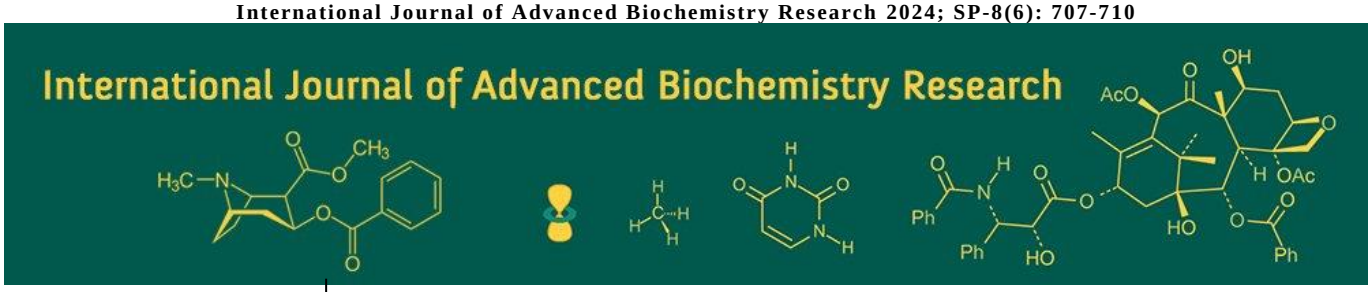

ISSN Print: 2617-4693
ISSN Online: 2617-4707
IJABR 2024; SP-8(6): 707-710
www.biochemjournal.com
Received: 16-04-2024
Accepted: 20-05-2024

Nitesh Yadav
Department of Veterinary Medicine, College of Veterinary Sciences, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, Haryana, India

Sunil Punia
Department of Veterinary Medicine, College of Veterinary Sciences, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, Haryana, India

Neelesh Sindhu
Department of Veterinary Clinical Complex, College of Veterinary Sciences, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, Haryana, India

Tarun Kumar
Department of Veterinary Clinical Complex, College of Veterinary Sciences, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, Haryana, India

Anju Poonia
Department of Veterinary Surgery and Radiology, College of Veterinary Sciences, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, Haryana, India

Maneesh Sharma
Department of Veterinary Clinical Complex, College of Veterinary Sciences, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, Haryana, India

Corresponding Author:
Sunil Punia
Department of Veterinary Medicine, College of Veterinary Sciences, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, Haryana, India

Haematobiochemical alterations in equines with various pulmonary diseases

Nitesh Yadav, Sunil Punia, Neelesh Sindhu, Tarun Kumar, Anju Poonia and Maneesh Sharma

DOI: https://doi.org/10.33545/26174693.2024.v8.i6Si.1403

Abstract
The equine respiratory tract is so highly specialized for exercise that even the slightest deviation from normal can limit a horse's career. The equine respiratory tract has several functions including intake of air for oxygenation of blood, thermoregulation by evaporative cooling, compensatory mechanism and play important role in defense mechanism. Respiratory tract affections of equines include both upper respiratory tract disorders like sinusitis, epistaxis, nasal polyps, strangles, guttural pouch empyema, rhinitis and lower respiratory tract disorders like bacterial and viral pneumonia (broncheo-alveolar and interstitial), pleuritis, neoplasia of lungs etc. Pneumonia the most commonly reported condition in working horses. The present study was conducted in equines suspected for respiratory tract affections reporting to Department of Veterinary Clinical Complex, College of Veterinary Sciences, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar or from an organized farm over a period of 5 months. Pulmonary affections in animals were diagnosed mainly with the help of diagnostic imaging techniques like radiography and ultrasonography. Clinico-pathological studies of pulmonary affections of equines in relation to haemato-biochemical parameters was evaluated and interpreted. Neutrophilic leucocytosis was observed in all the categories of pulmonary affections in the present study There were significant increase in ALP (216.5±19.027), BUN (17.91±1.554), globulin (4.0813±0.153) and triglycerides (45.012±5.414) value as compare to control groups. The mean value of creatinine (1.0875±0.183) and LDH (774.75±33.933) were decreases which may or may not be significant. Hypalbuminaemia was present in all clinically affected pulmonary diseases. The mean value of glucose (62.7±4.484) was significantly decreased mainly in case of fibrinous pleuritis at level of significance (P<0.05) as compare to control group.

Keywords: Equine, hemato-biochemical parameters and pulmonary affections

Introduction
Equines are the animals which grouped under odd toed foot animals in the order Perissodactyla. They are known by serving the community by transportation, and also in some country their meat has been taken as food (Sussi *et al.*, 2001) [16]. India possesses 1.46 per cent of the world's total horse population and stands 12th in the world for horses and 9th for donkeys, mules and hinnies (Parker, 2008) [13]. In earlier days, horses were mainly used for transport and communication but later they became popular as a companion and working animal throughout the world. Horses are the important part of livestock. According to 20th livestock census, total population of Horses, Ponies, Mules & Donkeys in the country is 0.55 million during 2019 which is decreased by 51.9% over previous Livestock Census (2012) (Basic Animal Husbandry and Fisheries Statistics, Government of India 2019). The horses have unique place in the Hindu mythology. In our country, horses mainly used for transportation in hills and fast running saddle horse used for riding and driving carriages used by different organisation/units like Police, Border Security Force. The most common diseases of equine animals are respiratory disease, lymphatic system disease, and GIT disease. Respiratory tract infections are considered the major problem of equine worldwide (Abdisa, 2018) [2]. The equine respiratory tract is so highly specialized for exercise that even the slightest deviation from normal can limit a horse's athletic career (Oke, 2010) [12]. The equine respiratory tract has several functions including intake of air for oxygenation of blood, thermoregulation by evaporative cooling, compensatory mechanism and play

important role in defense mechanism. Disorders of the respiratory system are second in importance only to those of the musculoskeletal system in limiting the athletic performance of the horse and causes major economic loss (Ainsworth, 2004) [4]. The disorders of the respiratory system are responsible for 42% of decreasing performance of athlete horses, second to diseases of the musculoskeletal system (Allen *et al*., 2006; Hewson and Arroyo, 2015) [5, 9]. Predisposing factors for infectious pulmonary disease have been reported to include long-distance transportation with inadequate rest periods, complications of oesophageal obstruction, inhalation of food or saliva, immunosuppression. Respiratory tract affections of equines include both upper respiratory tract disorders like sinusitis, epistaxis, nasal polyps, strangles, guttural pouch empyema, rhinitis and lower respiratory tract disorders like bacterial and viral pneumonia (broncheo-alveolar and interstitial), pleuritis, neoplasia of lungs etc. The infectious viral agents like Equine herpes virus, Equine influenza, Equine rhinitis virus, bacterial agents like *Streptococcus equi* (most common agent of Upper respiratory tract causing Strangles), *Rhodococcus equi* (most common agent of Lower respiratory tract causing Foal Pneumonia) and *Burkholderia mallei* (causing Glanders) and Parasitic agents like lungworm (*Dictyocaulus arnfeldi*, common agent of lower respiratory tract), *Parascaris equorum* commonly affect the respiratory tract of equines. The non-infectious causes include inflammatory airway disease due to exercise intolerance, recurrent airways obstruction due to allergens, neoplasia of trachea, lung, guttural pouch, recurrent laryngeal hemiplegia due to paresis, sinusitis, guttural pouch empyema, tracheitis, epistaxis, pleuritis etc. (Constable *et al*., 2017) [7].

Pneumonia the most commonly reported condition in working horses is basically the inflammation of the pulmonary parenchyma, which may be associated with viruses, mycoplasma, bacteria, (or combination of three), fungi, metazoan parasites, and physical and chemical agents (Constable *et al*., 2017) [7]. Pneumonia is one of the leading causes of morbidity and mortality in foals (Cohen, 1994) [6]. Primary pleuritis is usually due to a perforation of the pleural space and subsequent infection and secondary pleuritis is usually develops from infectious lung diseases. Diagnosis of respiratory tract infections can be made by detailed anamnesis with thorough clinical examination, both physiological and haemato-biochemical parameters, molecular tests like real time PCR for detection of viral pathogens, diagnostics imaging techniques like X-ray, ultrasonography and examination of upper respiratory tract by endoscope. Radiography is an excellent tool to use in conjunction with endoscopy when evaluating diseases of the respiratory tract (Couetil *et al*., 2013) [8]. Radiography is the best technique to characterise lesions deep within the lung when the periphery of the lung is normal. On radiographic examination of thoracic area presence of fluid in pleural spaces, pneumonic changes in lungs, pulmonary cyst may be observed. In recent times ultrasonography is most useful for imaging and discriminating soft tissues and more readily available than radiography to the equine practitioner for the diagnosis of respiratory diseases (Roy *et al*., 2003) [15]. Ultrasonography is useful in diagnosis of pulmonary congestion whereas in case of pleuritis and pleural effusion, hypoechoic fluid with echogenic bands is imaged in the pleural cavity. Ultrasonography is more sensitive than radiography in the diagnosis and characterization of pleural effusion, discrimination between atelectasis and lung consolidation, and detection of small superficial pleural or parenchymal abnormalities. The clinico pathological findings in affected animals include leucocytosis with neutrophilia, low haemoglobin concentration, monocytosis, thrombocytosis, hyperfibrinogenaemia, hyperproteinaemia and hypoalbuminemia. Also increased fibrinogenaemia and increased inflammatory cells in tracheal aspirates can be observed (Constable *et al*., 2017) [7].

Materials and Methods

The present study was conducted in equines suspected for respiratory tract affections reporting to Department of Veterinary Clinical Complex, College of Veterinary Sciences, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar or from an organized farm over a period of 5 months. The present study was conducted in equines suspected for respiratory tract affections. The animals under study were those reporting at Department of Veterinary Clinical Complex, College of Veterinary Sciences, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar for treatment or from an organized farm, over a period of 5 months. Animals exhibiting clinical signs like pyrexia, nasal discharge, coughing, dyspnea, increased vesicular to bronchial breath sounds, trouble breathing (dyspnea), increased respiratory rate (tachypnea), respiratory noise (inspiratory or expiratory), stance with abducted limbs and elongated neck, poor mucous membrane color, poor body condition score and respiratory distress with open mouth breathing etc were recruited in the study. Pulmonary affections in animals were diagnosed mainly with the help of diagnostic imaging techniques like radiography and ultrasonography. Clinico-pathological studies of pulmonary affections of equines in relation to haemato-biochemical parameters was evaluated and interpreted.

Anamnesis

Detailed anamnesis (as per owner) such as age, sex, parity, appetite, coughing, nasal discharge, oedema, respiratory distress etc. was recorded via a self-designed performa.

Clinical examination

Complete clinical examination of the patients was made that included recording of rectal temperature (° F), heart rate, respiration rate, coughing status, auscultation of lungs and heart for abnormal sound, mucous membrane appearance, mandibular lymph node swelling etc. On the basis of primary clinical signs and symptoms like history, nasal discharge, respiratory distress, abnormal lungs sound (crackles or wheezes), open mouth breathing etc., the involvement of respiratory system was considered for further examination. Clinical scoring was done separately as suggested for pulmonary cases by Ohnesorge *et al*. (1998) with some modifications. Parameters were scored from 0 to 4 and the sum of scores yielded a total cumulative score.

Haematological and Biochemical estimation

Collection, processing and preservation of blood samples

A total of five ml blood was collected from jugular vein of the affected patients of which two ml of blood was collected aseptically in K_3Ethylenediamine-tetra acetic acid (K_3EDTA) coated sterile vials for estimation of

Haemoglobin (Hb), Packed Cell Volume (PCV), Total Erythrocyte Count (TEC), Total Leucocyte Count (TLC), Differential Leucocyte Count (DLC) and three ml blood sample was collected in silica particles coated sterile vials and serum was separated after centrifugation at 3000 rpm for 5 minutes and stored at -20℃ in aliquots for further biochemical, electrolyte and blood biomarker estimation. All the samples were labelled and simultaneously recorded. The serum samples were used for analysis of Glucose, Total protein (TP), Serum Albumin, Albumin and Globulin ratio, Serum Creatinine, Lactate Dehydrogenase (LDH), Aspartate Amino Transferase (AST), Alkaline Phosphatase (ALP), Blood urea nitrogen (BUN), Sodium (Na^+), Potassium (K^+), Chloride (Cl^-) and Calcium (Ca^{++}).

Haematology

The Blood samples collected in vials coated with K_3EDTA were immediately analysed for complete haematological examination using fully automated haematology cell counter (MS4s, Melet Schloesing Laboratories and France).

The erythrocytic indices measured were haemoglobin (Hb) in g/dl, packed cell volume (PCV) in %. The leucocytic indices measured were total leukocyte count (TLC) in 10^3 /mm3, differential leukocyte count (DLC) including Neutrophils (N), Lymphocytes (L), Monocytes (M) and Eosinophils (E) in %.

Serum Biochemistry

Fully automated Random-Access Clinical Chemistry Analyser (EM Destiny 180, Erba Diagnostics Mannheim GmbH) was employed for estimation of biochemical parameters using kits procured from Transasia Biomedical Limited. Fully automated Easylyte® expand Na/K/Cl/Ca/Li analyser was also employed for estimation of plasma sodium, potassium and chloride parameters using kits procured from Transasia Biomedical Limited.

Results and Discussion

Table 1: Haematological and electrolyte findings (Mean ± SE) in equines affected with different pulmonary diseases

Parameters		Pneumonia (Mean ± SE)	Fibrinous Pleuritis (Mean ± SE)	Normal/Control (Mean ± SE)
Hb (%)		9.28±0.561[a]	10±0.563[a]	12.625±0.459[b]
TEC (10^6 µL)		7.31±0.616	7.66±0.452	6.75±0.126
TLC (10^3 µL)		13.95±1.786[b]	8.60±1.422[a]	7.33±0.147[a]
DLC (%)	N	81.54±3.299[b]	82±2.360[b]	36.75±1.262[a]
	L	15.54±2.105[a]	14.75±1.655[a]	32.75±1.633[b]
	M	1.54±0.243	1.25±0.163	1.5±0.176
	E	1.72±0.421	1.87±0.515	1.25±0.132
PCV (%)		32.72±2.10	31.05±2.132	34±0.475
Na^+(mEq/L)		131.3±1.09	130.4±0.93	135.3±0.92
K^+(mEq/L)		3.70±0.13	2.99±0.10	3.81±0.15
Cl^-(mEq/L)		94.82±1.56	98.13±2.24	95.13±1.49
Ca^{++}(mEq/L)		9.6±0.33[a]	9.312±0.37[a]	12.07±0.24[b]

[a, b] = Statistically significant between groups at $P<0.05$

Hb=Haemoglobin, TEC= Total Erythrocyte count, TLC= Total leucocyte count, DLC= Differential leucocyte count and PCV= Packed cell volume

Table 2: Biochemical alterations in equines (Mean ± SE) affected with different pulmonary diseases

Parameters	Pneumonia (Mean ± SE)	Fibrinous Pleuritis (Mean ± SE)	Normal/Control (Mean ± SE)
Glucose (mg/dl)	75.009±4.285[a]	62.7± 4.484[b]	77±1.035[a]
Total protein(g/dl)	7.11±0.185[a]	6.7013±0.225[a]	5.075±0.122[b]
Serum albumin(g/dl)	2.7545±0.090	2.62±0.241	3.075±0.141
Triglycerides (mg/dl)	65.4±4.335[a]	45.012±5.414[b]	22.375±2.658[c]
BUN (mg/dl)	18.26±1.558[a]	17.91±1.554[a]	12.25±1.064[b]
ALP(U/L)	251±14.016[a]	216.5±19.027[a]	123.25±4.839[b]
Serum Creatinine(mg/dl)	1.3809±0.103[a]	1.0875±0.183[ab]	0.925±0.079[b]
A:G ratio	0.7064±0.073	0.64±0.081	0.66±0.008
LDH(U/L)	840.355±110.512[a]	774.75±33.933[ab]	553.375±14.522[b]
AST(U/L)	674.818±314.073	458.638±48.420	240.25±10.555
Globulin(g/dl)	4.3564±0.162[a]	4.0813±0.153[a]	2.3375±0.062[b]

[a,b,c] = Statistical significant between groups at $P<0.05$;

LDH- Lactate dehydrogenase, AST- Aspartate aminotransferase, BUN- Blood urea nitrogen, ALP- Alkaline phosphatase, A: G=Albumin: Globulin

Haematological assessments are useful in the clinical diagnosis of infectious, parasitic and metabolic diseases. There were significant increase in ALP (216.5±19.027), BUN (17.91±1.554), globulin (4.0813±0.153) and triglycerides (45.012±5.414) value as compare to control groups. The mean value of creatinine (1.0875±0.183) and LDH (774.75±33.933) were decreases which may or may not be significant. Hypalbuminaemia was present in all clinically affected pulmonary diseases. The mean value of glucose (62.7±4.484) was significantly decreased mainly in case of fibrinous pleuritis at level of significance ($P<0.05$) as compare to control group. Neutrophilic leucocytosis was observed in all the categories of pulmonary affections in the present study. Neutrophils may increase in response to a number of conditions or disorders, including infections (bacteria, viruses, fungi, and parasites), injuries, inflammatory disorders. Similar observation was also observed by Lakritz *et al.* (1993) [10] in 23 foals between 1 and 7 months of age. Increased number of neutrophils is a necessary reaction by the body, as it tries to heal or ward off an invading microorganism or foreign substance (Aiello and Moses, 2016) [3]. Decreased haemoglobin concentration in

pneumonia and fibrinous pleuritis cases indicated anaemia, which may be due to chronic anorexia along with chronically affected animals. Erythropoietin is secreted because of systemic hypoxia and erythrocytosis occur as appropriate compensatory response which occurs in severe pulmonary and heart diseases (Aiello and Moses, 2016) [3]. Hypocalcaemia was observed in all the categories of pulmonary affection. Calcium is transported through the bloodstream as dissolved ions or bound to proteins such as serum albumin. According to Aiello and Moses, (2016) [3] hypocalcaemia can be observed along with hypoalbuminemia, and in present study there was significant decrease in albumin levels in all pulmonary affections categories. Globulin and albumin are positive and negative acute-phase protein respectively that increases in response to inflammatory stimuli. Significant hyperproteinaemia and hyperglobulinemia may be attributed due to positive acute phase response and hypoalbuminemia may occur due to negative acute phase protein nature of albumin. Hyperproteinaemia and hyperglobulinemia could be encountered with active and chronic inflammation, respectively (Reuss and Giguere, 2015) [14]. LDH is an enzyme required during the process of turning sugar into energy for cells and it is present in many kinds of organs and tissues throughout the body, including the liver, heart, pancreas, kidneys, skeletal muscles, lymph tissue, and blood cells. On the other hand, it has been demonstrated that the LDH level increases after an injury to the liver, skeletal muscle, cardiac muscle, and kidney (Meyer and Harvey, 2004) [11]. Out of nineteen cases of pulmonary affection respiratory distress i.e., dyspnoea was present in 94.7% (Including mild, moderate and severe) cases suggests a lower respiratory tract infection which led to hypoxia. In hypoxia anaerobic respiration occurs and body catalyses pyruvate to lactate and therefore it results in a high level of LDH. Hence, the significantly higher value of LDH in pulmonary categories might have been due to the severe respiratory distress and hypoxia. Compression of the atria by pleural fluid may cause an increase in venous pressure in the great veins, decreased cardiac return and reduced cardiac output (Constable *et al*., 2017) [7]. The significant high value of BUN and creatinine in all pulmonary categories might have been due to increase catabolism of body protein and could result as a response to infection while the increase in serum creatinine due to kidney dysfunction after infection (Constable *et al*., 2017) [7]. The non-significant increased activities of AST in all the pulmonary affections categories in present study could be attributed to the hepatic congestion and also due to acute inflammation. These enzyme elevations may have reflected some hepatocellular damage and centrilobular hypoxia.

Conclusion

In conclusion, haematological assessments serve as valuable tools in diagnosing infectious, parasitic, and metabolic diseases, as demonstrated by the significant alterations in various parameters observed in this study. Elevated ALP, BUN, globulin, and triglycerides, along with decreased creatinine and LDH levels, underscored metabolic shifts potentially indicative of disease severity. Hypoalbuminemia and neutrophilic leucocytosis were pervasive in pulmonary conditions, reflecting systemic inflammation and immune response. Concurrent findings of anaemia and hypocalcaemia further highlighted the multifaceted physiological impacts of respiratory distress. The elevated LDH levels in severe cases underscored its role as a marker of tissue damage exacerbated by hypoxia. These insights underscore the complexity of systemic responses in pulmonary diseases, necessitating comprehensive diagnostic strategies for effective clinical management.

References

1. 20th Livestock Census, DADF, Ministry of Fisheries, Animal Husbandry and Dairying, Government of India; c2020.
2. Abdisa T. Systematic Review: Effect of Strangle on Health of Equine Animals and its Prevention. Curr Trends Biomedical Eng & Biosci. 2018;16(1):555927
3. Aiello SE, Moses MA. 2016. The Merck Veterinary Manual, eleven editions. Merck & Co.pl. 3174 (0,457), 0-194.
4. Ainsworth DM, Hackett RP. Disorders of the Respiratory System. Equine Internal Medicine. 2004, 289-353.
5. Allen KJ, Tremaine WH, Franklin SH. Prevalence of inflammatory airway disease in national hunt horses referred for investigation of poor athletic performance. Equine Veterinary Journal. 2006;38(S36):529-34.
6. Cohen ND. Causes of and farm management factors associated with disease and death in foals. J Am Vet Med Assoc. 1994;204:1644-51.
7. Constable PD, Hinchcliff KW, Done SH, Grunberg W. A Textbook of the Diseases of Cattle, Horses, Sheep, Pigs, and Goats (11th Ed. Vol. 1), 2017.
8. Couetil LL, Cardwell JM, Gerber V, Lavoie JP, Leguillette R, Richard E. A. Inflammatory Airway Disease of Horses--Revised Consensus Statement. Journal of veterinary internal medicine. 2016;30(2):503-515.
9. Hewson J, Arroyo LG. Respiratory disease: diagnostic approaches in the horse. Veterinary Clinics: Equine Practice. 2015;31(2):307-36.
10. Lakritz J, Wilson WD, Berry CR, Schrenzel MD, Carlson GP, Madigan JE. Broncho interstitial pneumonia and respiratory distress in young horses: clinical, clinicopathologic, radiographic, and pathological findings in 23 cases. Journal of Veterinary Internal Medicine. 1993;7(5):277-88.
11. Meyer DJ, Harvey JW. Veterinary Laboratory Medicine. 3rd ed. Philadelphia, USA. Elsevier. 2004, p. 169-196.
12. Oke S. The Equine Respiratory System. The HORSE.com. 2017, 24958.
13. Parker S. Status and future of horse industry. In: Textbook of Equine Science. 3rd Edn. 2008;Chapter 2:28-33.
14. Reuss SM, Giguere S. Update on bacterial pneumonia and pleuropneumonia in the adult horse. Veterinary Clinics: Equine Practice. 2015;31(1):105-20.
15. Roy MF, Lavoie JP. Tools for the diagnosis of equine respiratory disorders. Vet Clin North Am Equine Pract. 2003;19(1):1-17.
16. Sussi C, Martuzzi AL, Catalano AL. Characteristics of Horse Meat Consumption and Production in Italy. Dept. PABVQSA Section Zootechnical Sciences and Quality of Animal Production, University of Parma, via del Taglio 8, I-43100 Parma, Italy, 2001.