

# RHINITIS OF SWINE. IX. FURTHER STUDIES ON AETIOLOGICAL AGENTS

By RONALD GWATKIN\*, LUCIA DZENIS\*, A. S. GREIG\*  
and C. GRINEWITSCH\*.

In two previous papers we (1, 2) recorded the production of an atrophic rhinitis indistinguishable from that occurring naturally and following the nasal instillation of nasal scrapings by instillations of cultures of *Pasteurella multocida*. Since our first paper, Flatla and Braend (3) reported that a species of *Pasteurella* seems to be the most important aetiological factor. They were able to produce atrophy of the turbinals.

Schofield (4) was able to produce varying degrees of atrophic rhinitis with our cultures in baby pigs and sections showed characteristic changes. These cultures had been isolated for varying lengths of time before use so it could not be expected that they would be as active as when first isolated.

The following experiments deal with the use of nasal material, Selas filtrates, pleuro-pneumonia-like organisms (PPLO) and *Pasteurella multocida*.

## METHODS

The methods employed were the same as in earlier work.

Pigs were removed from the sow at 4 to 7 days of age and placed in separate pens for each group in a house containing only these experimental animals. Suspensions of nasal material consisted of approximately 10 per cent of tissue ground up in salt-free broth and strained through gauze. Bacterial suspensions were made from 24-hour cultures of *P. multocida*. The third subculture after isolation was generally used and cultures were confirmed in the usual manner by fermentation reactions and tested for agglutinability. The density was about 7 x No. 1 McFarland's nephelometer. The cultures obtained from rhinitis cases were not agglutinogenic. The filtrates were made with a Selas 02 candle at the specified pressure and tested for sterility by cultural methods. Broth cultures of PPLO were used.

Five instillations of each product were given at daily intervals. The dose of each was 0.5 ml. divided between both nostrils. Any animals that died and those killed at the end of the experiment were examined for rhinitis and cultures were made from the turbinates on blood agar plates. Colonies were fished and subcultures seeded in lactose, maltose, glucose, mannitol, sucrose and salicin.

Heads were opened longitudinally so that when the septum was cut away the entire nasal cavity on each side could be observed. This gives a clearer picture of the turbinated structures and makes it easy to collect material for future use. When material had to be held for longer than a day it was frozen at -20°C.

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

## EXPERIMENTAL

*Experiment 66* — Nasal material and Selas filtrate of this material from artificially infected pigs. The material was obtained from an earlier experiment and had been held frozen. Six pigs were used. Three were given filtrate and three received the unfiltered suspension. They were killed for examination 64 days after the first infecting dose. The three filtrate pigs appeared normal and *P. multocida* was not recovered on culture. The three suspension pigs had well-marked rhinitis, the turbinated structures varying from complete to partial decalcification. These animals yielded *P. multocida* on culture.

*Experiment 67* — Transmission of infection by contact with culture-infected pigs. The culture used had been isolated from a pig in an earlier experiment. Seven 4-day-old pigs were used. Three were given 5 instillations of culture suspension and four were left in the pen as contacts. One of the infected pigs was killed 76 days after the commencement of the experiment. It showed some shrinkage and deformity of the turbinals and pus. *P. multocida* was isolated from the nasal passages. The remaining six were killed on the 88th day. The three principals and two of the three contact animals showed atrophic lesions and *P. multocida* was isolated from all. In this experiment, infection spread to the contact animals although the lesions in the infected animals were not very severe.

*Experiment 68* — Vaccination of young pigs with *P. multocida* prior to infection. A culture of *P. multocida* from an experimental pig was grown on beef infusion agar, washed off in 0.85 per cent salt solution, killed by the addition of 0.25 per cent formalin, cultured for sterility and brought to a density of 30 x No. 1 McFarland.

Twelve pigs from 2 litters were used, both lots being 2-days-old. Two of each litter were used to form 3 groups of 4 pigs. Four animals received one subcutaneous injection of 2.0 ml. at 5 days. Four were given 0.5, 0.75 and 1.0 ml. at 2, 5 and 9 days, respectively. Four were left as unvaccinated controls. They were infected by nose with 5 daily instillations of pooled material from experimental pigs, commencing when they were 16 days of age. They had been left with the sow until this time but were placed in 3 separate pens after being infected. They were killed 88 days after commencement of the experiment.

Three of the four animals that had received 3 instillations of vaccine developed rhinitis. One of the single vaccination group died too soon to be of value. Two of the 3 survivors had rhinitis. Only one of the four unvaccinated controls was affected. All six rhinitis-infected animals yielded *P. multocida* on culture. The organism was not recovered from any of the normal pigs. Identity of cultures was established in the usual manner. In this trial, the vaccinated animals fared worse than the unvaccinated controls.

*Experiment 69* — Pleuro-pneumonia-like organisms and *P. multocida*.

Ten 7-day-old pigs were used for this experiment. Four were given the usual nasal instillations of a broth culture of PPLO, three were given a suspension of *P. multocida*, and three were left uninfected. The two cultures had been isolated from the same nasal scrapings of a rhinitis-affected pig. Each group was in a separate, solid-walled pen. They were killed 70 days after commencement of the experiment.

The four pigs which had received PPLO remained normal. Two of the three *P. multocida* pigs had rhinitis. The three animals which had not been given either suspension but which had been held in an adjoining pen were normal. *P. multocida* was recovered from the two rhinitis pigs but from none of the others.

*Experiment 70—Pleuro-pneumonia-like organisms and uninfected controls.* Seven 7-day-old pigs were used in this experiment. Four were given 5 instillations of a broth culture of PPLO isolated from an experimentally infected pig. Three were held in an adjoining pen as controls. They were killed 77 days after commencement of the experiment.

Three of the PPLO pigs showed some pus in the nares but if there were any changes in the turbinates they were very indefinite. Three of the four yielded PPLO on culture. The three controls were normal and cultures were negative.

*Experiment 71 — Contact infection from culture-infected pigs.* Eight pigs were used in this trial, which was similar to Experiment 67. Eight 6-day-old pigs from the same litter were used. Three were given nasal instillations of *P. multocida* isolated from a rhinitis head from Manitoba. They were placed in a pen with three litter-mates. The other two pigs were given instillations of the nasal curettings from which the culture was isolated and were kept in a separate pen.

One of the culture-infected pigs died 46 days after the first infective dose. It had a well-marked bilateral atrophic rhinitis and *P. multocida* was isolated from it. The remaining seven were killed on the 87th day. The two surviving culture-infected pigs, two of the three contact pigs and one of the two nasal scraping pigs had rhinitis. *P. multocida* was isolated from the culture-infected and the two contact pigs which had developed rhinitis. This confirms the results of Experiment 67 in which the contact pigs also became infected.

*Experiment 72 — Rabbit-passaged material in pigs.* Rhinitis had been passed from rabbit to rabbit by nasal instillation of a suspension of rabbit turbinates for 8 passages. The eighth passage rabbit material and *P. multocida* isolated from this were each instilled into the nostrils of three 7-day-old-pigs. Three other pigs from the same litter were kept in an adjoining pen as a check on conditions in the house.

The nine pigs were killed 92 days after commencement of the expe-

riments. Two of the three that had received rabbit nasal material showed some atrophy. The three culture pigs were normal, as were the three that had been held as controls on air-borne infection. *P. multocida* was isolated from one rabbit in each of the first two groups but not from the controls. Passage through rabbits had not maintained virulence for pigs.

*Experiment 73 — Aerosols of PPLO, P. multocida and a mixture of the two organisms.* Eight 5-day-old pigs from one litter were used. The PPLO was that used in Experiment 69 and *P. multocida* had been isolated from a pig in Experiment 66.

The pigs were exposed in a tightly closed chamber under slight negative pressure and the infected spray was drawn off into a tank of formalin solution. Culture was blown in for one minute with a Collison spray. The chamber was left closed for another minute, the pigs were removed, and the chamber and attachments were disinfected with formaldehyde and aired before the next group was exposed.

They were killed 76 days later. One of the three PPLO pigs showed slight damage which could be called atrophic. The other two were normal. All three *P. multocida* animals had well-marked rhinitis with advanced decalcification and atrophy. The two animals which had received the mixture of cultures both showed some decalcification and deformity but this was not as advanced as the Pasteurella group. *P. multocida* was not isolated from any of these animals.

*Experiment 74 — Nasal material and P. multocida from a culture-infected pig.* This was a repetition and confirmation of earlier experiments. A litter of 8 pigs was used. Two were killed at 3 days and proved negative for PPLO and Pasteurella. Three of the remaining six were infected with nasal material from a pig in Experiment 67 which had been infected with culture. *P. multocida* isolated from this material was used to infect the other three animals.

They were killed 74 days after commencement of the experiment. All had a moderate but definite atrophic rhinitis and *P. multocida* was isolated from them.

*Experiment 75 — Intranasal vaccination of pigs with killed suspension of P. multocida.* A suspension of 24-hour agar culture of *P. multocida* was killed by the addition of merthiolate. The suspension had a density of 17 x No. 1 McFarland's nephelometer. It was shown to be sterile. Three 4-day-old pigs were given a nasal instillation of 1.0 ml. divided between both nostrils. This was repeated 4 days later. Two litter-mates were held as controls. Six days after the second injection the nasal mucus of both groups was tested for opsonocytotoxic activity. No difference was observed. They were then infected with a suspension of nasal material from which the vaccine culture had been isolated.

The pigs were killed 64 days after the first infective dose. The material

was evidently not very active as both unvaccinated animals and one of the three vaccinates were normal. The other two had rhinitis, but not to a marked degree. *P. multocida* was isolated from the three vaccinates but not from the two controls.

*Experiment 76* — *Frozen nasal material and PPLO isolated from this material.* Frozen scrapings from pigs in Experiment 70 and PPLO isolated from this material were used to infect five 7-day-old pigs with the usual five instillations. They were killed 70 days after the first infective dose. Two animals in the PPLO group were normal and one showed a suggestion of change in the turbinates but bacteriological examination showed that this animal had acquired *P. multocida* in some manner. The two which had received the source material developed definite rhinitis but did not yield *P. multocida* on culture. This may have been due to their having been killed later than the PPLO group on account of other tests being carried out on them.

#### SUMMARY

A Selas 02 filtrate did not produce rhinitis. The source material was proved to be infective.

In two experiments with pigs infected with *P. multocida*, litter mates left in the same pen developed rhinitis as a result of exposure to the infected pigs.

Fourteen pigs in 4 experiments were infected with PPLO isolated from proven infective material. Three showed pus in their nares but no atrophy. One, which had been exposed to an aerosol, showed a suggestion of atrophic change. A second one, which had been infected in the usual manner, also showed a slight degenerative change but *P. multocida* was isolated from the nose.

Subcutaneous and intranasal vaccination with chemically-killed *P. multocida* did not protect pigs against nasal infection 10 to 14 days after the first dose. The vaccinates appeared to be more susceptible than the controls. If such were actually the case, it may have been due to the short interval between vaccination and exposure.

Further confirmation was obtained that cultures isolated from culture-infected pigs were capable of producing rhinitis.

Nasal curettings of the eighth rabbit passage of material which had originally been very active in pigs produced only a moderate degree of atrophy in 2 of 3 infected pigs. A culture of *P. multocida* isolated from the 8th rabbit passage did not produce any change in 3 litter-mates.

#### ACKNOWLEDGEMENTS

The authors gratefully acknowledge the assistance of Mr. A. W. Peterson, Chief, Live Stock and Poultry Division, Canada Department of Agriculture, and his staff in obtaining field material; and the advice and support of Dr. Chas. A. Mitchell, Chief, Animal Pathology Division.

REFERENCES

1. GWATKIN, R., and DZENIS, L. RHINITIS of Swine. VII. Production of lesions in pigs and rabbits with a pure culture of *Pasteurella multocida*. Can. J. Comp. Med. and Vet. Sci. 17: 215-217, 1953.
2. GWATKIN, R., and DZENIS, L. Rhinitis of Swine. VIII. Experiments with *Pasteurella Multocida*. Can. J. Comp. Med. and Vet. Sci. 17: 454-464, 1953.
3. SCHOFIELD, F. A., Ontario Veterinary College, Guelph, Ont. Personal communication, April 1954.
4. FLATLA, J. L., and BRAEND, M. Infectious atrophic rhinitis in pigs. Studies on the etiology. XVth Internat. Vet. Congress Proceedings, Part. 1, Vol. 1: 180-185, Aug. 1953.

---

MARITIME VETERINARIANS MEET

Fifty-five veterinarians of the four Atlantic Provinces gathered for the Annual Conference of Maritime Veterinary Associations, June 22-24 at Mt. Allison University, Sackville, New Brunswick.

Dr. C. A. Mitchell, Dr. T. Childs, brought greetings from Divisions of Animal Pathology and Health of Animals Branch. Dr. J. Dufresne, President of C. V. M. A. spoke on "Public Relations." The speakers with their respective papers follow:

Dr. R. T. Gwatkin — "Diagnostic Tests"

Dr. J. H. Ballantyne — O. V. C. "Surgical Anatomy of Bovine Left Flank."

Dr. J. Stevens — Concord, N. H. 2 papers on Small Animal Virus Diseases, and Surgical Techniques.

Dr. G. W. Dashner, St. Stephen, N.B., Dr. H. H. Kelly, Charlottetown, P.E.I., and Dr. J. A. MacKay, New Glasgow, N.S., were members of a large animal panel with Dr. R. McG. Archibald, Truro, N.S., Moderator. Mr. F. G. Proudfoot of Nova Scotia, Dept. of Agriculture, spoke on Poultry Flock Management.

Two new items were used on this year's programme. Poultry Disease Diagnosis Panel had Dr. D. B. Butterwick, Fredericton, N.B., H. VanZuol, Truro, N.S., G. C. Fisher, Charlottetown, P.E.I., and J. F. Frank, Sackville, N.B. as demonstrators. S. E. Magwood and L. A. Donovan, Sussex, N.B. demonstrated a rumenotomy.

J. F. Frank, Sackville, is secretary of Committee for Arrangements, with the secretaries of three Provincial Associations, W. A. Roach, M. I. Lowrie, and R. McG. Archibald as members.