RHINITIS OF SWINE

VII. PRODUCTION OF LESIONS IN PIGS AND RABBITS WITH A PURE CULTURE OF PASTEURELLA MULTOCIDA

by RONALD GWATKIN*, LUCIJA DZENIS* and J. L. BYRNE*.

Earlier attempts to produce rhinitis in baby pigs by the use of bacterial cultures, singly and in combination, were unsuccessful (1, 2, 3) except that subcutaneous injection of *Pasteurella multocida* in the frontal area of mice produced penetration of the bone in one case and a thinning of that tissue in another. The injections killed the majority of the infected mice. When isolated from the two affected mice the culture did not reproduce the condition.

Jones (4) reported that *P. multocida* was rarely absent from early lesions of rhinitis and one of us (J.L.B.), in the course of 186 bacteriological examinations of nares of pigs, recovered *P. multocida* from 38 per cent of those showing affected turbinates and from 16 per cent of those without visible damage to the nasal structures.

Owing to the frequent occurrence of this organism, and in view of the action of streptomycin on the infection (5), it was decided to try again with a culture of *P. multocida* isolated from a field case of rhinitis, nasal material from which animal was later shown to be extremely active in baby pigs. Since there had been some suggestion in our earlier work that more than one factor might be concerned in the aetiology of this condition, a pure culture of *P. multocida* alone and combined with a filtrate made from the same source material was used for nasal instillation of pigs, as outlined in the following experiment.

Trial of Pasteurella multocida with and without filtrate obtained from the same source.— Cultures were made from nasal material being used as the infective material in a concurrent experiment and *P. multocida* was isolated from it. This organism produced acid in glucose, mannitol, and sucrose and did not ferment lactose, maltose and salicin. It was typed by Dr. G. R. Carter as Type B showing a few filaments, and the dissociation status was considered to be mucoid and fluorescent. The third subculture was used for the experiment after 24 hours incubation. It was grown on beef infusion agar, washed off with 0.85 per cent salt solution and the density adjusted to 7 times McFarland's No. 1 tube. The suspension was distributed in 5 tubes, one of which was used for the first instillation and the others held frozen at -20C for subsequent, daily instillations.

A filtrate was prepared from the nasal material from which the culture had been isolated. Two grams of the material were ground up in 20 ml. of

*Division of Animal Pathology, Department of Agriculture, Canada, Animal Diseases Research Institute, Hull, Quebec.

salt-free broth, filtered through cotton, filter paper and a Seitz filter disc on a B-D filter adapter. Cultures showed it to be bacteriologically sterile.

Seven pigs were removed from the sow when they were 4 days old, placed in 3 groups and given nasal instillations as under:—

Group 1 — Two pigs, Nos. 16 and 17, were given 1.0 ml. of equal parts of bacterial suspension and sterile broth divided between both nostrils.

Group 2— Three pigs, Nos. 18, 19 and 20, were given 1.0 ml. of equal parts of bacterial suspension and filtrate distributed between both nostrils.

Group 3— Two pigs, Nos. 21 and 22, were given 1.0 ml. of equal parts of filtrate and broth in the same manner.

These instillations were repeated on 4 following days, the mixtures being made just prior to use.

Two months later, Groups 1 and 2 were showing symptoms of rhinitis and, at that time, they were tested by the intradermal method with a heat-killed suspension and a broth filtrate of the homologous organism. Neither of these elicited any response.

The three groups were killed for necropsy 12 weeks after commencement of the experiment and results were as follows:—

GROUP I — Bacterial suspension and Broth

No. 16— No shrinkage of turbinates on right side, which appeared normal. The left side showed a little pus in the nares but no shrinkage.

No. 17— The turbinates on the right side were shrunken to about one half and there was pus in the ethmoid cells. There was also some decalcification on the left side and pus in the ethmoid region.

GROUP 2— *Bacterial suspension and filtrate*

No. 18— Both sides almost completely decalcified, ethmoid cells affected.

No. 19— Pus in turbinates on right side but no apparent shrinkage, although the turbinates appeared to be softer than normal. Pus in ethmoid cells. The left side showed some decalcification and shrinkage of the turbinal structures and there was pus in the ethmoid cells, as well as in the nasal passages.

No. 20— Completely decalcified on both sides, ethmoid cells were affected and contained pus.

GROUP 3— *Filtrate and broth*

Nos. 20 *and* 21— Entirely normal and showed no evidence of rhinitis.

All five pigs that had received culture developed rhinitis. Neither of the two animals that had received culture alone showed complete decalcification of the turbinated structures. In two of the three that received culture and filtrate the decalcification was complete or almost entirely so, but No. 19 corresponded to the first group in showing only slight reduction. Obviously, the numbers are not large enough for comparison. Both pigs in the filtrate and broth group were normal, confirming earlier experiments in which filtrates regularly failed to produce lesions. This group also shows that infection was not accidental-

ly introduced into the experiment. Cultures were made from the nares of these pigs and *P. multocida* was recovered from Nos. 18 and 20, the two badly affected animals.

In this experiment, rhinitis, indistinguishable from the usual type, was produced by *P. multocida*. Owing to an unfortunate interruption in our supply of pigs, we have been unable to repeat this work, but some confirmation is provided by the production of rhinitis in rabbits, with destruction of the turbinates as seen in pigs. A culture of *P. multocida* from an experimentally infected pig produced excellent lesions in a young rabbit which survived for a couple of weeks. The tendency was for most of these animals to die from an acute Pasteurella infection.

Nasal material from an artificially infected pig also produced rhinitis. This material has now been passaged in 4 lots of young rabbits and *P. multocida* has been isolated from all the affected animals. Details of these experiments will be given in a later paper.

SUMMARY

Rhinitis, indistinguishable from the usual experimental disease, was produced in baby pigs by nasal instillation of *Pasteurella multocida*, Type B, isolated from a field case of rhinitis.

There was some suggestion that a filtrate of nasal material increased the activity of the culture but, with such small numbers, this can only be considered for what it is worth.

Rhinitis was produced in rabbits by nasal instillation of cultures of *P. multocida* and by nasal suspensions. The latter material has been carried through four rabbit passages and the organism has been isolated from all affected animals in each passage.

ACKNOWLEDGMENTS

We are indebted to Dr. G. R. Carter, then on our staff and now with the Ontario Veterinary College, for typing strains of *P. multocida*, and to Dr. E. F. Johnston and Dr. A. A. Hanna of Carp for locating the field material used in this experiment.

REFERENCES

(1) GWATKIN, R., PLUMMER, P. J. G., BYRNE, J. L., and WALKER, R. V. L. Rhinitis of swine. III. Transmission to baby pigs. Canad. J. Comp. Med. and Vet. Sci. 13: 15-28, 1949.

(2) GWATKIN, R., and PLUMMER, P. J. G. Rhinitis of swine. IV. Experiments on laboratory animals. Canad. J. Comp. Med. and Vet. Sci. 13: 70-75, 1949.

(3) GWATKIN, R., PLUMMER, P. J. G., BYRNE, J. L., and WALKER, R. V. L. Rhinitis of swine. V. Further studies on the aetiology of infectious atrophic rhinitis. Canad. J. Comp. Med. and Vet. Sci. 15: 32-38, 1951.

(4) JONES, T. L. Streptomycin and other agents used in infectious atrophic rhinitis of swine. J. Amer. Vet. Med. Assn. 121: 192-194, 1952.

( ) GWATKIN, R., and DZENIS, L. Rhinitis of swine. VI. Topical application of streptomycin in artificially infected pigs. Canad. J. Comp. Med and Vet. Sci. 16: 330-332, 1952.