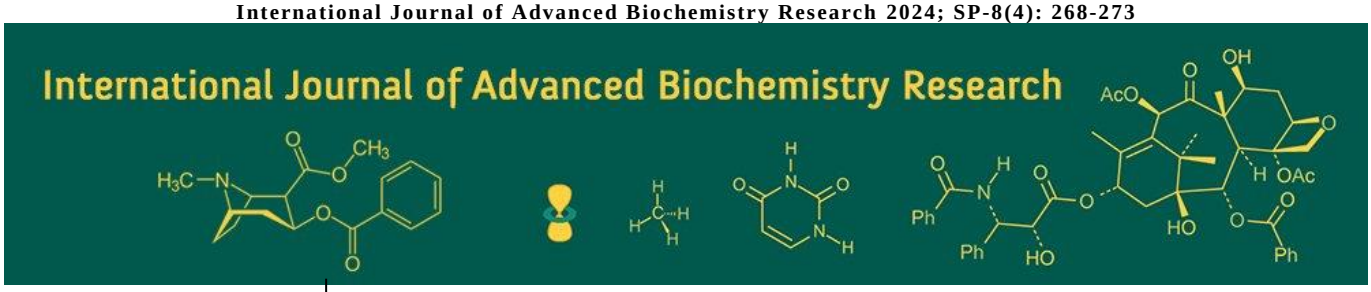

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Isolation and histopathological examination of *P. multocida* in pneumonic cases of Buffalo

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Abstract
A total 180 cases of buffaloes slaughtered in the slaughter house of Unnao and Barabanki district of Eastern Uttar Pradesh was surveyed during the month October, 2022 to March, 2023. The lungs exhibiting gross pathological lesions was put into 10% neutral formalin for histopathological studies and other part of lung for bacteriological studies. On Gross examination maximum cases of congestion and hemorrhages in lung were observed followed by emphysematous conditions and pulmonary oedema in the slaughtered animal in both the districts. Microscopic sections of lungs were examined and the major histologic findings are vascular and bronchiolar congestion along with severe emphysema seen in some cases. Some of the Emphysematous lung showed hyperplasia of the bronchiolar wall and peribronchial lymphoid tissue and exudates in the alveoli. In the bacteriological study, a total samples which showed gross lesions in slaughtered buffaloes in both the districts were collected. Among the tested samples (34 and 26 samples from Unnao and Barabanki districts respectively), were identified as *Pasteurella spp.* depending on their cultural/colony characters, morphology and biochemical properties *viz* Indole, Methyl red, Catalase production, Urea hydrolysis and Citrate utilization. Out of 34 isolates, 32 showed positive results for Indole test, 24 positive for catalase, 24 isolates, were positive for Urea hydrolysis, 23 isolates considered as positive for Methyle red. All isolates tested negative for gelatin liquefaction but 30 isolates were found positive for citrate utilization. Hence, so many unhealthy/diseased buffaloes are being slaughtered in the slaughtered houses for human consumption and that meat should not be consumed or may be condemned as not fit for human consumption due to infections.

Keywords: Isolation, histopathological, P. *multocida*, Buffalo

Introduction
One of the most serious and common health problems affecting buffaloes is lung diseases. In lung, infection localisation is supported by the thick connective tissue septa, but if they thicken and oedematize, they may restrict airways during expiration, leading to an imbalance between inspiratory and expiratory volumes (Argade *et al.,* 2019) [2]. Lung disease, which can be acute or chronic, can lead to debility and death, which can result in significant financial losses. During carcass inspection, pathological evidence of severe infectious illnesses or disease states has been found. The most prevalent causes of lung affection are parasitic diseases like hydatidosis and verminous pneumonia, bacterial diseases as Pateurellosis, tuberculosis.

One of the main reasons of death in the bovine species is difficulties with the respiratory system (Patrick, 2009) [14]. According to Duff and Galyean (2007), complicated respiratory disease is a multifactorial syndrome that arises as a result of intricate interactions between the host, the environment, and the pathogen. One of the major infectious diseases in the globe is pasteure Department of Veterinary Pathology,llosis. Little attention has been given to the disease since it shares many similarities with respiratory illnesses that affect cattle, buffalo, and sheep. Pasteurella causes severe illnesses such as mastitis and septicemia, if left untreated, can cause a high mortality rate in cattle and buffaloes (Araghy, 2007) [1].

One of the main bacterial infections responsible for these clinical symptoms is *Pasteurella multocida* (*P. multocida*) (Welsh et al., 2004) [23]. Both ante-mortem and post-mortem investigations should record any issues with meat cleanliness and possible consumer health risks. According to Salem *et al.* (2011) [19], meat inspection data are a valuable information

source in this situation and are crucial for epidemiology and preventive veterinary medicine. Through the production of animal products like milk, meat, wool, and other items, livestock is a substantial contributor to India's overall agricultural economy.

When an animal's immune system is compromised by factors like overcrowding, transportation, drought, bad weather, malnutrition, nasopharyngeal colonisation, dehydration during weaning, and concurrent respiratory infections, pneumonic pasteurellosis (also known as enzootic pneumonia) can occur. The nasopharyngeal and oral mucous membranes of clinically healthy cattle, sheep, and goats harbour this bacterium, an opportunistic pathogen. According to Ewers *et al.* (2004) [7] and Trevor *et al.* (2008) [22], the majority of *Mannheimia spp*. is opportunistic infections that are frequently isolated from asymptomatic carriers. Due to the significant financial losses and associated expenses of care and prevention, respiratory illnesses are a critical issue that breeders face more than veterinarians.

The diagnosis of bacterial pneumonia in buffaloes presents a substantial difficulty to the clinician. The doctor commonly uses a diagnostic laboratory since clinical indicators by themselves are not always diagnostic.

In order to determine whether or not the animal had any disease or disease condition that deemed the carcass unfit for consumption, the local veterinary authority and municipality were now required to conduct inspections of the abattoir. Traditional slaughter houses and slaughtering facilities as found in India's municipalities and city corporations. An ideal abattoir, according to Torres (2007) [21], should have ante-mortem and post-mortem carcass examination, hygienic management, and condemnation of ill animals. To assure the highest quality consumable animal products. So, in this study the animals included from the different slaughter houses that had respiratory symptoms or were suspected to have pasteurellosis.

Considering the above facts, the present study was designed to study the prevalence and isolation of pneumonic lesions in buffalo slaughtered in slaughter houses and its gross and histopathological changes of localized or generalized diseases/disease conditions of lungs in the slaughtered buffaloes.

Materials and Methods

Collection of material

Morbid materials: The tissue samples collected from organized slaughter house of Unnao and Barabanki Districts were preserved in 10% buffered formalin and processed in Department of Veterinary Pathology, ANDUAT, Kumarganj, Ayodhya. Samples were collected for Six months from October, 2022 to March, 2023. Gross pathological lesions were recorded with the aid of an experienced pathologist following FAO meat inspection manual (Herenda *et al.*, 1994) [9].

Gross pathology

Gross lesions in different visceral organs were noted immediately after opening the carcass at the slaughterhouse. The tissue samples were collected from cases showing gross lung lesions

Histopathological study

Formalin-fixed tissues were processed by the routine acetone-xylene technique, impregnated, and embedded in paraffin wax. Sections were cut at 4-5 µm thickness with the help of a semi-automatic rotary microtome.

The sections were stained with haematoxylin and eosin (H&E) stain following the conventional procedure (Luna, 1968) [13].

Demonstration of bipolar organisms

The smears of heart blood and lungs were collected at the time of the post-mortem examination and stained with Leishman's stain for demonstration of bipolar organisms.

Isolation of *P. multocida*

Blood agar (BA) and MacConkey agar (MCA) were used as primary culture media for the preliminary isolation of organisms from the samples according to methods described by Cruickshank *et al.* (1980) [4] and Quinn *et al.* (1994) [17].

Identification of *P. multocida* Isolates

P. multocida isolates were identified by commonly used and available biochemical tests as per methods described by Cruickshank *et al.* (1980) [4] with some minor modifications.

Leishman staining

We prepared a diluted blood smear on a clean and dry microscopic glass slide and dried it in air. Now, cover the well-dried, thin blood smear with undiluted Leishman stain solution by counting the drops of Leishman stain. Let it stand for 2 minutes; then the methanol present in the stain fixed the smear on the glass slide. After 2 min, add a double volume of distilled water or phosphate buffer solution to mix the contents by gently swirling or blowing. Incubate the slides at 37 °C for at least 10 min. Due to this the blood cells became stained. Wash the slides thoroughly with phosphate buffered solution for 2 minutes or until they turn violet-pink in color. Air dried the slides. Then the smear was mounted with mounting media. Then it was air-dried for a few hours, and then the slide was observed under the oil immersion objective lens of the microscope. And then the different blood cells were observed under the microscope as follows:

Colony characteristics

The colony characteristics like size, shape, elevation, surface, edges, colour, opacity and stickiness were thoroughly investigated.

Results and Discussion

The buffalo is the dairy, draught and meat animal of Asia. Buffalo plays a pivotal role in the livelihood of many people in the country as well as in the glove especially in the tropical and subtropical zones. Different lesions of lungs in the buffalo are frequently observed in the abattoirs and diagnosed during the post mortem examination of carcass. Thus the present study was carried out to investigate the pathological lesions of lungs in the buffalo slaughtered in the slaughter house of Unnao and Barabanki district of Uttar Pradesh for human consumption.

Isolation of *Pasteurella multocida*

To determine the presence of *Pasteurella multocida* in bovine pneumonia the samples were collected from lungs and heart aseptically by method of singeing.

Examination of direct impression smear

Direct impression smear was also prepared on a microslide and stained by Leishman's stain. In slides stained by Leishmans stain typical bipolar rods were observed (Figure 1). Which is comparable to what we found, Tigga *et al.*

(2014) [20] and Desem *et al.* (2023) [6] also discovered similar Gram-negative coccobacilli.

Culture of *Pasteurella multocida* from pneumonic cases

Blood agar

After 24 hours incubation of bacteria in brain heart infusion broth, a loopful of broth was streaked on Blood agar plate and incubated at 37 ºC for 24 hrs. In some cases non haemolytic, medium sized, yellowish grey mucoid colonies with sweetish odour were observed. Examination of smear from bacterial colony. Smears were prepared from the culture of isolated non-haemolytic and individual colony in Blood agar plate and stained by Grams method of staining. Thirty-four isolates were observed as Gram negative coccobacilli.

Mac Conkey agar

The individual colonies were picked from blood agar plates, further streaked on Mac Conkey agar plate and again incubated for 24 hrs. The cases where bacterial growth was observed were considered as negative for *Pasteurella spp*. and only remaining 34 cases where no bacterial growth was observed were assumed to be *P. multocida* and were characterized by biochemical test (Figure 2, 3).

Biochemical characterization of *P. multocida*

The thirty-four isolates were identified by biochemical tests *viz* Indole, Methyl red, catalase production, Urea hydrolysis and citrate utilization. The results of biochemical tests are as shown in Table 01. The thirty-four isolates were identified by biochemical tests *viz* Indole, Methyl red, catalase, Urea hydrolysis and citrate utilization. The isolates were further characterized for their biochemical activity by the carbohydrate fermentation lest (Cruickshank *et al.* 1980) [4] with some minor modifications.

For Indole test out of 34 isolates, 32 showed deep red colour at the surface of the reagent indicating a positive results (Figure 4). When tested for catalase, in 24 cases effervescence were produced indicates positive for catalase (Figure 5). Urea hydrolysis was observed for 24 isolates and only 10 isolates were indicating a negative reaction for urea hydrolysis. Twenty three isolates were inoculated in Glucose phosphate peptone water (GPPW) and incubated at 37ºC for 24 hours. After that 5 drop of Methyle red solution was added and tube showing a bright red colour were considered as positive. All isolates tested negative for gelatin liquefaction but 30 isolates found positive for citrate utilization. In carbohydrate fermentation reactions 28, and 19 isolates were positive for glucose and sucrose fermentation respectively. The similar result was seen by Prabhakar *et al.* (2012) [16] and Rawat *et al.* (2019) [18], who also reported that the indole test was negative in *P. multocida* but positive in *P. haemolytica*.

Most *P. multocida* isolates were included in our study fermented sucrose, galactose and maltose. Avril *et al.* (1990) [3], and Pillai *et al.* (2013) [15] also reported similar findings. Kumar *et al.* (1996), however, noted variability in the fermentation of mannitol and sucrose. According to Wael *et al.* (2014) [24] and Tigga *et al.* (2014) [20], the majority of isolates of *P. maltocida* failed to ferment lactose, which is consistent with our findings.

Gross examination of lungs

A total number of one hundred eighty cases of buffalo slaughtered in the slaughter house of Unnao and Barabanki district of Uttar Pradesh was recorded during the months from October, 2022 to March, 2023.

Microscopic pathology of pneumonic lesions

Microscopic sections of sixty pneumonic lungs were examined and the major histologic findings were as outlined. Histopathological examinations of 60 pneumonic lungs grossly classified as bronchopneumonia revealed exudates in the alveoli, bronchioles and bronchi; congestion of blood vessels hemorrhage and sometimes hyperplasia of associated bronchial lymphoid issues. In the present study maximum cases of hemorrhage and congestion observed in slaughtered animal this might be due to seizures and struggling during slaughter (Islam *et al*., 2014) [11]. Similar to present finding maximum cases of haemorrage and congestion also observed by Islam *et al*. (2014) [11]. The cases of bronchopneumonia with gross pathology indicative of suppurative pneumonia had abundant neutrophils and few macrophages within the lumen of bronchi, bronchioles and alveoli. Vascular and bronchiolar congestion along with severe emphysema seen in some cases. Some cases lungs showed emphysema along with fibrosis and ruptured alveoli**.** In some cases sevear emphysematous lung showed faint pink color fluid in the giant alveoli (Figure 6). Varying degree of goblet cell hyperplasia and proliferation of bronchiolar epithelium along with oedema of lamina propria were observed. At places there was degeneration and desquamation of bronchi epithelium. In few cases multifocal areas of necrosis of the lung parenchyma along with alveoli was seen (Figure 7). Several foam cells and plasma cells were observed in the alveoli in some cases. Microscopically in the lung tissues with gross appearance of fibrinous bronchopneumonia the predominant exudates were fibrin with few neutrophils and macrophage in the alveoli and bronchi along with congestion and fibrosis. Mild goblet cell hyperplasia and infiltration of lymphoid cells was also observed. Congestion and hemorrhages were noticed. Microscopic sections of lungs grossly classified as interstitial pneumonia were characterized by oedema infiltration of macrophages and lymphocytes into the interalveolar spaces and -peribronchiolar area. Sevear mononuclear cell infiltration and vascular congestion and necrosed alveoli seen in cases of interstitial pneumonia. Thick interalveolar septa also seen in some cases. Alveolar walls were distended and some of their lining cells were destroyed. In some cases vascular and bronchial congestion leads to emphysema along with formation of giant alveoli (Figure 8). The solitary case of granulomatous pneumonia tested negative for acid fast organism but had a well-defined lesion surrounded by mononuclear cells, multinucleated giant cell and fibrous capsule. The distribution of microscopic lesions in the lung tissues comprising emphysema, congestion, hemorrhage thickness of alveolar septa, leukocytic infiltration and fibrosis were seen (Figure 9). The result of present findings coinside with the finding of Gebrehiwot *et al.* (2015) [8]. In some cases there was congestion of blood vessels along with necrosis and sloughing of bronchiolar epithelium. Cases of fibrosarcoma also observed during the study period along with hemorrhage in lungs.

Histopathological findings of present study more or less simulated with the study of earlier workers (Haridy *et al.*, 2006) [10].

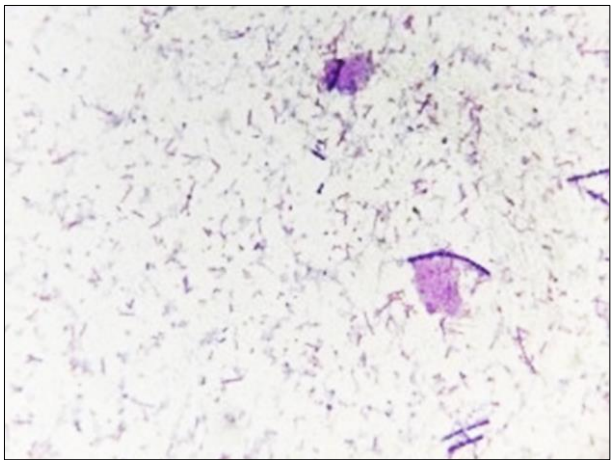

Fig 1: Typical bipolar rods of *Pasteurella spp*. by using Leishman's stain x1000.

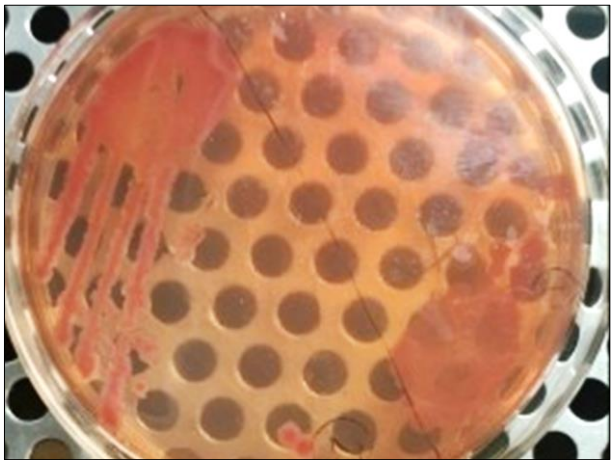

Fig 2: Growth On MacConkey lactose agar indicates negative for *Pasteurella* species

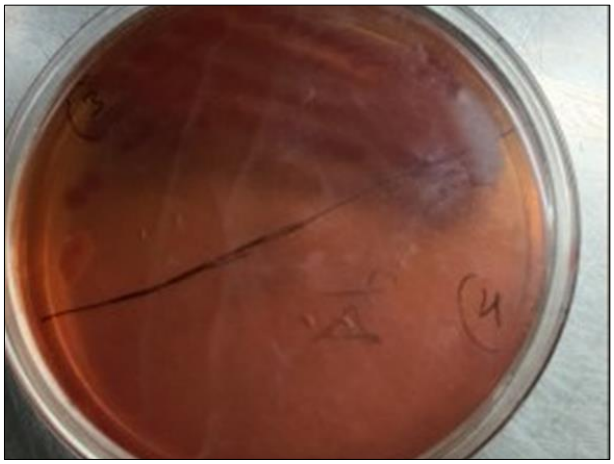

Fig 3: No growth On MacConkey lactose agar suspected for *Pasteurella* species.

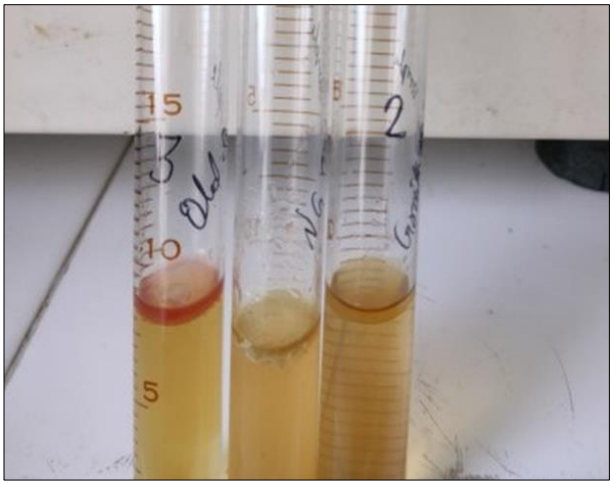

Fig 4: Red colour ring indicates positive for Indole test along with control

Fig 5: Catalase positive in *Pasteurella spp*.

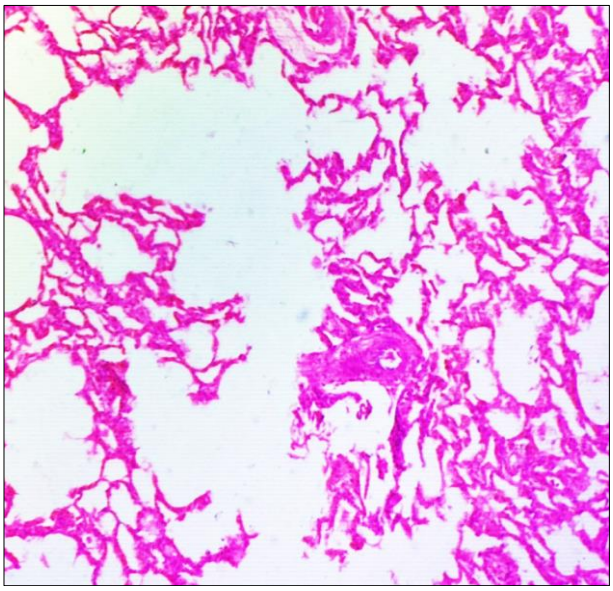

Fig 6: Severe emphysema of the lung along with faint pink color fluid in the giant alveoli. H&E X 100

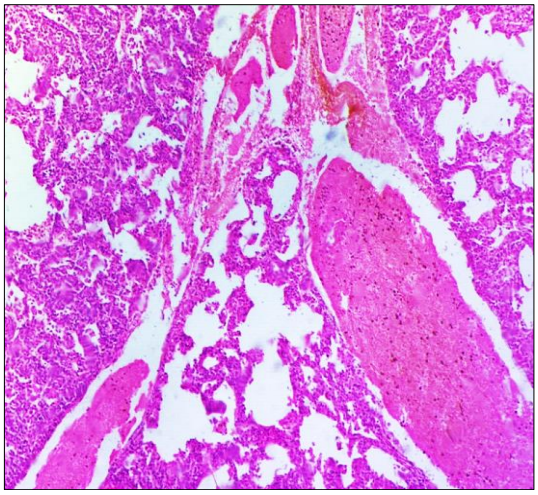

Fig 7: Extensive congestion of the blood vessels of the lung along with necrosis of the alveoli. H&E X 100

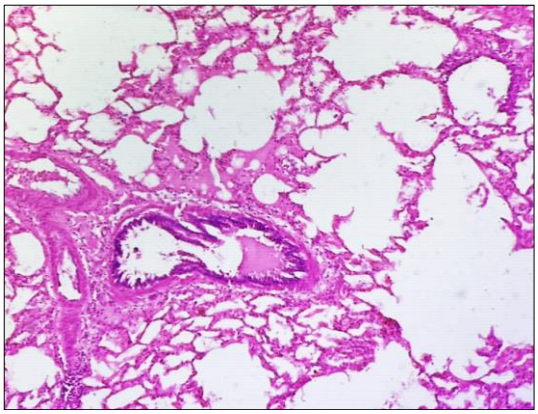

Fig 8: Vascular and bronchiolar congestion leading to emphysema of the lung characterized by formation of giant alveoli. H & E X 100

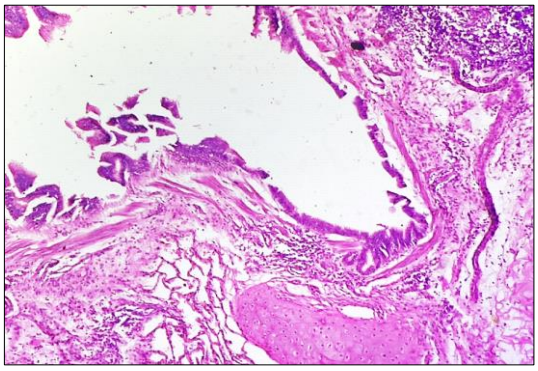

Fig 9: Hemorrhage and mononuclear cell infiltration along with fibrosis in the lung. H&E X100

Table 1: Shows different biochemical tests performance for *Pasteurella spp*

S. No.	Biochemical tests	Positive samples	Negative samples
1.	Indole	32	02
2.	Catalase	24	10
3.	Urea Hydrolysis	24	10
4.	Methyl red	20	14
5.	Glucose	28	06
6.	Sucrose	19	13
7.	Citrate Utilization	30	04

Conclusion

Pasteurella multocida was the main bacteria that was isolated from the lungs in the present study, causes pneumonia in young cows and buffaloes, so it may be a reaction to this organism's opportunist content in secondary invasion. The primary cause of pasteurellosis was *Pasteurella multocida*, while other bacteria are only frequently found in the nasal cavity alongside Pasteurella. The results of the study shows that pasteurellosis can occur in any season, with winter seeing the highest incidence due to environmental pressures like cold weather. Macroscopic lesions and bacterial infection are followed by other symptoms, such as those of repiratory disease brought on by endotoxin and pyrogen release. In the Slaughter House in Unnao and Barabanki districts, pasteurellosis in cattle and buffalo is not the focus of veterinary attention, but it is a potential source of economic loss. The findings support the hypothesis that it is higher in cattle than in buffalo at younger ages due to a poorer immune system and that it is higher in colder seasons due to stressful and congested housing conditions.

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