THE PROTECTIVE VALUE OF AN AUTOGENOUS BACTERIN AND TOXOID AGAINST EXPERIMENTAL *CORYNEBACTERIUM PYOGENES* INFECTION IN MICE

By

S. FALADE and O.A. DUROJAIYE,
Department of Veterinary Pathology,
University of Ibadan, Nigeria

SUMMARY

Bacteria and toxin from a borth culture of a porcine strain of *Corynebacterium pyogenes* were lethal to white Swiss mice within 24th of inoculation. The pyogenic factor was shown to be a component of the bacterial cells and not of the toxin. Mouse-protection tests using a formolised bacterin and formolized and heat-inactivated toxoide revealed that only the formolized toxoid conferred protection to experimental challenge. It is suggested that this toxoid may be of value in protecting animals against the natural infection.

INTRODUCTION

The role of *Corynebacterium pyogenes* in livestock diseases has been reviewed by several workers including McCracken and McCaughey (1973), Ueda *et al.* (1972), Smith *et al.* (1971), Suzuki *et al.* (1973), Smith (1966), Smith and Reynolds (1911) and Collier (1956). Several attempts have been made to control infections with this organism, particularly those occuring as outbreaks. Such methods include antibiotic therapy, Surgery, segregation and slaughter of badly affected animals and improved hygiene.

Corner *et al.* (1965), found the use of an autogenous bacterin consisting of a killed suspension of hemolytin streptococci 75%, and staphylococci 25%, and a commercial bacterin* consisting of pyogenic streptococci 50%, *C. pyogenes* 30%, *Staphylococcus aureus* 10% and *Escterichia coli* 10% in the control of swine abscasses caused by these orgenisms was of value. Oladosu and Felade (1976), investigated an outbreak of multiple abscesses involving a hard of 350 Duroc pigs at Ikorodu Farm Settlement, Lagos State of Nigeria involving *C. pyogenes.* This paper presents the findings of a preliminary attempt to assess the protective value of a *C. pyogenes* bacterin, heat-inactivated toxoid and formolized toxoid prepared from this porcine strain in experimentally infected white Swiss mice.

MATERIALS AND METHODS

Preparation of Toxoid

A 24th broth culture of the *C. pyogenes* strain was centrifuged and the supernatant recentrifuged and checked for sterility by plating on blood ager. An eliquot of this supernatent was washed twice in 0.85% saline and was heated at 65°C for 1hr. (heat-inactivated toxoid). To the remainder, an equal volume of 0.3% formalin in phosphate buffered saline, pH 7.4, was added (formolized toxoid).

Preparation of Bacterin

The bacterial deposit from above was washed twice in 0.85% saline and a viable count performed by the method of miles and Misra (1938) giving a result of 1.4 x 10^7 bacteria per ml. The bacterial suspension was then treated with an equal volume of 0.3% formalin in phosphate buffered saline, pH 7.4 (bacterin). The bacterin was checked for sterility.

To determine the challenge dose, graded doses of bacteria and toxin 0.1, 0.2, 0.3, 0.4, and 0.5ml were inoculated intraperitoneally into two groups of mice; that mice died within 24th. However, tenfold dilutions of 0.1ml amount of both toxin and bacteria produced no deathe of mice. It was therefore decided to use 0.1 ml as challenge done for toxin and bacterial suspension throughout the investigation.

Five groups of mice weighing between 10.15g were immunized intraperitoneally as follows:-

* Mixed Bacterin (Bovine) Formula No. 2
Pitman-Moore Co., Indinapolis Ind.

Group A: 6 mice each immunized with 0.1 ml of bacterin.
Group B: 6 mice each immunized with 0.1ml of formolized toxoid.
Group C: 6 mice each immunized with 0.1ml of heat-inactivated toxoid.
Group D: 6 mice (unimmunized)
Group E: 6 mice (unimmunized).

The mice were kept under observation until the 11th day when they were challenged by the intraperitoneal route. Three mice in groups A, B, C, and D each received 0.1 ml of the bacterial suspension, and the remaining three in these groups each received same amount of toxin. All the six mice in group E were left as the unchallenged control. Dead mice n the respective groups were examined for post-mortem lesions at intervals. Surviving mice were later challenged on the 20th day with 0.2ml of bacterial suspension and toxin as shown in Table 1. On the 40th day survivors were killed with chloroform and examined for post-mortem lesions.

RESULTS

All unimmunized mice in Group D died within 24th of innoculation. Post-mortem examination revealed pulmonary, hepatic and splenic congestion and hemorrhages, intercostal acchymosis, and hypertrophy of the liver and adrenal glands. The observations on the immunized mice are summarized in Table 1.

Post-mortem findings on the 21st Day in the toxin-challenged mice were mainly those of tomaemia and in the bacteria-challenged group, multiple abscesses were seen in the lung, kidneys and peritoneum, with marked congestion of

TABLE 1

Deaths and Survivals in immunized and control mice experimentally challenged with *C. pyogenes* bacterial suspension and filtrable toxin

Group	*Day 0 Vaccination No.*	*Day 1 (One) Survival*	*Day 11*	*Day 12 Survival**			*Day 19 Survival*	*Day 20*
				a.	*b.*	*c.*		
A	6	6		2	3	5	5	
B	6	6		3	—	3	4	
C	6	6		2	—	2	2	
D	6	—		—	—	—	—	
E	6	6		—	—	6	6	

Day 12 Survival	*Day 26 Survival*	*Day 30 Survival*	*Day 40 Survival*
1	1	1	1
4	3	3	3
2	—	—	—
—	—	—	—
		6	6	6	6

CHALLENGE (FIRST DOSE)

CHALLENGE (DOUBLE DOSE)

* *Group A*
a — bacteria immunized challenged with bacteria
b = bacteria immunized challenged with toxin
c = Total survival

Groups B,C.
a = Toxoid immunized challenged with toxin
b = Toxoid immunised challenged with bacteria.

the liver and spleen. All surviving mice killed on the 40th day had no significant postmortem findings with the exception of one mouse in Group A which had peritoneal abscesses yielding a pure culture of *C. pyogenes*.

DISCUSSION

Both the bacterial suspension and the centrifuged toxin were highly lethal for mice. The fact that abscesses were observed in the bacteria-challenged groups of mice only, indicated that the pyogenic factor was associated with the bacterial cells and not the toxin. Similar observations were made by Lovell, Foggie and Pearson (1950).

Based on the number of surviving mice on the 40th day, the formolized toxoid conferred a greater degree of immunity than the bacterin. The heat-inactivated toxoid was of no value in protecting mice against experimental challenge with either bacteria or toxin and, whereas the bacterin protected mice against toxin challenged, the formolized toxoid was relatively ineffective in protecting mice challenged with the bacterial suspension. An attempt is being made to assess the efficacy of the formolized toxoid in Duroc breeds of pigs.

ACKNOWLEDGEMENTS

Authors are grateful to Professor K. C. Sellers, Head of Department of Veterinary Pathology, University of Ibadan for his criticism of the text and to Mr. P. T. Okoh for secretarial assistance.

REFERENCES

COLLER, J.R. (1956) Abscesses of the pharyngeal region of swine. Bacteriological examination of exudates. Am. J. Vet Res. 17, 640—642.

CONNER, G.H; HOEFER, J.A. and ELLIS, D.E. (1965) Evaluation of Bacterins for Control of Swine abscess. J.A.V.M.A. 147, 479—483.

DERBYSHINE, J.B. and MATTHEWS, P.R.J. (1963) Immunilogical studies with *Corynebacterium pyogenes* in mice. Res. Vet. Sci. 4, 537—542.

LOVELL, R., FOGGIE, A. and PEASON, J.K.L. (1950) Field trials with *Corynebacterium pyogenes* alum precipitated toxoid. J. comp. Path. Ther. 60, 225.

McCRACKEN, A. and McCAUGHEY, W. J. (1973) A survey of abscesses in bacon weight pigs. Brit. Vet. J. 129, 359—361.

MILES, A.A. and MISRA, S.S. (1936) The setimation of the bactericidal power of blood. J. Hyg. (Camb.) 38, 732.

OLADOSU, L.A. and FALADE, S. (1976) Chronic and intractable porcine abscesses associated with *Corynebacterium pyogenes* and Norardia species in the Lagos and Oyo State of Nigeria. Proc. Nig. Vet. Med. Ass/West-Area Branch.

SMITH, J.E. (1966) Corynebacterium species as Animal Pathogens. J. appl. Bact. 29, 119—130.

SMITH, R.E. REYNOLDS, I.M., CLARK, G.W. and MILBURY, J.A. (1971) Fetoplasental effects of *Corynebacterium pyogenes* in sheep. Cornell vet. 61, 573—590.

SMITH, R.E. and REYNOLDS, I.M. (1971) *Corynebacterium pyogenes* Keratitis in a newborn calf. Cornell vet. 61, 491—595.

SUZUKI, T., KANEKO, S., SAITO, A., FURUYA, B: FFUJII, O. and KATO, K (1973). Patholigical and bacteriological and bacteriological studies on purulent diseases of pigs. J. Jap. Vet. Med. Ass. 26, 188—192.

UEDA, S., KABUTO, A and SATO, S. (1972) An outbreak of suppurative diseases caused by *Corynebacterium pyogenes* on a pig farm. J. Jap. vet. med. Ass. 25, 125—127.