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Research Article

CYTOPATHOLOGICAL STUDIES ON PNEUMONIC LESIONS OF SLAUGHTERED SHEEP WITH SPECIAL REFERENCE TO OVINE PULMONARY ADENOMATOSIS

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Abstract: Cytopathological investigations were conducted on the bronchoalveolar lavage and impression smears collected from the slaughtered sheep showing pneumonic lesions at Deonar abattoir, Mumbai, Maharashtra. Using a standard protocol, the systematic random sampling method was used to note the gross lung lesions and collect bronchoalveolar lavage, impression smears, and representative tissue samples for histopathology. Different cytological values of BALF and impression smears of lung tissues found positive for ovine pulmonary adenomatosis by histopathological findings were compared with bronchopneumonia, bronchointerstitial pneumonia, and interstitial pneumonia. The recovery percentage (44.6%) and total cell count (30.76±2.49cells/µl) of BALF in OPA cases were high compared to other pneumonia cases. The plasma cell counts of BALF (20.00±5.63) and lung impression smear (14.20±2.70) in the OPA- positive cases was significantly higher than the other forms of pneumonia. The different cytological values of the pneumonic sheep lungs with particular reference to the ovine pulmonary adenomatosis were placed for the record.

Keywords: *Ovine pulmonary adenomatosis, Bronchoalveolar fluid, Plasma cell, Impression smear*

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Introduction

The pneumonic conditions and various lung diseases could be studied using sampling BALF [1]. Different types of tests that can be performed on the BALF could be assessing the cell number, differential count, isolation and identification of etiological agents, and determination of immune proteins. OPA is one of the critical respiratory diseases of sheep which is generally subclinical and infects any age group. The disease's incubation period may vary from months to years or shorter (6–8 months) in non- endemic herds. Clinically the condition may cause respiratory distress emaciation and make the animal susceptible to secondary bacterial infections in the lungs. This disease is responsible for severe economic losses due to mortality and morbidity, resulting in reduced growth rate, body weight, and milk and wool production. The assessment of the differential cell profiles of BALF is potentially valuable in diagnosing disease conditions as it may be an accurate predictor of cellular changes occurring in the lungs [2] since scarce information was available about the BALF values and impression smear cytology of pneumonic lungs as per review of the literature. The current investigation was designed to study bronchoalveolar fluid and impression smear cytology of lungs showing pneumonic lesions with reference to pulmonary adenomatosis in slaughtered sheep at Deonar abattoir, Mumbai, during the period from July 2022 to November 2022.

Material and Method

A total number of 425 sheep lung samples were examined for pneumonic and adenomatous lesions. Out of which, 35 had gross lesions, samples like bronchoalveolar fluid were collected from affected lungs, and an impression smear was collected after incising affected lung tissue. The bronchoalveolar lavage was collected as described by Burrels *et al.,* (1993) [3], with slight modifications using Ryle's tube (10 FG, 3.3 mm O.D., and 105 cm length) to collect BALF. Along with the trachea, the lungs were removed and examined macroscopically for pneumonic and adenomatous lesions. After that, Ryle's tube was pushed into the lungs via the trachea until the obstruction developed. Then 40 ml sterile normal saline was loaded in the sterile syringe, connected with Ryle's tube, and injected into the lungs. The fluid was then aspirated back following a gentle massage of the lobes for 10 to 30 seconds and transferred into the sterile 50 ml centrifuge tube. The samples were brought to the laboratory using ice packs. The samples collected for the BALF study were examined for colour, and consistency, and the volume of this recovery fluid was recorded. The total nucleated cell count (TCC) of the BALF samples was carried out manually as per the standard procedure using Neubauer's chamber. The counts were represented as cells per µL of BALF. Then these samples were centrifuged at 2,000 rpm for 15 minutes, and after centrifugation, the supernatant was decanted, and sediment from the centrifuge tube was used for further studies. The sediment was smeared on a clean glass slide; the smear was fixed in methanol, air-dried, then stained with Giemsa to allow for further cell count. Staining characteristics and morphology were taken as the basis for the cell count. Differential cell count was calculated as per the guidelines given by Dawson *et al*., (2005).

All 35 samples were subjected to histopathological studies. Twelve samples were found positive for OPA. Nine cases of interstitial pneumonia, eight bronchointerstitial pneumonia, and six cases of bronchopneumonia were found. However, the histopathological study was separate from the present article, explicitly focusing on cytopathology.

Table-1 Volume of recovered fluid in different cases

SN	Type of case	Volume of BAL fluid recovered	Fluid recovery rate (%)
1	OPA	17.84ml	44.60%
2	Bronchopneumonia	16.7ml	41.75%
3	Interstitial pneumonia	13.64ml	34.10%
4	Bronchointerstitial pneumonia	15.9ml	39.75%

Table-2 Statistical data -Differential cell count of BALF direct smear for OPA and other cases (Mean ± S.E.)

Type of case	Neutrophil	Lymphocyte	Alveolar macrophages	Eosinophil	Plasma cells
Ovine pulmonary adenomatosis	101.60±32.98[1]	54.00±19.70[1]	224.40±29.33[1]	0.00±0.00[1]	20.00±5.63[2]
Bronchopneumonia	146.40±22.93[1]	78.60±13.39[1,2]	174.40±12.30[1]	0.60±0.004[1]	0.00±0.00[1]
Interstitial pneumonia	67.60±5.17[1]	121.80±5.36[2]	209.40±3.84[1]	0.40±0.002[1]	0.60±0.006[1]
Bronchointerstitial pneumonia	77.00±6.22[1]	117.60±11.35[2]	202.60±10.78[1]	0.80±0.005[1]	2.40±0.002[1]

Table-3 Statistical data-differential cell count of impression smear cytology of lung tissue (Mean Standard error)

Type of cases	Neutrophil	Lymphocyte	Alveolar macrophages	Eosinophil	Plasma cells
OPA	115.00±29.96[1]	56.20±21.50[1]	214.20±32.12[1]	0.40±0.004[1]	14.20±2.70[2]
Bronchopneumonia	150.00±24.43[1]	68.60±11.50[1]	180.00±17.11[1]	0.60±0.002[1]	0.80±0.02[1]
Interstitial pneumonia	76.00±8.16[1]	129.80±6.27[2]	193.60±13.44[1]	0.20±0.001[1]	0.40±0.002[1]
Bronchointerstitial pneumonia	121.80±17.85[1]	106.00±10.96[1,2]	170.40±25.10[1]	0.20±0.002[1]	1.80±0.06[1]

Statistical Analysis

Data obtained during, the cytological examination of bronchoalveolar fluid were subjected to one-way ANOVA statistical analysis using the IBM SPSS statistics processor version 26, and the means of total cell count and differential leukocytes count of the BALF were compared by assuming the equal variance of LSD, Tukey, Tukey B. The level of significance was reported at 5% level of significance ($p<0.05$).

Result and Discussion

The recovered lavage fluid had an average volume of 17.84 ml, 44.6% of the total normal saline infused in all the lungs studied during the present investigation. These findings were slightly lower than that recorded by Voigt *et al*., (2007) [4], who have an average volume of recovered fluid as 45.8 out of 100 ml instilled Normal saline. Whereas, Ezeasor *et al*., (2021) reported a 69.5% recovery rate of lavage fluid in slaughtered goats. Berrag *et al*., (1997) [5] and Deshpande *et al*., (2021) [6] reported a 31.2% and 65% of fluid recovery rate in live goats. A total of three different types of pneumonia and ovine pulmonary adenomatosis were recorded, including bronchopneumonia, interstitial pneumonia, and bronchointerstitial pneumonia. The fluid recovery rate for each case of pneumonia is given in [Table-1].

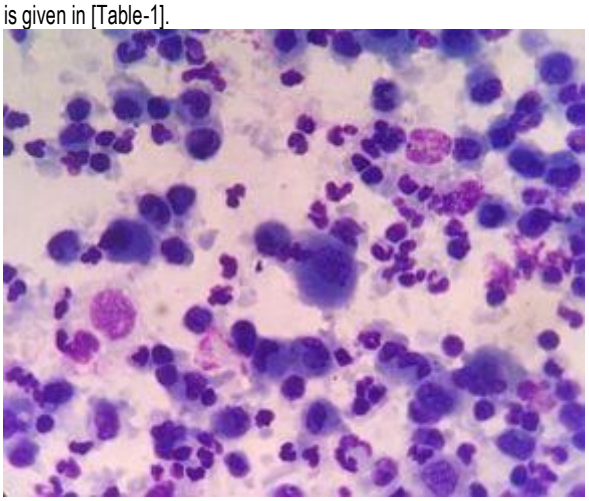

Fig-1 BALF cytology: Presence of neutrophilic response indicated by maximum neutrophilic cellularity in the background of alveolar macrophages and very few lymphocytes and plasma cells. Giemsa stain. X400.

Direct smear cytology of BALF fluid

The average percentage of differential cell count from direct smear cytology for ovine pulmonary adenomatosis and other cases are given in [Table-1]. Plasma cell count was significantly higher, and lymphocyte count was significantly lower in cases of OPA as compared to instances of all other categories of pneumonia and bronchointerstitial pneumonia respectively. In OPA cases, alveolar macrophages were the predominating cells with Mean± S.E as 224.40±29.33 cells (56%), neutrophils being the second highest with 101.60±32.98 cells (25.25%) followed by lymphocyte with 54.0±19.70 cells (13.5%), plasma cells with 20.00±5.. 63 cells (5%) and count of eosinophils was minimum with 0.00±0.00 cells (0%) out of total 400 cells counted. The above results agreed with Dawson *et al*., (2005), who recorded differential cell counts for different cases of pulmonary disease like Maedi, adenomatosis, and parasitism and compared them with normal lung BALF samples of slaughtered sheep. For OPA, the differential cell count percentages of alveolar macrophages, neutrophils, lymphocytes, eosinophils, and miscellaneous cells was recorded as 65.8, 17.9, 4.8, 8.9, and 0%, respectively. Mahile *et al*., (2022) [7] also recorded differential cell count for alveolar macrophages, neutrophils, lymphocytes, and eosinophils as 45%, 17.5%, 37%, and 0 %, respectively, in the case of OPA.

For lungs with bronchopneumonia cases, differential cell count showed higher values of neutrophils with 146.40±22.93 (36.5%) cells, and alveolar macrophages 174.40±12.30 (43.5%) cells, lymphocytes count was 78.60±13.39 (19.5%) cells, and eosinophils was 0.60±0.004 (0.15%) cells and plasma cells 0.00±0.00 (0%) cells. Mahile *et al*., (2022) [8] also observed higher percentage of neutrophils (46%), followed by alveolar macrophages (34%), lymphocytes (18%) and eosinophils (0.2%) in case of suppurative bronchopneumonia. Jarikre *et al*., (2016) also recorded differential cell count in different types of pneumonia in goat with higher percentage of neutrophils (41.1%), followed by alveolar macrophages (39.2%), lymphocytes (15.1%) and eosinophils (2.1%) in case of bronchopneumonia.

For interstitial pneumonia, Mean ± S.E. count of alveolar macrophages was recorded as 209.40±3.84 (52.25%) cells, for lymphocyte it was 121.80±5.36 (30.25%) cells, neutrophil was 67.60±5.17 (16.75%) cells, plasma cells 0.60±0.006 (0.15%) cells and eosinophil 0.40±0.002 (0.1%) cells. Mahile *et al*., (2022) also reported similar pattern of cell values with percentage of alveolar macrophages (55%), lymphocytes (30%), neutrophils (14%), and eosinophils (0.2%) in case of interstitial pneumonia. Oruc *et al*., (2011) [9] recorded differential cell values of 23.0±0.97, 17.3±0.87, 111±2.64, 2.22±0.36, and 10.3±0.67 for alveolar macrophage, neutrophil, lymphocyte, eosinophil, and ciliated epithelium respectively, in sheep infected with interstitial pneumonia. Compared to the other categories, interstitial pneumonia had a higher count of lymphocytes at ($p<0.05$).

For broncho-interstitial pneumonia, higher Mean ± S.E. values of alveolar macrophages were recorded as 202.60±10.78 (50.5%) cells, followed by lymphocyte i.e., 117.60±11.35 (29.25%) cells. For neutrophils, eosinophils, and plasma cell count were 77.00±6.22 (19.25%) cells, 0.80±0.005 (0.2%) cells, and 2.40±0.002 (0.6%) cells, respectively. Jarikre *et al*., (2016) reported alveolar macrophages, lymphocytes, neutrophils, and eosinophils as 205± 10.2, 90.50± 8.4, 72.30±13.0, and 5.1±2.5, respectively. An increase in the neutrophilic cellular pattern in BALF could be noted in can be found in, asbestosis, acute respiratory distress syndrome (ARDS), aspiration pneumonia, subacute hypersensitivity pneumonitis, as well as in many pulmonary infections.

The present study noted the neutrophilic pattern, specifically in bronchopneumonia and OPA. Bronchopneumonia could be caused due to mixed etiology, characterized by high neutrophil and macrophage count. Chronic inflammatory conditions result in a lymphocytic pattern or increased plasma cell pattern, as noted in cases of OPA compared to other pneumonia types in the present investigation. Stanzel *et al.* (2012) observed that such a lymphocytic pattern was typical in chronic granulomatous lung diseases or some drug reactions. More specifically, plasma cells were not present generally in BALF of human beings; if noted along with foamy macrophages and increased lymphocyte count, then drug-induced pathology could be suspected. These observations may or may not be accurate in the case of sheep lung diseases, which required further BALF and impression smear cytology studies in the ovine population.

Impression smear cytology from affected lung

An impression smear was taken from the cut portion of the affected lung tissue by gently touching the clean glass, sliding over it, and air drying. After fixation with methanol, it was stained with Giemsa stain for cytological evaluation. Mean values with the standard error for differential cell count were depicted. In OPA-positive cases; cytology revealed the highest mean values of alveolar macrophages as 214.20±32.12 (53.5%) cells followed by neutrophils with 115.00±29.96 (28.75%) cells, lymphocyte was slightly low with 56.20±21.50 (14%) cells, plasma cells were increased with mean values 14.20±2.70 (3.5%) cells, and eosinophils with 0.40±0.004 (0.1%) cells. A high number of neutrophils in the cytology of OPA contributes to the acute inflammatory response provoked by the animal body in response to the secondary bacterial infection to remove the stimulus from a particular site. OPA cases being of viral etiology and chronic cases, take a much longer time to establish the condition. After that, in immunocompromised animals, secondary bacterial infection can easily superimpose the first infection. Sequelae often include secondary bronchopneumonia, abscesses, and fibrous pleural adhesions. Bronchopneumonia cases revealed a higher mean± S.E. value of neutrophils with 150.00±24.43 (37.5%) cells, alveolar macrophages with a mean value of 180.00±17.11(45%) cells, lymphocytes with a mean value of 68.60±11.50 (17%) cells, eosinophil with mean value 0.60±0.002 (0.15%) cells and plasma cells with mean value 0.80±0.02 (0.2%) cells.

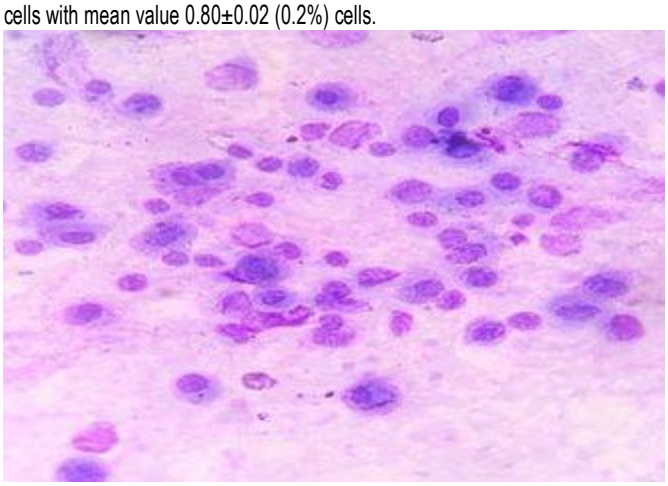

Fig-2 BALF cytology: Presence of lymphocytic response along with plasma cells indicated by maximum lymphocytic cellularity in the background of alveolar macrophages and few plasma cells. Giemsa stain. X400.

Interstitial pneumonia cases have revealed a much lesser mean value of neutrophils with 76.00±8.16 (19%) cells, an increased value of lymphocytes with 129.80±6.27 (32.25%) cells, and alveolar macrophages.e.193.60±13.44 (48.25%) cells, plasma cells with 0.40±0.002 (0.1%) cells, and eosinophils have mean values of 0.20±0.001 (0.05%) cells. In cases of bronchointerstitial pneumonia, higher mean values of alveolar macrophages were seen at 170.40± 25.10 (42.5%) cells, followed by lymphocytes with a mean value of 106.00±10.96 (26.5%) cells, mean value of neutrophil was slightly lesser than bronchopneumonia with 121.80±17.85 (30.25%) cells, plasma cells have a mean value of 1.80±0.06 (0.45%) cells, and eosinophil has a mean value of 0.20±0.002 (0.05%) cells.

Total cell count of BALF fluid

Mean values of total cell count with standard error of different types of cases encountered in the present research are given. All these values are expressed in n×10³/cumm. In OPA cases, the mean total cell count was 30.76 ±2.49, slightly higher than that recorded by Mahile *et al*., (2022), who had recorded TCC values of 27.08±3.5(×10³/cumm) in OPA.

In this study, the cytology of BALF was not studied for normal lungs, but by comparing the results of the present study, it was concluded that the total cell count was much higher for affected lungs than normal ones. For bronchopneumonia cases, the mean value of TCC has increased to 31.50±1.99×10³/cumm. In interstitial pneumonia, the mean value of TCC was 30.00±1.23×10³/cumm; for bronchointerstitial, the mean value of TCC was 28.95±0.26 ×10³/cumm. Similar findings were recorded by Mahile *et al*., (2022) of TCC with Mean ± S.E for OPA, suppurative bronchopneumonia, fibrinous bronchopneumonia, interstitial and bronchointerstitial as 27.88±3.5, 30.08±1.13, 31.23±1.94, 22.5±5.4, 28.85±2.03×10³/cumm in goat respectively.

The statistical analysis of TCC values was performed using IBM-SPSS version-26 by applying one way ANOVA test revealed that TCC values in different types of pulmonary conditions in the present study were statistically non-significant at p>0.05. The reason behind it might be the co-existence of two or more infection, viral and bacterial infection, which interferes with the cytological values and thus generate non-significant results.

Table-4 Statistical analysis of total cell count on BALF samples (Mean±Standard error)

SN	Type of case	TCC (Unit)
1	OPA	30.76±2.49[1]
2	Bronchopneumonia	31.50±1.99[1]
3	Interstitial pneumonia	30.00±1.23[1]
4	Bronchointerstitial pneumonia	28.95±0.26[1]

In all cases, the total cell count of BALF was increased. In direct smear cytology of OPA cases, alveolar macrophages were the predominating cells with Mean± S.E as 224.40±29.33(56%), neutrophils being the second highest with 101.60±32.98(25.25%) followed by lymphocyte with 54.0±19.70 (13.5%), plasma cells with 20.00±5.63(5%) and count of eosinophils was minimum with 0.00±0.00(0%) out of total 400 cells counted.

In OPA-positive cases, impression smear cytology revealed the highest mean values of alveolar macrophages, having 214.20±32.12, followed by neutrophils with 115.00±29.96, lymphocytes were slightly low with 56.20±21.50, plasma cells were increased with mean values 14.20±2.70, and eosinophils with 0.40±0.004. The high number of neutrophils in the cytology of OPA contributes to the acute inflammatory response provoked by the animal body in response to the secondary bacterial infection to remove the stimulus from a particular site. OPA cases being of viral etiology and chronic cases, take a much longer time to establish the condition. After that, in immunocompromised animals, secondary bacterial infection can easily superimpose the first infection.

Other cases like bronchopneumonia revealed a higher mean value of neutrophils and Interstitial pneumonia with an increased mean value of lymphocytes and alveolar macrophages. In bronchointerstitial pneumonia, higher mean values of alveolar macrophages were seen. The total cell count for the OPA case was recorded as 30.76±2.49. For other cases like bronchopneumonia, Interstitial pneumonia, and bronchointerstitial pneumonia, TCC was 31.50±1.99,30.00±1.23 and 28.95±0.26, respectively. Thus, there is an increase in the TCC count for any infection in the lung tissue. The statistical data of TCC studied for different cases found in the present study are not dependent on each other; thus, they are statistically non- significant at p>0.05.

Conclusion

The current study concluded that the statistically significant high count of plasma cells in BALF and lung impression smears was noted in the OPA-positive cases compared to other subclinical pneumonic lesions of slaughtered sheep lungs. The total cell counts of both BALF and lung impression smear revealed statistically nonsignificant variation in different pulmonary lung lesions of sheep. However, BALF cytology and lung impression smear revealed limited information for differential diagnosis of lung diseases.

Further scope

Further scope is still there to study the various age groups, sex, and respiratory disease conditions in sheep affecting the lungs to get the in-depth cytopathological values of sheep lung diseases for future diagnosis, prevention, and control strategies.

Application of research: Cytopathological studies on pneumonic lesions of slaughtered sheep for ovine pulmonary adenomatosis

Research Category: Veterinary Pathology

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Study area / Sample Collection: Mumbai Veterinary College, Mumbai, 400012

Cultivar / Variety / Breed name: Ovine Sheep

Conflict of Interest: None declared

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Ethical Committee Approval Number: Nil

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