

قسم : طب الحيوان أمراض الدواجن - كلية الطب البيطري - جامعة أسيوط .
رئيس القسم : أ.د / ابراهيم محمد حسن سكر .

دراسات عن حدوث الالتهاب الشعبي الرئوي في عجول التسيوين
الجاموس في أسيوط . مصر ج ٤٠ : ٤
٢- الأهمية الاكلينيكية لدراسة البروتينات الكلية في الدم وكذلك
عناصرها المختلفة وذلك باستخدام عملية التحليل الكهربى
في العجول المصابة

نور الدين خضر ، على السباعى ، محمد سمير

تم اجراء هذا البحث على عدد ١٢ عجل جاموسى تتراوح اعمارهم من ٩ - ١٢ شهر ويتراوح
وزنها من ١٠٠ - ١٥٠ كجم . عدد ٨ عجول منها كانت مصابة بالالتهاب الشعبي الرئوي تم
اختبارها من قطيع بلغ عدده ما يزيد على ٢٠٠ رأس من حيوانات موجودة بمزرعة الحواشكة لتسمين
العجول أما الأربعة عجول الأخرى فكانت سليمة اكلينيكيًا واستخدمت كضوابط .
تم تقدير كمية البروتينات الكلية وكذلك عملية الفصل الكهربى للبروتينات المختلفة وذلك بواسطة
استعمال أفلام الاجاروز وقد لوحظ الآتى :-
• نقص عام وطفيف في كمية البروتينات الكلية وكذا في كمية الالبومين .
• وقد لوحظ في بعض الحالات ارتفاع نسبة الألفا جلوبيولين خاصة الألفا - ٢ -
وفي حالات اخرى لوحظ نقصها . اما الجاما جلوبيولين فقد ارتفعت بنسبة ملحوظة
في معظم الحالات المرضية التي فحصت .

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INVESTIGATION ON AN OUTBREAK OF ENZOOTIC BRONCHPNEUMONIA IN BUFFALO
CALVES IN ASSIUT, EGYPT, A.R.E.
II - CLINICAL SIGNIFICANCE OF BLOOD SERUM PROTEIN LEVELS AND ELECTROPHORETIC
SERUM PROTEIN FRACTIONATION IN INFECTED CALVES

(With 2 Tables & 6 Figures)

By

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SUMMARY

This investigation was carried out on a total twelve calves. Eight buffalo calves 9-12 months of age were isolated showing severe respiratory distress, while four animals (apparently healthy) served as control.

Horizontal zone electrophoresis on agarose slide was adopted as a mean of indirect diagnosis and interpreting the significance of the serum protein fractions found in such cases. Marked hypoalbuminaemia was evident in all the eight cases examined. In some cases obvious rise in the alpha globulins specially the alpha-2 (27.2%), and in others obvious decrease in the alpha globulin fractions. Most of the cases examined showed marked rise in the gamma globulin fractions.

INTRODUCTION

Respiratory affections in fattening calves are widely spread throughout the world. This problem considered of great financial importance in the veterinary practice in Egypt, in particular the meat producing stations. In cattle respiratory affections is a very complex syndrome responsible for the occurrence of many deaths in the animals being particularly affected. It involves stress factors, bacterial and viral infections (EL-SEBAIE, IBRAHIM, HOFMANN, and AMER, 1983).

Little informations are available regarding the plasma protein contents and its fractionation in buffalo calves suffering a respiratory distress, however, CORNELIUS, (1960) and SCHALM, (1975) accepted that stress in several form altered the total plasma protein concentrations.

In pneumonia in humans, rheumatic fever, tonsillitis and streptococcal infections hypoalbuminemia were detected (CHANUTIN and GJESSING, 1946).

Because of the high resolving power of zone electrophoresis, this is the technique applied in our laboratory. The present study was planned to describe the use of horizontal electrophoresis on agarose slide to study and identify the serum protein fractions found in buffalo calves suffering from respiratory distress. This could serve as a new and more accurate technique for the indirect diagnosis be adopted in veterinary practice.

MATERIAL and METHODS

Twelve buffalo calves 9-12 months of age, and an average 150 kg. body weight were comprised in this study. Eight served as an unhealthy animals as they exhibited the typical symptoms of severe respiratory affections, while four apparently healthy calves act as control throughout the period of the investigation. They were isolated from a herd of over 200 heads buffalo calves belonging to the General Meat Organisation at El-Hawatka station, in the nearby Assiut, Upper Egypt. They had been proved by EL-SEBAIE *et al.* (1983) upon examination of forty-one buffalo calves from the affected herd to harbour *Pasteurella multocida* upon bacteriological examination. The authors were also able to isolate Infectious Bovine Rhinotracheitis (IBR), Mucosal Disease (MD), and Para Influenza Type 3 (PI3) upon viral serotyping.

Proteins of the blood serum samples were estimated using test kits supplied by Boehringer Mannheim, (W. Germany). Regarding serum protein fractions horizontal zone electrophoresis on agarose slide was applied on the same day of blood collection. Using Hamilton syringe 0.8 . of serum to the sample wells of the agarose film was pipetted, processed for approximately 25 minutes using 95 ml. universal barbital buffer of 8.6 pH in each chamber of the cell. At the completion of the electrophoretic separation, the film was then placed in 200 ml. of the amido black 10 B working stain for 15 minute removed from the stain solution and then rinsed in 200 ml. of 5% acetic acid clearing solution, using magnetic stirrer for 30 seconds. The film was then completely dried for 20 minutes, allowed to cool at room temperature, then washed in the 5% acetic acid clearing solution to clear the excess stain prior to drying for one minute with agitation. It is then transferred to a second stirr-stain dish containing clean 5% acetic acid solution, rinsed again for one minute until the excess stain is removed and dried for 15 min. Densitometry of the stained film was performed with the DCD-12 Digital Computing Densitometer (Beckman Instrument Company), fitted with 520 nm. interference filter. Gaussian curves were constructed for each protein fraction.

RESULTS

It is evident from our obtained results (Table 2 and Fig. 1,2,3,4,5 & 6) that marked hypoalbuminaemia was observed in all infected calves. Five fractions were recorded in both, apparently healthy and infected calves (Table 1,2 and Fig. 1,2,3,4,5 & 6), the latter showed varying degrees. No distinal changes were observed for the total serum protein level, with the slight hypoproteinaemia found in same infected calves.

As shown in our results (Table 2 and Fig. 1, Animal No. 1) a noticed increase in both alpha-1 and alpha-2 globulins in comparison with the apparently healthy calves. It is also interesting to record here an obvious decrease in the alpha-2 globulin fraction in calves No. 4,5 and 6 (Table 2 and Fig. 3,4 and 5) as reached down to 8.4%, 12.5% and 10.4% respectively from a normal values of 12.45 to 17.3% for the apparently healthy calves.

A distinct and marked rise in the gamma globulin fractions was evident in all the infected calves in particularly ones showed decrease in the alpha globulin fractions.

DISCUSSION

Concentrations of total serum protein in the calves suffering severe respiratory distress in our gained results (Table 2 and Fig. 1,2,3,4,5 & 6) were evident to show a slight decrease in comparison with the apparently healthy calves. This could be attributed to the stress in its several forms that might be present, whether in the form of bacterial toxins of *Pasteurella multocida* or in the form of viral agents as by PI3, MD and IBR as described by EL-SEBASIE *et al.* (1983). The general unthriftiness may affect worsely the hepatic parenchyma resulting in failure of the protein synthesis. These results seem to agree with those reported by CORNELIUS (1960) who considered febrile diseases to be the most common reasons for hypoproteinaemia.

Serum samples of bronchpneumonic calves (Table 2 and Fig. 1,2,3,4,5 & 6) showed an obvious drop in the quantity of albumin fraction. The same findings were detected in humans by CHANUTIN and GJESSING (1946). The mechanism of albumin synthesis in the animal body was studied by SCHALM (1975) who stated that the control of albumin synthesis lie in the colloidal osmotic pressure changes. CORNELIUS (1960) attributed hypoalbuminaemia to the stress situation to which the infected animals being subjected that mostly accompanied by depression of its synthesis in the liver resulting in greater losses of albumin than globulins.

Of particular interest to mention that our results illustraed in (Table 2, Fig. 1) for the diseased calf No. 1, showed marked increase in the alpha globulins specially the alpha-2 fraction, while distinct decrease was dovious. in calves No. 4,5 and 6 (Table 2, Fig. 3,4 & 5). WINZLER (1955) stated that glycoproteins in the alpha-2 globulin zone is in great concentration and seem to show the greatest change in disease. The author added that this increase could be accounted for the inflammatory processes in the acute period of the disease specially when accompanied by necrosis. Furthermore, the auther considered the increase in the alpha-2 globuln rich in glycoproteins is characteristic of genuinely cellular inflammatory states and is related to the degree and extent of inflammation. Another

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explanation given by GJESSING *et al.* (1947) that the increase in the alpha-2 globulin fraction depended mostly to an increase in the lipoprotein components. The exact mechanism by which alpha-2 globulin fraction decreases has never been satisfactory explained and little investigations were carried out, however the authors suggest that the seat of control might be in the liver.

It has been shown from our results given in (Table 2, Fig. 3,4 & 5) that marked hypergammaglobulinaemia was noticed (Calves No. 4,5 & 6). These results seem to be in close agreement with those reported by AFFONSO, MITIDIERI, RIBEIRO and VILLELA (1960) who stated that the increase in gamma globulins represent in parts an increase in the antibodies, the possibility of an immune response to tissue breakdown products. Another explanation given by SCHALM (1975) that febrile illness cause a loss of nitrogen associated with a rise in energy output and an increase in adrenocortical activity resulting in diversion of amino acids to antibody formation an gluconeogenesis.

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Table (1): Percent Composition Of Blood Serum Proteins and Their Fractionations
in Apparently Healthy Buffalo Calves

Animal No.	Total Proteins g/l	Albumin			Globulins	
		%	%	%	%	%
In	78.05	39.90	17.00	15.50	12.20	15.40
2	85.75	42.10	13.00	17.30	15.90	10.70
3	79.80	45.15	18.60	14.40	11.60	10.35
4	87.15	40.83	17.21	12.45	15.15	14.36

Table (2): Percent Composition of Blood Serum Proteins and Their Fractionations
in Calves Suffering Bronchopneumonia

1	75.24	25.50	22.50	27.20	8.20	16.80
2	77.21	24.10	16.50	11.00	12.40	36.00
3	73.71	27.50	20.40	11.20	18.40	22.50
4	69.03	29.70	21.70	8.40	12.60	27.50
5	74.28	24.60	20.70	12.50	11.80	31.20
6	68.75	27.80	25.20	10.40	15.70	21.90
7	74.82	24.50	20.80	16.70	16.30	22.80
8	73.56	25.60	21.90	17.40	15.60	20.40

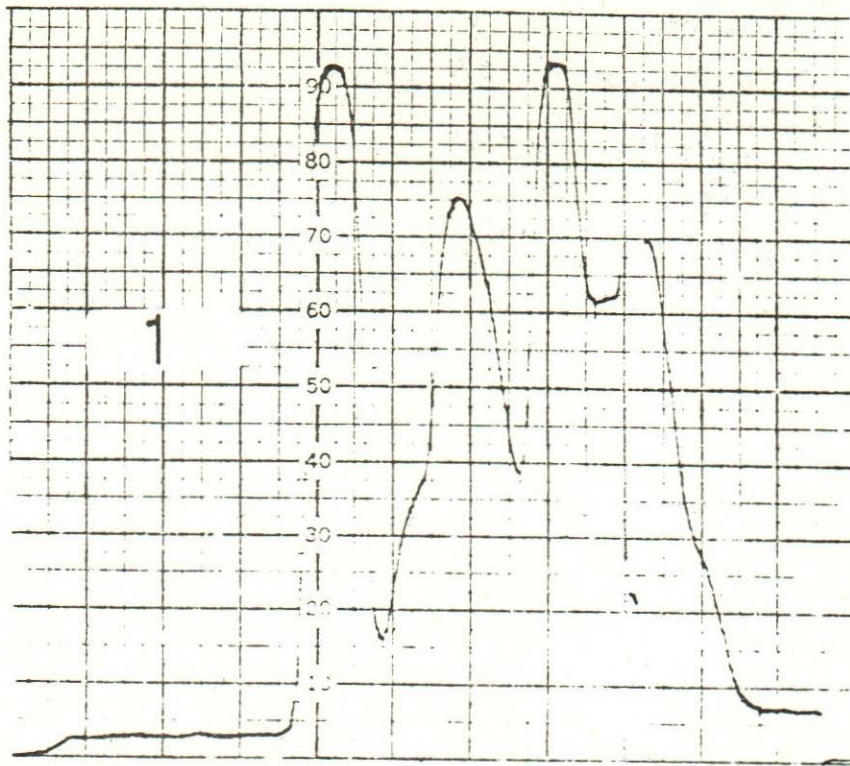


Fig. 1. Electrophoretic Pattern of A Buffalo Calf Suffering Bronchopneumonia. Note the Marked Increase in the -2 Globulin Fraction.

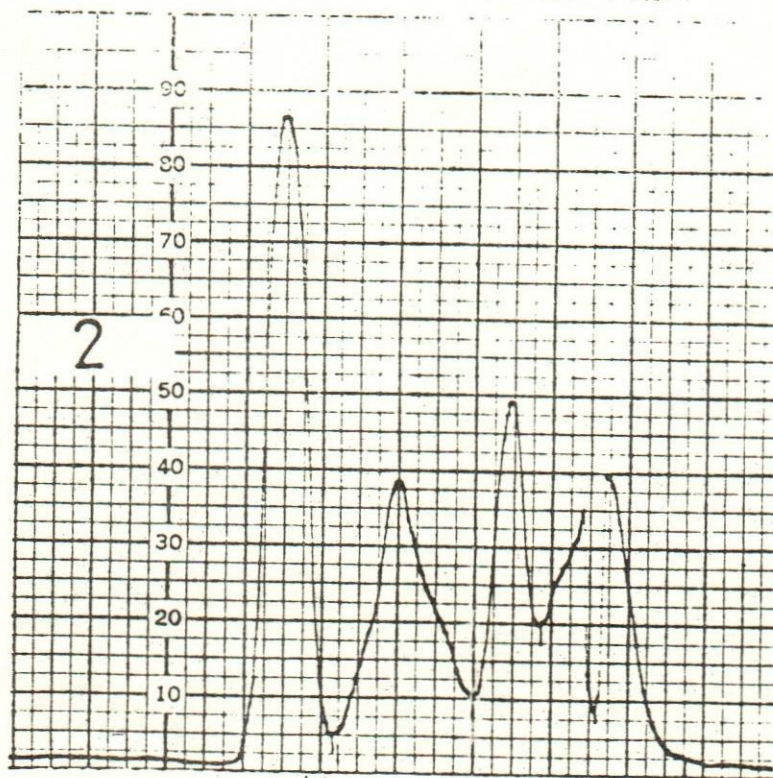


Fig. 2. Electrophoretic Pattern of A Buffalo Calf Suffering Bronchopneumonia.

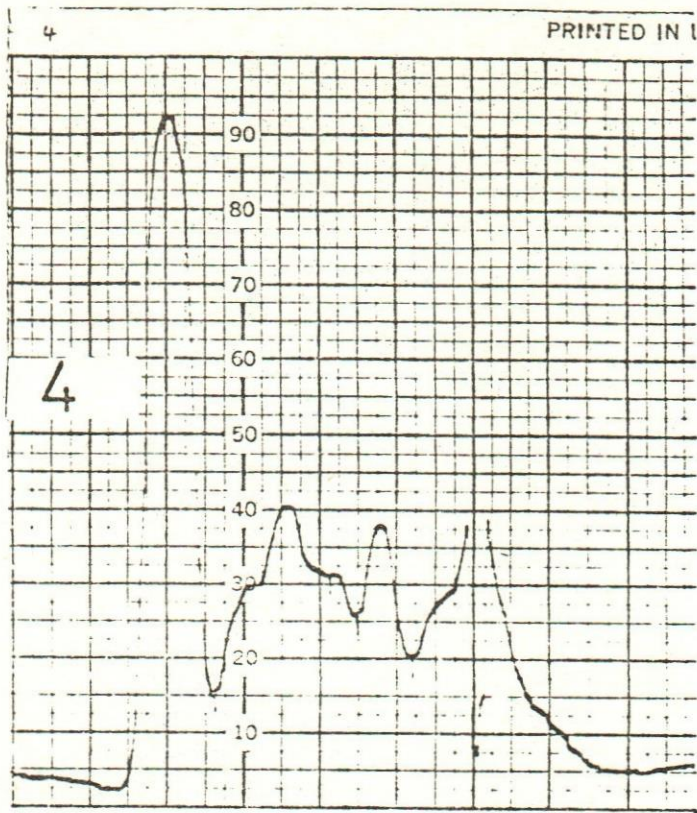


Fig. 3 . Electrophoretic Pattern of A Buffalo Calf suffering Bronchopneumonia . Note the Marked Decrease in the α -2 Globulin Fraction
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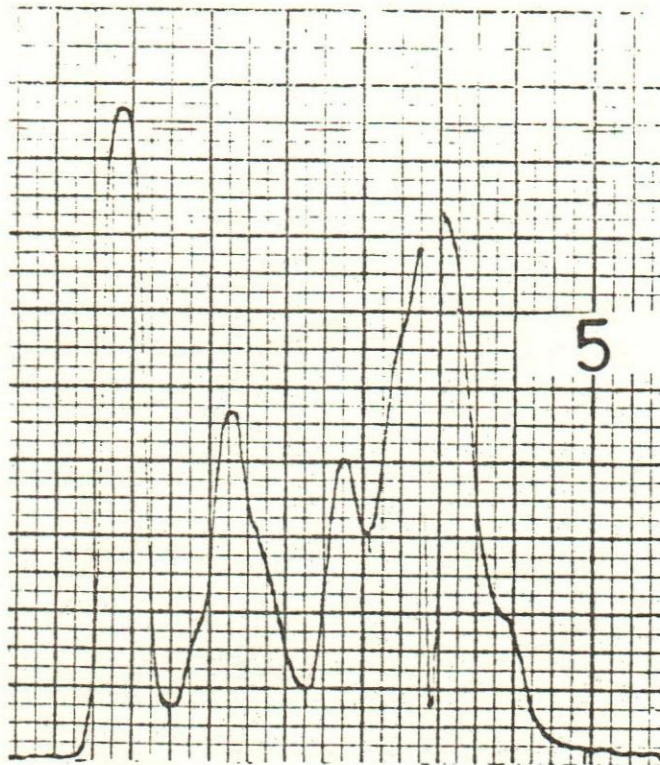


Fig. 4 . Electrophoretic Pattern of Serum Proteins in A Buffalo Calf Suffering Bronchopneumonia . Note the High level of γ -Globulin Fraction .

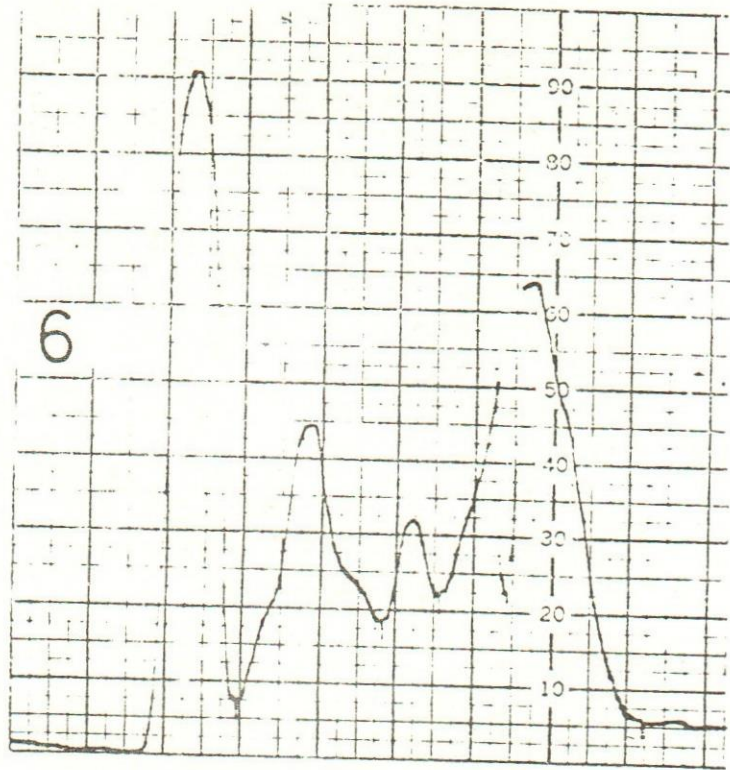


Fig. 5. Electrophoretic Pattern of Serum Proteins in Buffalo Calf Suffering Bronchopneumonia. Note the low level of α -2 and the high level of the γ -Globulin Fractions.

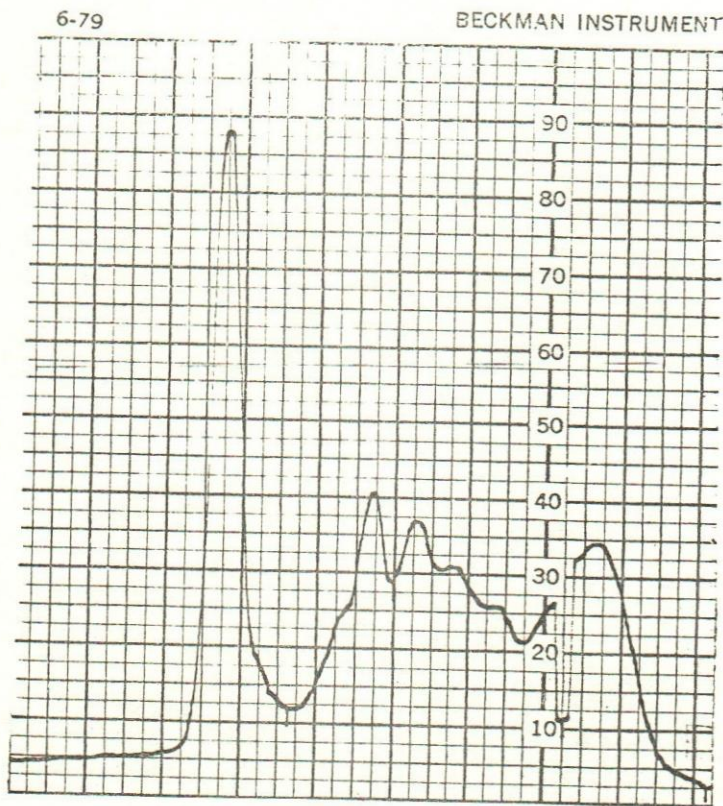


Fig. 6. Electrophoretic Pattern of Serum Proteins in Apparently Healthy Buffalo Calf.

