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Detection of Staphylococcus aureus, Streptococcus spp., and *Mycoplasma spp*. with the study of hematological picture associated with pneumonia in calve

Muna Ali Hussein[3], Bashar Sadiq Noomi[*2]

[1] Directorate of Salah al-Din Education, Tikrit , Iraq
[2] Department of Microbiology , Collage of Veterinary Medicine , Tikrit University, Tikrit , Iraq.

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Corresponding Author:
Name: Dr. Bashar S. Noumy
E-mail: vetbashar1981@gmail.com
Tel: : +9647716184477

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ABSTRACT

The current study aims to determine the dissemination of *Staphylococcus* spp., *Streptococcus* spp., and *Mycoplasma* as causes of calves pneumonia, for this purpose 50 nasal swabs were collected for bacterial culture and DNA extraction, accompanied that 50 blood sample were collected from the same animals and 20 blood samples were collected from healthy calves. The results showed that *Staphylococcus*, *Streptococcus*, and *Mycoplasma* detected at the rates of 52%, 32%, and 26% receptively. From blood's parameter, the results showed a significant decrease in hemoglobin concentration, Red blood cell count, and packed cell volume while a significant increase in total leukocytic counts

1. Introduction

Pneumonia is a common problem in cattle, affecting groups or individuals of all ages and types. It is inflammation of lung tissue, it caused by bacteria, viruses, or parasites, and may be acute, chronic, or progressive. [1].

Many complications of pneumonia may occur like pleuritis, pulmonary abscess, lung consolidation, and acute fibrinous pneumonia. [2]. Pneumonia is caused by a complex interaction between the environment (which produces stress), microorganisms, and the host's immune response. The most common agents of pneumonia in sheep and lambs are *Mycoplasma ovipneumoniae*, *Mannheimia haemolytica*, and *Pasteurella multocida* [3]. Other causative agents include *Escherichia coli*, *Pasteurella multocida*, *Streptococcus pyogenes*, *Streptococcus pneumonia*, *Klebsiella pneumonia*, *Proteus vulgaris*, and *Corynebacterium pyogenes* [4].

Calves may be infected with pneumonia through contamination of environment and contaminated water and food, also the rodents and wild birds play a role on

transmission of infection [5]. . Cough, fever, loss of appetite, and depression are the main signs of pneumonia. The cough may be continuous and mucopurulent nasal discharge appears [2].

2. Material and methods

Animal subjected in the current study: 50 calves (in age from 2 weeks to 6 months) suffering from respiratory signs.

Samples: nasal swabs for bacterial study

Bacterial culture: the nasal swabs cultivation on tryptone soya broth at 37° C for 24h. then subcultured on mannitol salt agar and blood agar. Gram's stain and groups of biochemical tests were applied according to [6]. Genetic methods for detection of *Mycoplasma*

DNA extraction for *Mycoplasma* detection: DNA template was prepared by reactivation of subculturing from tryptone soya broth into brain- heart infusion broth, then DNA extracted by boiling lysis method and according to (5).

Primers: In the current study pairs of primers were used(Table 1).

Table (1): primers used in the current study

Primer	Sequence (59R39)	Fragment size (bp)
(Mycoplasma genus-specific GPO3	*(F) 5'GGGAGCAAACACGATAGATACCCT3'*	270
	5'TGCACCATCTGTCACTCTG-TTAACCTC 3'	

Reaction mixture: 25 µl reaction mixture containing 200 µM of dNTPs, 0.2 µM of each primer, 1.875 mM of $MgCl_2$, and 1.25 U of Taq DNA polymerase, and 50 ng of DNA was added. Thermo-cycler program as in table (2)

Table (2): Thermocycler program applied in the current study

Stage	Temperature (c)		Time	No. of cycles
First Denaturation	95		60second	1
Denaturation step	95		60second	35
Primer-annealing step	*Mycoplasma genus-specific GPO3*	55	60 second	
DNA extension step	72		2mints	
Final DNA extension	72		5 mint	1
End Temperature	4			

Hematological study: 70 blood samples (50 blood samples collected from infected calves and 20 blood samples collected from healthy calves) applied [7]. and include

a- hemoglobin concentration (Hb)
b- red blood cell count (RBCs)
c- packed cell volume (PCV),
d- total leukocytic counts

3.Results and discussion

According to the results of the bacterial culture and PCR test, 64% (32:50) were positive to tests conducted in the current study. The appearance of negative samples to tests conducted in the current study, that's may due to other causes of pneumonia-like Gram-negative bacteria, parasites, and fungi [8].

Result of PCR test: out of 50 nasal swabs *Mycoplasma* spp. detected in 13 samples with the rate of 26% (13:50) and give band with molecular weight 270bp as in Figure (1).

According to results of bacterial culture *Staphylococcus aureus* spp. Detected at the rate of 32% (16: 50). As in table (3).

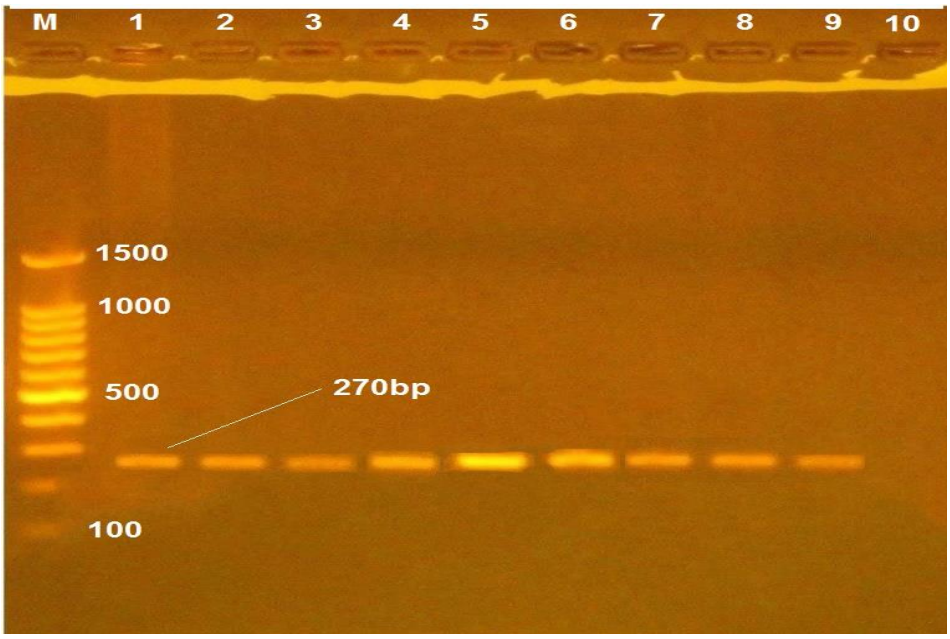

Figure (1): The results of the PCR test. M: 100bp DNA marker, wells 1-7: positive samples of *Mycoplasma* spp. with the band in size 456b

Table (3): Results of bacterial culture and PCR test

Type of isolates	No of isolates
Negative tests conducted in the current study	18
Staphylococcus aureus	8
Mycoplasma	6
Streptococcus spp	5
Staphylococcus aureus + *Streptococcus* spp.	11
Staphylococcus aureus + *Mycoplasma*	7

The isolation of *Staphylococcus* and *Streptococcus* as causes of pneumonia agreed with [5, 6, 9]. With different in isolation ratio, these difference may be due to differences in culture technique, seasonal and location of study, age of the animals, and environmental and management conditions Results of hematological study: Table (4) showed significant differences in

all parameters understudied between the control and infected group.

Table (4): compere between the hematological parameter of the control group and infected group.

Parameters	Control group	Infected group
Hemoglobin concentration (Hb)	14.30±0.56	11.12±0.35
Red blood cell count ($\times 10^6$ /µl)	13.21± 0.19	10.68±0.21
Packed cell volume (PCV),	43.20±0.62	36.70±1.9
Total leukocytic counts ($\times 10^3$/µl)	7.94±2.01	13.06±1.9

The significant decrease in the RBCs parameter (RBCs count, Hb, and PCV) refers to microcytic-hypochromic anemia. This may occur due to an inflammatory process that leads to hyperplastic trapping free iron and hence increases iron storage in phagocytic cells that are lead to a decrease in the production of RBCs in the bone marrow and the reduction in Hb synthesis [10]. The significant increase in the WBCs count in the current study refers to acute inflammation due to bacterial infection, thus causes tissue damage and stimulation of cytokine production [11, 12]. This could be attributed to those infectious agents and products of tissue injury stimulate a variety of cells to release growth factors, cytokines, and other mediators of inflammation that act as prompt stimuli and are all interrelated in causing the increase in total white blood cells count and more production, proliferation, maturation and bone marrow release of mature and immature neutrophils (13, 14).

Conclusion

Staphylococcus aureus is the main causative agent of pneumonia in calves. Pneumonia caused by *Staphylococcus*, *Streptococcus,* and *Mycoplasma* lead to a significant decrease in hemoglobin concentration, Red blood cell count, and packed cell volume while significant increase in total leukocytic counts.

References

1- Naveed M, Javed MT, Khan A and Krausar R (1999): Hematological and bacteriological studies in neonatal lambs with reference to neonatal lamb mortality. Pakistan Vet. J., 19(3): 127-131.

2- Ayling, R.D. and Nicholas, R.A.J., 2007. Mycoplasma Respiratory Infections. In: Aitken, I.D. (Ed.), Diseases of Sheep, 4th ed. Blackwell, Oxford. 231–235.

3- Damassa, A.J., Wakenell, P.S., Brooks, D.L., 1992. Mycoplasmas of goats and sheep. The Journal of Veterinary Diagnostic Investigation, 4, 101–113.

4- Jarveots, T.; Suuroja, T; Lepp, E. (2000). The aetiology and patho-morphology of respiratory disease in calves, Estonia agriculture University, pp. 214-222.

5- Amstutz, H. E. ; Anderson, D. P. ; Benzel, H. A. (2000). The Merck veterinary manual, bovine respiratory disease complex, 8th. Ed. By merk & co. INC. pan - American copyright convention.

6- Quinn, P. J.; Cartet, M. E.; Markey, B. K.; Donellt, W. J.; Leonnard, F. C.; Maghire, D. (2002). Veterinary Microbiology and Microbial disease. Blackwell publishing company.

7- Feldman BF, Zinkl JC and Jain NC (2000): "Schalm's Veterinary Hematology", 5th

(ed.), Lippincott Williams & Wilkins, Philadelphia, London. Feldman BF, Zinkl JC and Jain NC (2000): "Schalm's Veterinary Hematology", 5th (ed.), Lippincott Williams & Wilkins, Philadelphia, London

8- Radostits, O. M; Henderson, J. A; Blood, D. C; Arundel, J. T. and Gay, C. C.(2007). "Veterinary Medicine: A Textbook of the Diseases of Cattle, Sheep, Pigs, Goats, and Horses". 11th Ed; W.B. Saunders Elsevier. UK. Chapter 18: 966-993.
9- Andrews, A. H.; Blowey, R. W.; Bboyd H. and Eddy, R. G. (2004). Bovine medicine disease and husbandry of cattle, calf respiratory disease, 2nd. Ed. By blackwell publishing company.
10- Aytekin İ, Mamak N, Ulucan A and Kalinbacak A (2011): Clinical, hematological, biochemical and pathological findings in lambs with Peste des Petits Ruminants. Kafkas Univ. Vet. Fak. Derg., 17(3): 349-355.
11- Sayed AS, Ali AA, Mottelib AA and Abd El-Rahman AA (2002): Bronchopneumonia in buffalo- calves in Assiut governorate. Studies on bacterial causes, clinical hematological and biochemical changes associated with the disease. Assiut Vet. Med. J., 46(92): 138-155.
12- Obasi, O.L., Raji, M.A., Adogwa, T., Natala, A.J., 2001 Gross pathological lesions in bacterial pneumonia of ovine and caprine hosts in Zaria, Nigeria. Global Journal of Pure and Applied Sciences 7 (1), 57-60.
13- in culture-negative pneumonic lung tissues of bighorn sheep (Ovis canadensis) from outbreaks in the western USA, 2009–2010. *Journal of Wildlife Diseases*, *50*(1), 1-10.
14- Townsend, K. M., Frost, A. J., Lee, C. W., Papadimitriou, J. M., & Dawkins, H. J. (1998). Development of PCR assays for species-and type-specific identification of Pasteurella multocida isolates. *Journal of clinical microbiology*, *36*(4), 1096-1100.

الكشف عن المكورات العنقودية الذهبية والمكورات العقدية والميكوبلازما ودراسة الصورة الدموية المصاحبة للالتهاب الرئوي في العجول

منى علي حسين [1]، بشار صادق نومي[2]

[1] مديرية تربية صلاح الدين – تكريت – العراق
[2] فرع الطب الاحياء المجهرية – كلية الطب البيطري- جامعة تكريت– تكريت - العراق

الخلاصة

الهدف من الدراسة الحالية الى تحديد انتشار المكورات العنقودية الذهبية والمكورات العقدية والميكوبلازما للالتهاب الرئوي في الاغنام وتحديد الصورة الدموية المصاحبة لذات الرئة. ولهذا الغرض جمعت 50 مسحة أنف لغرض الزرع الجرثومي واستخلاص الحامض النووي، كما جمعت 50 عينة دم من نفس الحيوان و 20 عينة دم من حيوانات سليمة لغرض اجراء الصورة الدموية .أظهرت النتائج أن المكورات العنقودية والمكورات العقدية والميكوبلازما شخصت بنسبة 52٪ و 32٪ و 26٪ على التوالي. كما أظهرت نتائج الدراسة الحالية انخفاض معنوي في تركيز الهيموجلوبين وعدد خلايا الدم الحمراء وحجم الخلايا المكدسة مع زيادة معنوية في تعداد الكريات البيض الكلي