Vet. Med. J., Giza. Vol. 40, NO. 2 (1992): 9-13.

A CASE REPORT ON THE ISOLATION OF ACTINOBACILLUS LIGNIERESII FROM LAMBS

By

G. ABD EL-GABER* , Z.M. EL-SAYED* and N.A. EL-DANAF**

*Animal Health Research Institute , Dokki , Giza.
**Animal Reproduction Research Inst., Al-Ahram , Giza.

(Received : 17.2.1992)

INTRODUCTION

A. lingnieresii produces a disease, which in many respects resembles actinomycosis. The disease is sporadic in nature and of low incidence, but some individual enterprises encounter high recoveries and significant losses (**Wilson et al. 1983** and **Kimberling , 1988**).

Actinobacilli were isolated from different animal species and ducks wherease Allen (1976) recovered *A. actinoides* from calves suffering from bronchopneumonia. Among equines, **Webb et al.** (1976) isolated *A. equuli* from an aborted foetus but **Carman** and **Hodges** (1982) recovered *A. suis* from different ages of ill horses. In swine, **Pedersen** (1977) reported *A. equuli* and *A. suis* from piglets and adult ones. On the other hand, **Kielstein et al.** (1981) isolated these biovars from pigs suffering from broncho-pneumonia. Meanwhile in ducks, *A. lignieresii* was reviewed by **Bisgaard** (1975).

This organism produces lesions in sheep involving the skin especially in the head region (**Krieg and Holt, 1984**). In lambs of 2 to 8 weeks old, **Rahaley and White** (1977) isolated organisms resembling *Histophilus ovis* in association with synovitis, septi caemia and/or abscessation. Similar organisms were also reviewed from 2 sheep flocks with a high neonatal mortality rate. They suggested that these organisms may be better classified in the Genus Actinobacillus.

The authorities informed that morbidity and mortality rates among newly born lambs (5-7 days old) had been occurred 25 times in different labours during 4 successive months. These lambs originated from a herd of 25 dams that showed recent labours which were located at a farm in Kaliobia Governorate.

Affected lambs were suffering from rise of temperature (41 - 41.5°C) and acute inflammation of the gum of the lower jaw. Later on, they stopped suckling, then died within a week. No cases had been reported in the vicinity.

The present work was achieved by investigation of the causative

bacterial agent from the mouth lesions of newly born lambs, studying its pathogenicity in susceptible laboratory animals and its sensitivity to available antibotics.

MATERIAL AND METHODS

Lesions from the gum of the lower jaw of 2 diseased newly born lambs were collected for bacteriological examination.

Isolation and identification of the causative agent was carried out according to the criteria described by Wilson et al. (1983); and Krieg and Holt (1984) as follows:

The samples were cultivated aerobically and anaerobically on nutrient broth, sheep blood agar and MacConkey agar for 24 - 72 hrs at 37°C. Also, several subcultures on liquid and solid media were performed.

Smears from the lesions and suspected colonies were stained by Gram's and modified Ziehl-Neelsen's stains. At the same time, the colonies were stained by wet India ink preparations.

The following biochemical tests were used for identification of the isolates: Fermentation of sugars (glucose, lactose, maltose, mannitol, sucrose, galactose, mannose, trehalose, arabinose, dulcitol, inositol and sorbitol), indole, methyl red, Voges-Proskauer, citrate and gelatin liquifaction.

Baby guinea pigs (3 - 5 days old) as well as adult ones were inoculated for investigating pathogenicity of the isolates. A group of newly born guinea pigs was infected by the oral route and another one was inoculated subcutaneously with 2×10^9 CFU. Adult guinea pigs were inoculated either subcutaneously or intraperitoneally with the same dose, then with 20×10^9 CFU.

Sensitivity tests were performed on the basis of Cruickshank (1972); and Russell and Quesnel (1983).

RESULTS AND DISCUSSION

Viral and mycotic examination of the samples routinely were found to be negative. *A. lignieresii* was recovered from the tissues of the gum. This could be regarded as a first record of its isolation from sheep in Egypt.

As recorded by Wilson et al. (1983); and Krieg and Holt (1984), the isolated organism was Gram negative bacillus (exhibited irregular staining), pleomorphic, facultative anaerobic with coccal elements which often lied at the poles giving the characteristic "Morse code" form. The organism was non-sporing, non-capsulated and non-motile. By modified Ziehl Neelsen's stain, the clubs appeared red and the bacilli were blue. Wet

India ink preparations exhibited small amounts of extracellular slime around the bacilli.

The isolates produced non-haemolytic, greyish colonies of 2 mm diameter when grown on sheep blood agar for 24 hours at 37°C. After 72 hours, the organism showed paint brush haemolysis at 4°C. On MacConkey agar plates, lactose fermenting colonies of the same size were observed 24 hours post-incubation at 37°C.

On liquid medium, the organism formed sticky growth on primary isolation from the tissues while on repeated subcultures, the organism produced uniform turbidity accompanied by a slight deposit.

The organism produced acid only from glucose, lactose, maltose, mannitol, sucrose, galactose, mannose and trehalose. It does not ferment arabinose, dulcitol, inositol and sorbitol. The isolates were indole negative, methyl red negaive, Voges-Proskauer positive and citrate positive. It does not liquify gelatin.

Administration of 2 X 10^9 CFU of this organism in baby guinea pigs was non-pathogenic by the oral route. Meanwhile, it was lethal after 24 hours when inoculated subcutaneously. Adult guinea pigs that inoculated subcutaneously or intraperitoneally with the same number of bacteria were not affected. On the other hand, those inoculated by 20 X 10^9 CFU subcutaneously survived and those injected intraperitoneally died after 24 hours. This means that, its pathogenicity in guinea pigs depends mainly on the number of administered bacteria and their route of administration in relation to age of guinea pigs.

Recovery of Actinobacillus from guinea pigs was carried out by **Lentsch and Wagner (1980)** who isolated *A. lignieresii* and *A. equuli* from guinea pigs. At the same time, **Boot et al. (1983)** recorded 88 isolates from 69 out of 279 guinea pigs (25.0%) belonging to the Pasteurella - Actinoacillus group and concluded that its members must be considered potentially pathogenic for guinea pigs.

P.M. examination of dead baby guinea pigs showed gelatinous oedema with severe subcutaneous inflammation, enlargement and congestion of regional lymph nodes and general congestion of internal organs. Dead adult ones post-intraperitoneal inoculation showed a septicaemic picture represented by congestion of all internal organs with presence of diffuse amount of peritoneal fluid. Congested liver and enlarged spleen were surrounded by fibrinous membrane. Congestion also included lungs, kidneys, suprarenal glands and both testicles.

The organism was re-isolated from subcutaneous exudate, peritoneal fluid, heart blood, liver,

spleen, lungs and kidneys of dead baby and adult guinea pigs. On the other hand, Lentsch and Wagner (1980) isolated *A. lignieresii* and *A. equuli* from oropharynx, conjunctiva and middle ear of guinea pigs. Boot et al. (1983) also isolated such organism from pneumonic lung, enteritic jujenum and inflammed mammary glands of guinea pigs.

The main predisposing factors that caused the disease in newly born lambs were due to undeveloped immune system and stress factors. During labour, infection of lambs may arised from genital passages (Krieg and Holt, 1984) and/or contaminated soil (Kimberling, 1988). In relation to post-delivery, transmission of infection may be attributed to licking offsprings by dams whereas the organism lives commensally in their mouth (Phillips, 1961) and/or through suckling mastitic udders (Laws and Elder, 1969 b).

Among lambs, rise of body temperature was due to septicaemia caused by this organism or may account for secondary invadors. Mortality of lambs might be due to developed mouth lesions which were painful enough to hinder their suckling or due to septicaemia.

Frequency of appearance of morbid cases may be accounted to bad hygienic measures, persistance of causative agent on the premices or to stress factors.

Localization of *A. lignieresii* in this herd without its transmission to neighbour farms was proved by Krieg and Holt (1984) who reported that actinobacilli were usually of sporadic nature, but occasionally a group of animals may be affected when a common trigger factor was present. That is to say that the infection is endogenous in origin (Cruickshank, 1972).

In vitro sensitivity tests, the authors found that this organism was sensitive to Tetracycline Hcl (18 mm in diameter), Nitrofurantoin (15), Nalidixic acid (14), Oxytetracycline (14). Meanwhile, it was resistant to Ampicillin, Amoxicillin, Erythromycin and Gentamicin.

Additional field studies would provide informations on tracing this syndrome to apply antibiotics for the treatment of diseased lambs but this was out of authors hands.

SUMMARY

Actinobacillus lignieresii was incriminated as a cause of mouth lesions in newly born lambs suffering from oral acute inflammations. This is the first record of its isolation in Egypt. The organism was found to be pathogenic for guinea pigs. The isolates were sensitive to Tetracycline Hcl, Nitrofurantoin, Nalidixic acid and Oxytetracycline respectively.

ACKNOWLEDGEMENT

Authors thanks are due to Prof. Dr. E.M. Sayour, Professor Emer-

itus, Department of Bacteriology, Animal Health Research Institute for his advice, supervision and tracing identification of the isolates in this syndrome.

REFERENCES

Allen, J.G. (1976): Broncho-pneumonia in calves in Western Australia associated with Actinobacillus actinoides. Aust. Vet. J., 52 (2), 100.

Bisgaard, M. (1975): Characterization of atypical Actinobacillus lignieresii isolated from ducks with salpingitis and peritonitis. Nordisk Veterinaermedicin, 27 (7/8), 378-383.

Boot, R.; Oosterom, J. and Walvoort, H.C. (1983): Recovery of members of the Pasteurella-Actinobacillus gorup from guinea pigs. Lab. An., 17 (4), 285-289.

Carman, M.G. and Hodges, R.T. (1982) : Actinobacillus suis infection of horses. New Zealand Vet. J., 30 (6), 82 -84.

Cruickshank, R. (1972): Medical Microbiology. 11th Ed., Printed in Great Britain by T. & A. Constable LTD., Hopetoun street. Printers to the University of Edinburgh.

Kielstein, P.; Bocklisch, H. and Zepezauer, V. (1981): Haemorrhagic necrotizing pleuropneumonia of swine caused by actinobacilli. Archiv fur Experimentelle Veterinarmedizin, 35(6), 879-902.

Kimberling, C.V. (1988): Jensen and Swift's Diseases of sheep. 3 rd Ed., Philadelphia.

Krieg, N.R. and Holt, J.G. (1984): Bergey's manual of systematic bacteriology. Volume 1, 570 575.

Laws, L. and Elder, J.E. (1969b): Mastitis in sheep caused by Actinobcillus ligniere sii. Aust. Vet. J., 45, 401 - 403.

Lentsch, R.H. and Wagner, J.E. (1980): Isolation of Actinobacillus lignieresii and Actinobacillus equuli from laboratory rodents. J. Clin. Microb., 12 (*3), 351-354.

Pedersen, K.B. (1977): Actinobacillus infections in swine. Nordisk Veterinaermedicin, 29 (3), 137-140.

Phillips, J.E. (1961): The commensal role of Actinobacillus lignieresii. J. Path. Bact., 82, 205.

Phillips, J.E. (1964): Commensal actinobacilli from the bovine tongue. J. Path. Bact., 87, 442.

Rahaley, R.S. and White, W.E. (1977): Histophilus ovis infection in sheep in Western Victoria. Aust. Vet. J., 53 (3), 124-127.

Russell, A.D. and Quesnel, L.B. (1983): Antibiotics; Assessment of antimicrobial activity and resistance. Academic Press Inc. (London). Copyright by the society for applied bacteriology, technical series No. 18. Printed in Great Britain by St. Edmunds, Suffolk.

Webb, R.F.; Cockram, F.A. and Pryde, L. (1976): The isolation of Actinobacillus equuli from equine abortion. Aust. Vet. J. 52, (2), 100-101.

Wilson, G.; Miles, A. and Parker, M.T. (1983): Topley and Wilson's; Principles of bacteriology, virology and immunity 7th Ed., Volume 2, 43-45.