THE CYTOLOGY OF THE BRONCHOALVEOLAR LAVAGE (BAL) FLUID IN HEALTHY SMALL EAST AFRICAN GOATS

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SUMMARY

Bronchoalveolar lavage fluid (BAL) was performed on 20 clinically normal Small East African goats, aged between 9 and 15 months and weighing between 10 and 20 kgs. A tracheotomy was surgically prepared and normal saline infiltrated through a canula into the lungs. BAL fluid was recovered by suction using a 30-ml syringe. Total cell counts and differential cell counts were performed on the harvested BAL fluid. Analysis of the lavaged fluid found the total cell counts to range between 6400 and 11200 cells per cubic millimeter and differential cell counts indicated that the lymphocytes were the predominant cell type (93.12%) macrophage ranked second in population (6.00%) while neutrophils ranked third in population (1.86%). Eosinophils and basophils were not represented in the lavaged fluid. The study has provided a preliminary data of the normal cytology of the bronchoalveolar lavage fluid in the Small East African goats.

INTRODUCTION

In Tanzania goats rank second to cattle in population, five and twelve million heads respectively (FAO, 1990); and their meat is very popular. Thus there is a lot of interest in utilising them further for meat production for domestic and export market, also goats provide milk, hair, skin and are used in a number of social functions such as payment of dowry. Additionally, goats are frequently used as experimental animals in biomedical research.

Goat productivity is hampered by a number of diseases including helminth infestations, especially haemonchosis, respiratory and diarrhoea diseases (Kusiluka and Kambarage, 1996). Respiratory diseases are known to be of great challenge in small ruminant productions (Akerejola *et al.*, 1979), causing about 33 percent mortalities (Chawla et al., 1982; Vihara *et al.*, 1986). Even when the diseases do not cause deaths, subclinical conditions do pose a significant challenge to the farmers in terms of poor performance of the animals. However, such epidemiological studies have not been carried out in Tanzania. Kusiluka and Kambarage (1996) contend that the situation is not different from other tropical and subtropical regions investigated so far.

Several diagnostic aids have been developed to reveal the status of distal airways, among such techniques is bronchoaleolar

lavage. The technique involves instillation of normal saline into the alveolar and is retrieved back along with cellular and acellular debri. Bronchoalveolar lavage (BAL) technique is a useful tool in the sampling of the lower respiratory tract both for clinical indications and for research-related investigations (Reynolds, 1987; Linder and Rennards, 1988; Klech and Hunter, 1990) and it is almost a routine diagnostic tool in chronic obstructive pulmonary diseases (COPD) (Lapointe *et al.*, 1993).

The purpose of this study was therefore to provide, apparently for the first time, a normal cytological picture of the bronchoalveolar lavage fluid of goats. The information obtained has provided a baseline against which clinical and pathological processes can be assessed.

MATERIALS AND METHODS

Twenty Small East African goats aged between 9 and 24 months were purchased from different sources in Morogoro Municipality and BAL fluid was collected from each goat using the protocol described below.

The animals were put under general anaethesia using xylazine and ketamine intravenously at a dosage rate of 0.2mg/kg and 11mg/kg respectively. A ventral incision was made in the neck region exposing the trachea through the incision. A small incision was made in between the tracheal rings and swabbed to clear off of blood. A sterile catheter (3 mm in diameter) was passed through the incision to the bronchus. Normal saline (80 – 100ml) was introduced through the catheter using a 30-ml syringe and then drawn by suction. Between 30 – 60% of the infused fluid was harvested.

Analysis of fluid

The harvested fluid was centrifuged at 2000 revolutions per minute (rpm) for 10 minutes. Carefully using a Wintrobe pipette, the supernatant was separated from the pellet. The sediment obtained was divided into two portions, one to be used for the total cell count and the other one for differential cell count.

Total cell count

One portion of the pellet was suspended in 5 mls of Hank's balanced solution. From this solution, total cell counts were determined by the use of a haemocytometer. The number of cells was recorded as number of cells per cubic millimeter.

Differential cell counts

Smears made from the second portion of the pellet were air-dried and fixed with methanol. Fixed smears were stained with giemsa for 30 to 45 minutes. Using the straight edge counting method differential cell counts were performed on stained smears.

RESULTS

The percentage recovery of BAL, fluid ranged from 32% to 60% with a mean of 52.5% and the results are given in Table 1. Differential cell counts for healthy goats indicated

that the dominant cell type was the lymphocyte (88% to 98% with a mean of 91.7%). Phenotypically, three subpopulations of lymphocytes were identified as small, large and activated lymphocytes. Of the three subpopulations, large lymphocytes were in the majority (67.1%) followed by small lymphocytes (22.2%) and the least represented was the activated lymphocytes (13.4%. Macrophages accounted for 6.6% followed by neutrophils 1.86%, eosinophils were only observed in two goats out of the twenty goats and accounted less than 1% in those goats. Basophils were not represented in the BAL fluid of healthy goats.

The results for the differential cell counts for BAL fluid are provided in Table 2. The total cell counts for BAL fluid ranged from 800 to 12200 cell per cu. mm. With an average of 9090 cells per cu. mm., the results are given in Table 3.

DISCUSSION

The amount of fluid recovered after instillation of normal saline in the lungs of healthy goats ranged between 30 and 60% of the instilled volume. Results obtained in the present study compares with previous studies in other mammalian species (Rebar et al. 1990; Sweeney et al. 1992; Clark et al 1995). It has been observed that with subsequent lavages, the volume recovered tends to increase (Sweeney et al. 1992). In the present study only one lavage was performed, hence the relatively low recovery of BAL fluid.

Results of the differential cell counts of BAL fluid for healthy goats indicated that lymphocytes are the predominant cell type (92%), followed by macrophages (6.65%) neutrophils (1.55%) while basophils and eosinophils were hardly observed. Lymphocytes were further categorised phenotypically into three categories, namely, small, large and activated lymphocytes. The results indicated that of the three categories, large lymphocytes predominant (65%) followed by small lymphocytes (21%) whereas activated lymphocyte population.

To the best of our knowledge, results for the differential cell counts for healthy goats have not been reported before. The present results for the goat, however, appear to differ significantly from differential cell counts reported for the dog. (Eleanor *et al.*, 1993), sheep (Burrels, 1985), horse (Sweeney *et al.*, 1992; Larson and Busch, 1985; Derksen *et al.*, 1989), cats (Hawkins *et al.*, 1994) which show that macrophages are predominate in the BAL fluid.

Table 1: Volume of saline infused in the lungs of clinically normal goat and the percentage of volume recovered.

GOAT No.	VOLUME INFUSED (MLS)	VOLUME RECOVERED (%)
BAL/01	100	60
BAL/02	85	55
BAL/03	90	55
BAL/04	100	57
BAL/05	100	49
BAL/06	80	55
BAL/07	90	57
BAL/08	95	40
BAL/09	100	46
BAL/10	100	57
BAL/11	90	60
BAL/12	100	58
BAL/13	95	46
BAL/14	80	57
BAL/15	90	60
BAL/16	100	59
BAL/17	100	45
BAL/18	85	32
BAL/19	90	43
BAL/20	100	55

Table 2: Differential cell counts for healthy goats

GOAT No.	LYMPHOCYTES (%)			TOTAL	MACROPHAGES	NEUTROPHILS	EOSINOPHILS	BASOPHILS
	small	large	activated					
BAL/01	27	69	2	98	2	0	0	0
BAL/02	41	20	31	92	6	2	0	0
BAL/03	52	80	5	90	8	2	0	0
BAL/04	60	30	4	94	5	1	0	0
BAL/05	10	70	10	90	8	2	0	0
BAL/06	8	60	22	90	7	3	0	0
BAL/07	16	62	15	93	6	1	0	0
BAL/08	9	69	14	91	6	3	0	0
BAL/09	22	65	8	93	6	1	0	0
BAL/10	18	68	7	93	5	2	0	0
BAL/11	15	60	14	89	10	1	0	0
BAL/12	17	65	10	92	8	0	0	0
BAL/13	11	51	26	88	8	3	1	0
BAL/14	18	66	9	93	5	2	0	0
BAL/15	20	57	12	89	10	1	0	0
BAL/16	14	64	16	94	5	1	0	0
BAL/17	9	70	9	88	8	3	1	0
BAL/18	27	55	8	90	9	1	0	0
BAL/19	16	59	18	93	6	1	0	0
BAL/20	28	60	6	94	5	1	0	0

Table 3: Total cell counts for the healthy goats

GOAT No.	TOTAL CELL /cu mm
BAL/01	10400
BAL/02	6800
BAL/03	11200
BAL/04	800
BAL/05	10400
BAL/06	8200
BAL/07	6400
BAL/08	8000
BAL/09	10800
BAL/10	12000
BAL/11	9400
BAL/12	12200
BAL/13	11200
BAL/14	8400
BAL/15	9600
BAL/16	11200
BAL/17	7200
BAL/18	9000
BAL/19	10200
BAL/20	8400

The current findings in the goat that macrophages are not the major cell type in the BAL fluid appear to be in agreement with previous scanning electron microscopy studies of the distal airways of healthy goats (Kahwa *et al.*, 1997). In the previous study, large areas of pulmonary parenchyma had to be searched to be able to locate a single macrophage.

The paucity of alveolar macrophages observed in the goat in the present study could be due to the fact that the goat has a significant population of pulmonary intravascular macrophages (PIMs) which reside in the vascular bed (Atwal and Saldahna, 1985, Atwal *et al.*, 1994).

Results for the total cell count of BAL fluid in the goat indicate a total cell count ranging between 6000

and 12000 cells /cu.ml. There is no existing data on the normal ranges of the total cell counts of BAL fluid in the goats for comparison. However, the present results obtained for the goat are far below the normal total cell counts of BAL fluid for the sheep, which is reported to range from 10^5 to 10^6 cells/ cu.ml. (Burrles, 1985) and in cats $10.6x10^6$ (Hawkins *et al.*, 1994). However, the total cell count for BAL fluid reported in the present study compares with total cell count of peripheral blood of the goat of $7.74x10^3$ (Jones and Krebs, 1972).

The present study has provided apparently for the first time, a cytological picture of the BAL fluid in healthy goat. The differential cell counts have shown that the lymphocytes are the dominant cell type as opposed to the macrophages observed in other domestic mammalian species. The present finding suggests that the lung defense mechanisms of the goat may be different from other domestic mammalian species. However more research is required especially on the lymphocyte subpopulations observed in the present study.

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