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**EVALUATION OF ENTEROTUBE II ROCHE FOR
DIAGNOSIS OF SOME BACTERIAL CAUSES
IN NEONATAL CALF PNEUMOENTERITIS**
(With 2 Tables & 1 Fig.)

By

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تقييم استخدام الانتيروتيوب II روش في تشخيص
بعض المسببات البكتريولوجية للإنتهاب الرئوي المعوي في
العجل الفريزيان

مراد اسماعيل ، حلمى صديق ، على السباعى ، ليلى صلاح الدين

أجري هذا البحث في مزرعة الأبقار الفريزيان بمنطقة بني مر بأسبوط حيث تم في هذا البحث دراسة الصفات الاكلينيكية والتشريحية والمسببات البكتريولوجية لعدد 30 حالة من حالات الإنتهاب الرئوي المعوي في العجل الفريزيان حديثة الولادة (12-1 أسبوع) وعُدد ه حالات نافقة بسبب ذات المرض مع وجود 10 حالات سليمة استعملت كضوابط للبحث وقد اتضح من البحث ما يلي : أن أهم أنواع البكتريا المسببة لهذه الحالات كانت بكتريا القولون والباستريلا والبكتريا العنقودية والسبحية والكنيسيليا والستروباكتريا والانتيروباكتريا - وباستخدام تجارب الحساسية للميكروبات المعزولة وجد أن استخدام جرعتين يوميا من الكاناميسين بمعدل 5 مجم -كجم من وزن الحيوان المريض في العضل بالإضافة الي المنشطات والمحاليل يؤدي الي تحسن ملحوظ لمثل هذه الحالات بعد مروره أيام من العلاج - واستخدام الانتيروتيوب II روش في تشخيص الانتيروبكتريا يوفر كثيرا من الوقت والتكاليف وذلك بالمقارنة بإتباع الطرق الروتينية للتشخيص .

SUMMARY

Clinical, bacteriological and postmortem investigations were applied on 30 newlyborn frezian calves suffering from pneumoenteritis in Bani-Mor friezian farm. The study revealed that E.Coli; Pasteurella; Staphylococci; Streptococci; Klebsiella ozaene; Citrobacter freundii; Enterobacter aerogenes and Enterobacter cloacae species were associated with this problem. Using invivo and invitro sensitivity tests, it was shown that Kanamycin injection in a dose of 5 mg/kg.b.wt. twice daily was very efficient in controlling such cases. The study revealed also that the use of Enterotube II Roche for the diagnosis of Enterobacteriaceae infection was very helpful in reducing time and costs.

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INTRODUCTION

Calf enteritis was currently studied by many authors, too many to refer. However, recent studies in this respect were conducted by MORIN, et al. (1979) and ALLEN & WHITE (1985). A variety of laboratory examinations was carried out (according to the available facilities) which in general constitute a laborious task to the examiners.

The aim of the present study was planned to evaluate comparatively the application of a new method recently introduced in the field of bacteriological examination. The study will include also the clinical signs and causes of calf pneumoenteritis at a farm around the city of Assiut beside the application of in vitro sensitivity tests on the isolated strains.

MATERIAL and METHODS

1 - Animals:

A total number of 40 newly born friezian calves (1-12 weeks age) in Bani-Mor friezian farm at Assiut Governorate were the subject of this study. Out of them 25 were suffering from signs of pneumoenteritis, 5 freshly died of this condition and 10 healthy calves as a control group.

2 - Samples:

- a) From the alive diseased cases swabs were collected aseptically from upper respiratory tract and rectum for bacteriological examinations.
- b) From fresh dead cases, lung, liver and intestinal specimens were collected aseptically for bacteriological study after their postmortem examination.

Smears from each samples and specimen were cultivated at 37°C for 24 hs., then cultivated on Blood agar and MacConkey agar at 37°C. Pure Colonies were identified using routine methods described by CRUICKSHANK (1962) and Enterotube II Roche.

3 - Enterotube II Roche*:

The tubes were inoculated with the bacterial suspension (prepared from colonies) and incubated for overnight at 37°C. The results depended on reconstituents of the media in the tube and inturn change in its colour. The reactions were read according to the interpretation tables then the identification of the bacteria was obtained by referring to the identification table using the analytical profile index.

* F. Hoffmann La Roche Company, Basle, Switzerland.

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4 - Susceptibility test discs**:

For the in vitro sensitivity test the following antibacterial agents were used per disc: Kanamycin 30 ug, Erythromycin 15 ug, Streptomycin 10 ug, Pencillin G 10 I.U. and Compound Sulphonamide 300 ug. Kanamycin was then given by injection of each diseased animal intramuscularly with a dose of 5 mg/kg.b.wt. twice daily for 7 days.

RESULTS

In infected cases, the most significant abnormal clinical signs were rise of body temperature (up to 41 C), anorexia, mucopurulent nasal discharge, mouth breathing, painful cough, watery diarrhoea with offensive odour, dehydration and recumbancy in 4 cases. The main P.M. findings in autopsied cases (5) were emaciation, congested intestinal and respiratory mucosae, accumulation of mucopurulent nasal discharge, pleuritis with pleural adhesions and congestion of thoracic and mesenteric lymph nodes. The isolated microorganisms from alive and dead cases are illustrated in Table 1 and Fig. 1.

Results of Enterobacteriaceae identification either by ordinary methods or by using Enterotube II Roche revealed that the results were 100% similar in both methods.

Results of in vitro sensitivity tests are given in Table 2. From the table, it is evident that Kanamycin and Erythromycin are the most sensitive antibiotics. Using one of them (Kanamycin) in vivo, progressive recovery was observed after five days following twice daily i.m. injection of 5 mg/kg b.wt. of the drug, in addition to 3 C.C adrenaline S/C, 3 C.C ADE i.m and 250-500 C.C normal saline i.v. injections once daily to each diseased animal.

DISCUSSION

Clinical signs and P.M. picture of calf pneumonia and enteritis reported in this work were more or less similar to that previously mentioned by BLOOD and RADOSTITS (1989) who identified E.Coli as one of the causes of calve enteritis.

E.Coli was one of the isolated bacteria from calve pneumoenteritis (Table 1). This result was in coincidence with that of several workers (CORBOZ and BECKER, 1973; ALLEN and WHITE, 1985 and LEHNER, et al. 1985) who isolated E.Coli from dead and diseased diarrhoeic calves. Citrobacter Freundii and Klebsiella ozaenae were also

** Oxoid LTD. Basingstoke - Hampshire - England.

isolated; a result which was nearly similar to that reported by CRUICKSHANK (1962) who mentioned that certain species of Citrobacter freundii had been suspected of causing enteric infection. On the other hand the author reported also that Klebsiella pneumoniae was isolated from fatal cases of lobar pneumonia. The author added that Klebsiella ozaenae was a non-pathogenic microorganism.

CRUICKSHANK (1962) and BITSCH, et al. (1976) isolated Pasteurella organisms from diseased animals and lung of dead pneumonic calves; a finding which supports our results.

Enterobacter aerogenes was isolated in the present study from lung and liver of dead animals with pneumoenteritis. This result is supported by CARTER (1979) who considered that such organism is a cause of animal diseases especially mastitis.

The identification of Enterobacteriaceae using routine steps (as morphology, biochemical, serology ... etc) consumes a somewhat long time beside its high costs. Using Enterotube II Roche and its interpretation table for diagnosis of Enterobacteriaceae is thus important as regards time and costs. Available literature lacks much on the comparison between the routine methods and Enterotube II Roche for diagnosis of Enterobacteriaceae. The present result could be considered as a preliminary study for application of Enterotube II Roche for the detection of Enterobacteriaceae in animal diseases.

Based on in vitro results, treatment of diseased animals by intramuscular injection of kanamycin in a dose of 5 mg/kg b.wt. twice daily cleared that a progressive recovery was observed after 5 days as compared with the control healthy calves.

REFERENCES

- Al-Darraji, A.M.; Cutlip, R.C.; Lehmkühl, D.; Graham, D.; Kluge, J.P. and Frank, G.H. (1982): Experimental infection of lambs with bovine syncytial virus and Pasteurella haemolytica. Clinical and microbiological studies. Am. J. Vet. Res., 42: 236-240.
- Allen, S.D. and White, R.D. (1985): Dairy calf diarrhoea. Incidence of infective agents in Northern Utah and Southeastern Idaho. Agri. Practic, 6: 23-24; 28-31.
- Bitsch, V.; Friis, N.F. and Krogh, H.V. (1976): Microbiological study of pneumonic calf lungs. Acta Veterinaria, 17: 32-42.
- Blood, D.C. and Radostits, M. (1989): Vet. Medicine. 7th Ed. A text book of the diseases of cattle, sheep, pigs, goats and horses. Published by Bailliere Tindall, a division of Cassell Ltd, 1st Anne's Road, Eastbourne BN21 3UN.
- Carter, G.R. (1979): Diagnostic procedures in Vet. Bacteriology and mycology 3rd Ed. Charles, C. Thomas publisher, Springfield. Illinois. U.S.A.
- Collier, J.R.; Chow, T.L.; Benjamin, M.M. and Deem, A.W. (1960): The combined effect of infectious bovine rhinotracheitis virus and Pasteurella haemolytica on cattle. Am. J. Vet. Res., 21: 195-198.

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- Cruickshank, R. (1962): Handbook of bacteriology. A guide to the laboratory diagnosis and control of infection: 10th Ed. E and S. Livingstone Limited, Edinburg and London.
- Corboz, L. and Becker, M. (1973): Neonatal diarrhoea in the calf; enterotoxic activity of E.Coli isolated from dead diarrhoeic calves. Schweizer Archiv fur Tierheilkunde 4: 149-159.
- Lehmkuhl, H.D. and Gough, P.M. (1977): Investigation of causative agents of bovine respiratory tract disease in beef cow calf herd with an early weaning program. Am. J. Vet. Res., 38: 1717-1720.
- Lehner, B.; Baumgartner, W.; Nowotny, N.; Awad-Masalmeh, M. and Hinaidy, H.K. (1985): Evidence from faecal consistency in the identification of causes of calf diarrhoea. Praktische Tierarzt, 66: 308-317.
- Morin, M.; Lariviere, S.; Lallier, R.; Begin, M.; Roy, R. and Ethier, R. (1979): Neonatal calf diarrhoea; pathology and microbiology of spontaneous cases in dairy herds and incidence of the enteropathogens implicated as etiological agents. Vet. infectious diseases organization, 347-367.
- Pirie, H.M.; Pringle, G.R.; Allan, E.M. and Kennedy, G.J. (1981): Acute fatal pneumonia in calves due to respiratory syncytica virus. Vet. Rec., 104: 411-416.

Table 1: Type and site of isolated bacteria from diseased and dead calves.

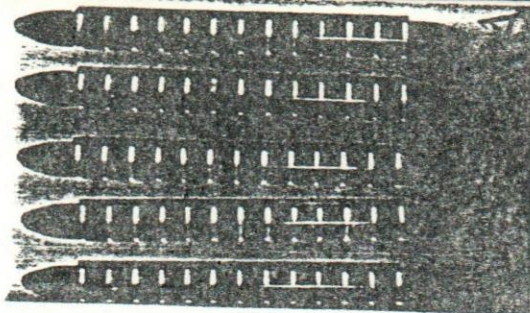
Animal	Type of bacteria	Site of isolation
Diseased	<u>E. Coli</u>	Rectal swabs
	Staph. Spp, Strept. Species Pasteurella Spp.	Upper respiratory tract swabs
Dead	<u>E.Coli</u> , <u>Citrobacter freundii</u> , <u>Enterobacter cloacae</u> , <u>Klebsiella</u> <u>Ozaenae</u>	Intestinal tract
	<u>Enterobacter Aerogenes</u> and <u>Klebsiella Ozaenae</u>	Liver
Dead	<u>E.Coli</u> , <u>Citrobacter freundii</u> <u>Klebsiella Ozaenae</u> , <u>Enterobacter</u> <u>aerogenes</u> and <u>Pasteurella Spp.</u>	Lung

Table 2: Type of bacteria and its in vitro sensitivity tests

Type of Disc	Microorganism					
	Citroaacter +	Pasteurella	Clostridia	E. Coli	Staph.	Strept.
	Enterocacter Spp.	Spp.	Spp.		Spp.	Spp.
Zanasylin 30 ug	+++	+++	+++	+++	++	+++
Erythromycin 15 ug	+++	++	++	+++	+++	+++
Streptomycin 10 ug	++	+	++	++	+	+
Penicillin G.10 IU	+	+	+	+	+	++
Compound Sulphonamide 300 ug	++	++	+	+	++	+

Abbreviations:

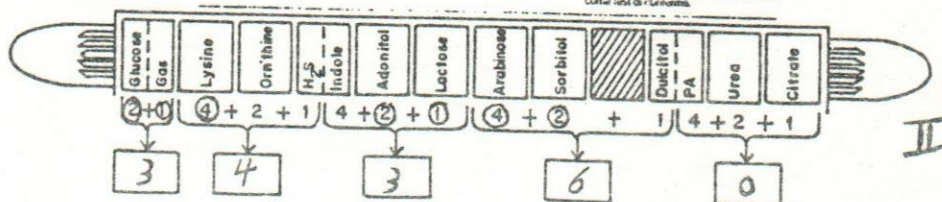
- ++++ Very highly sensitive
- +++ Highly sensitive
- ++ Moderately sensitive
- + Slightly sensitive.



<Enterotube> II Roche

Trade Mark

Voges-Proskauer (VP) utilized as
confirmatory test only
Voges-Proskauer (VP) wird nur als
Zusatztest verwendet
Voges-Proskauer (VP) utilisé seulement
comme test complémentaire
Voges-Proskauer (VP) utilizzato solamente
come test di conferma



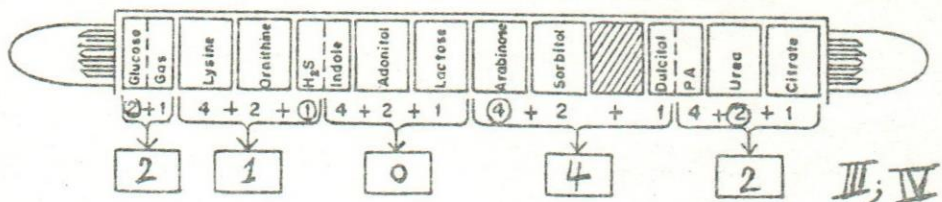
Patient name _____ Date _____ Organism identified _____

Klebsiella ozaenae

<Enterotube> II Roche

Trade Mark

Voges-Proskauer (VP) utilized as
confirmatory test only
Voges-Proskauer (VP) wird nur als
Zusatztest verwendet
Voges-Proskauer (VP) utilisé seulement
comme test complémentaire
Voges-Proskauer (VP) utilizzato solamente
come test di conferma



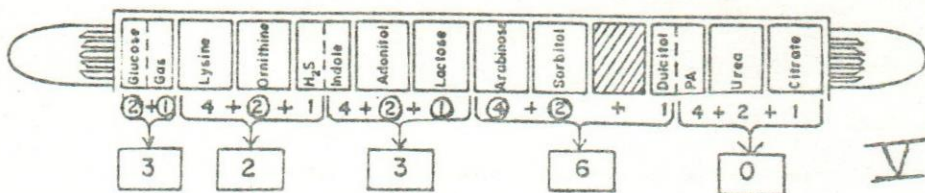
Patient name _____ Date _____ Organism identified _____

Citrobacter freundii

<Enterotube> II Roche

Trade Mark

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Voges-Proskauer (VP) wird nur als
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comme test complémentaire
Voges-Proskauer (VP) utilizzato solamente
come test di conferma



Patient name _____ Date _____ Organism identified _____

Klebsiella ozaenae; *Enterobacter aerogenes*; *Enterobacter cloacae*

Diagramatic scheme for Enterotube II Roche