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**PASTEURELLA MULTOCIDA SEROTYPE 1: A
ASSOCIATED WITH RESPIRATORY INFECTION OF
DOMESTIC RABBITS IN A HOLDING COLONY*¹**

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CARTER & BAIN reported that, among 27 cultures of *Pasteurella multocida* from infections in rabbits, 11 belonged to CARTER's type A, 3 to type D and the remaining cultures were not typable. Recently a useful method was devised for serological typing of *P. multocida* by NAMIOKA & MURATA^{6,7}. NAMIOKA & BRUNER indicated that *P. multocida* cultures from various animals were classified into 12 serotypes by combining the capsular types and O groups. They proposed that serotypes of the organisms should be designated on the basis of the O and K antigens. Moreover, they stated that there is a relationship between serological types and host distribution. There is no doubt that, by characterizing the serotypes on the basis of the capsular and O antigens, the relationship between pasteurellosis and the organisms becomes more apparent than previously demonstrated. However, no strains of *P. multocida* of rabbit origin have yet been typed on the basis of the O and K antigens.

This paper deals with serotype and biochemical characteristics of *P. multocida* from diseased rabbits of a colony and the effect of the injection of antibiotics on the isolation of Pasteurella from the rabbits.

MATERIALS AND METHODS

Specimens from dead or killed rabbits were cultured on blood agar and MacConkey agar plates. The specimens examined are shown in table 1. Specimens from some cases were cultured in an enrichment medium. Isolates of *P. multocida* were preserved in cooked meat media for further investigations.

Initial identification of *P. multocida* was made on the basis of colonial appearance, morphology, no growth on MacConkey agar, indol production, growth characteristics on Kligler iron agar media and occasionally penicillin sensitivity. Fermentation test of sugars

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was made in CTA media (trypticase (BBL) 20 g, L-cystine 0.5 g, sodium sulfite 0.5 g, NaCl 5 g, agar 3.5 g, 0.2% phenol red 10 ml, water 1,000 ml, pH 7.3) described by NAMIOKA. Final reactions were recorded on the 7th day. Serological typing was made according to the methods of NAMIOKA & MURATA^{5,6}.

RESULTS

An outbreak of respiratory disease among rabbits and antibiotic treatment
Sporadic deaths occurred in February, 1964 in a colony of approximately 300 adult rabbits in a laboratory in Sapporo. This colony was not a breeding, but a holding colony. In March, about 20 rabbits died of respiratory disease exhibiting a purulent nasal discharge occasionally stained with blood, labored breathing, emaciation and a rough coat. Some diseased rabbits showed diarrhea. The disease progress was rather chronic. Thus, during 4 months from February to May, 42 or more rabbits died and more than 8 were sacrificed as a result of respiratory disease. Macroscopical lesions were observed most frequently in the lungs of the rabbits. Fibrinous pneumonia of various stages was found in the rabbits. Severe cases showed purulent pleuro-pneumonia and pyothorax. The trachea was inflamed and frequently contained a frothy, blood-stained exudate. Some cases exhibited a fibrino-purulent pericarditis and peritonitis. The spleen showed only slight swelling. Two of the diseased rabbits examined had a subcutaneous abscess either in the abdominal or mandibular region. The symptoms and pathological changes observed during the outbreak were similar to those of "snuffles" described by TANAKA and other investigators.

In the same animal house, about 200 guinea pigs had been kept. No disease was recorded in the guinea pigs. Moreover, no effort was made to check *Pasteurella* contamination of the animals.

Antibiotic treatment for therapy and prophylaxis was commenced at the end of March. Oral administration of oxytetracycline and tetracycline was unsuccessful, because the rabbits did not eat the medicated feed. Intramuscular injections of streptomycin were performed on all rabbits of the colony for 3 consecutive days from the 4th to the 6th of April (60 mg total per rabbit). In addition similar injections of penicillin were made for 7 days from the 23rd to the 29th of the month (a total of 420,000 units per rabbit). Other sanitary procedures such as isolation of diseased rabbits and disinfection of cages were also carried out.

In April, a total of 19 rabbits died or were sacrificed. In May, 8 rabbits died or were destroyed. This study involves observations of the colony during the period ending May 31.

Isolation of *P. multocida* from diseased rabbits Table 1 shows the frequency of *P. multocida* isolation from each organ in a total of 21 rabbits that died or were destroyed. Some specimens were discarded because of contamination with *Proteus* organisms. However, the lung specimens were cultured in all cases. Some of the cases showed systemic infection. Pure growths of *Pasteurella* were obtained from 5 of 6 lung specimens of positive cultures. All the *Pasteurella*-negative lungs which were cultured, after the antibiotic treatment, yielded *Escherichia coli*, streptococci, *Klebsiella*-like organisms and other bacteria, but no cultures of *Bordetella bronchiseptica*. Growth of *Bordetella* was not recognized in any specimen.

TABLE 1 *Isolation of Pasteurella multocida from diseased rabbits*

| ISOLATION OF <i>P. MULTOCIDA</i> | NO. OF POSITIVES/NO. OF SPECIMENS EXAMINED | | | | | | | | | | NO. OF POSITIVES/NO. OF RABBITS EXAMINED | | |
|---|--|---------|-------|--------------------|--------|-------|---------|---------------------|-------|---------|---|------------|-----------------|
| | Lungs | Trachea | Heart | Nasal discharge | Spleen | Liver | Kidneys | Mesenteric nodes | Urine | Abscess | Dead | Sacrificed | Total |
| Before the first injection of antibiotic | 5/7 (71.4%) | 4/6 | 2/6 | 1/1 | 2/6 | 1/6 | 2/6 | 0/4 | 0/2 | 2/2 | 4/4 | 3/3 | 7/7 (100%) |
| After the first injection | 1/14 (7.1%) | 1/13 | 2/14 | 2/12 | 0/9 | 1/13 | 0/11 | 0/7 | 0/4 | | 3/12 | 0/2 | 3/14 (21.4%) |

It was evident that the antibiotic treatment reduced the number of *Pasteurella* isolations from diseased rabbits as shown in table 1, although the control lot of rabbits was not prepared. Only 3 (21.4%) of the 14 cases examined yielded the organisms after the treatment. Of the cases, one gave *Pasteurella* only from the heart via enrichment culture, another only from the nasal discharge and the remaining one showed systemic infection. It should be noted that the last one died about 3 weeks after the penicillin treatment.

TABLE 2 *Biochemical and serological characteristics of Pasteurella multocida isolates**

| TEST | REACTION |
|--|----------|
| Methyl red, Voges-Proskauer, H ₂ S production, Growth in Simmons' ammonium citrate media, Urease production | } - |
| Indol production, Reduction of nitrates to nitrites | + |
| Fermentation of | |
| Xylose, Glucose, Sorbitol, Sucrose, Galactose, Fructose, Mannose, Mannitol | } + |
| Lactose, Dulcitol, Arabinose, Dextrin, Inulin, Maltose, Rhamnose, Raffinose, Salicin, Inositol, Trehalose | } - |
| Serotype 1 : A (O group-1, Capsular type-A) | |

* Seventeen strains from 6 rabbits were employed for biochemical tests and 4 from 4 rabbits for serological typing.

Biochemical characteristics and serotype of *Pasteurella* isolates As shown in table 2, the isolates had uniform biochemical characteristics. Their serotype was identified as 1 : A. High sensitivity of the isolates to penicillin, streptomycin, chloramphenicol, erythromycin, tetracycline and kanamycin was confirmed by the disc method.

Pathogenicity tests One of the isolates which had been kept in cooked meat media was inoculated into broth media. The overnight broth culture was injected into mice, rabbits and pigeons. Three mice, aged 6 weeks, were given, intraperitoneally, a dilution of the broth culture containing approximately one cell of *Pasteurella*. One of the mice died from septicemia within 7 days. In another experiment 2 of 6 mice treated similarly died within 2 weeks. Four rabbits (about one kg) were divided into 2 groups of 2 each. Each rabbit of one of the groups was injected subcutaneously with a dose of 10⁶ cells, and 2 rabbits of another group received 10⁸ respectively. These 4 rabbits showed severe symptoms. One rabbit of each group died from septicemia within 7 days. Two rabbits survived had widespread subcutaneous abscesses and *Pasteurella* was recovered only from the lesions 31 days after the inoculation. Two adult pigeons were refractory to intramuscular inoculation with a dose of 10³ cells of *Pasteurella*. One of the birds had an abscess in the muscle of the

site of injection, but no isolation of *Pasteurella* was recorded at necropsy 20 days after the inoculation. From these observations, it can be seen that the isolate was pathogenic to mice and rabbits, although the observations were based on small numbers of test animals.

DISCUSSION

According to NAMIOKA & MURATA serotype 1:A of *P. multocida* was isolated from pneumonia of swine in Canada, Formosa and Japan. The type was also isolated from sepsis of mice in Japan. From this study it is evident that the type caused respiratory infection and subcutaneous abscess in rabbits.

According to HAGEN, both sulfaquinoxaline (225 g per ton) and furazolidone (nf-180 50 g per ton) in the rations reduced the number of deaths due to primary pneumonia, and eliminated *Pasteurella* from the bacterial flora of the lung tissues. While this was true in the unweaned young rabbits, it was not the case in the mature females. Moreover, the addition of sulfamethazine or of aureomycin failed to reduce the number of pneumonia deaths or *Pasteurella* isolations. Thus he concluded that the addition of furazolidone and/or sulfaquinoxaline to the growing ration of domestic rabbits may be useful in the production of animals relatively free from pasteurellosis, if offspring weaned from such a regimen are housed in uncontaminated environment.

On the other hand, there have been few reports on the effect of the injection of antibiotics on pasteurellosis in adult rabbits. After the onset of the antibiotic injection in this study, *Pasteurella* isolations were reduced from 100 to 21.4%. The *Pasteurella*-negative lungs gave other species of bacteria, although they had pathological changes almost similar to those observed before the antibiotic treatment. This indicates that the antibiotics injected eliminated *Pasteurella* from the lung tissues of diseased rabbits, but did not enable them to recover from the respiratory disease in its advanced stages. Moreover, about 3 weeks after the last antibiotic injection, one rabbit died from a systemic infection of *P. multocida*. This means that the efficacy of the treatment was insufficient under the conditions of this study.

SUMMARY

Pasteurella multocida strains were isolated from adult domestic rabbits suffering from respiratory disease in a holding colony. The strains had uniform biochemical characteristics and were of serotype 1:A (NAMIOKA & MURATA, 1961). The isolate was pathogenic to mice and rabbits. The injection of streptomycin and penicillin reduced the number of *Pasteurella* isolations from diseased rabbits.

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