— Note —

Carrier State of *Pasteurella pneumotropica* in Mice and Rats

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Localization of *Pasteurella pneumotropica* was investigated in the respiratory tract, conjunctiva and vagina of 5-week-old, 10-week-old and retired asymptomatic mice and rats. The highest isolation rate of the organisms was obtained in the pharyngolarynx, showing 85 to 97.5 % in carrier mice and 100 % in carrier rats. Numbers of the organisms in this site were 10^{3-5} and 10^{7-8} organisms/g tissue in 4-week-old mice and rats, respectively. Isolation rates in the nasal cavity and trachea of the both animals were not so high as those in the pharyngolarynx, but usually higher than those in the external nares. The organisms were rarely isolated from the lung. Isolation of the organisms from the conjunctiva was common in rats, especially in young ones, but rare in mice. About 30 % of carrier mice and 50 to 100 % of carrier rats harbored the organisms in their vaginas.

It is well known that *Pasteurella pneumotropica* is distributed in several species of animals such as mouse, rat, hamster, guinea pig and dog [1, 10, 11], sometimes causing pneumonia [1-3, 9, 10], subcutaneous abscess [11, 15, 16, 18], mastitis [8, 15], conjunctivitis [1, 13, 16], metritis [1], abortion [17] and urocystitis [1]. However, carrier state of the organisms in these animals have not been sufficiently studied.

The present study was performed to investigate localization of the organisms in asymptomatic mice and rats which had been known to be common natural hosts of the organisms [1, 2, 5, 9, 10].

Forty each of 5-week-old, 10-week-old and retired mice and twenty each of rats

of the same ages were obtained from a respective breeding colony which had been known to be contaminated with *P. pneumotropica* but not with other noticeable pathogens such as *Salmonella* spp., *Corynebacterium kutscheri*, Tyzzer's organisms, *Mycoplasma* spp., Sendai virus, mouse hepatitis virus and ectromelia virus. Their external nares, nasal cavity, pharyngolaryngeal, tracheal, lung, vaginal and conjunctival specimens were submitted to cultivation for *P. pneumotropica*. Immediately after the animals were sacrificed with chloroform, all specimens, except for lung material, were obtained by swabbing the mucus membrane of the organs with fine cotton swabs moistened with sterile broth. They were cultured directly on 5 % horse

Table 1. Isolation of *P. pneumotropica* from mice

Age of mouse	No. of mice		No. of positive isolation / No. of carriers examined						
	Examined	Carrying the organisms	External nares	Nasal cavity	Pharyngo-larynx	Trachea	Lung	Conjunctiva	Vagina
5-weeks	40	37 (92.5)	12/37 (32.4)	17/37 (46.0)	36/37 (97.1)	16/37 (43.2)	0/37	2/37 (5.4)	6/20*
10-weeks	40	40 (100.0)	20/40 (50.0)	26/40 (65.0)	39/40 (97.5)	21/40 (52.5)	0/40	1/40 (2.5)	14/40 (35.0)
Retired	40	35 (87.5)	6/35 (17.1)	11/35 (31.4)	30/35 (85.7)	11/35 (31.4)	1/35 (2.9)	3/35 (8.6)	13/35 (37.1)

Note : () : Per cent

* : Reading could not be made on all the samples owing to swarming of *Proteus* sp. over the culture medium.

Table 2. Isolation of *P. pneumotropica* from rats

Age of rat	No. of rats		No. of positive isolation / No. of carrier examined						
	Examined	Carrying the organisms	External nares	Nasal cavity	Pharyngo-larynx	Trachea	Lung	Conjunctiva	Vagina
5-weeks	20	20 (100.0)	8/20 (40.0)	11/20 (55.0)	20/20 (100.0)	18/20 (90.0)	0/20	13/20 (65.0)	8/10*
10-weeks	20	20 (100.0)	11/20 (55.0)	13/20 (65.0)	20/20 (100.0)	8/20 (40.0)	0/20	13/20 (65.0)	4/ 8*
Retired	20	20 (100.0)	10/20 (50.0)	15/20 (75.0)	20/20 (100.0)	14/20 (70.0)	0/20	6/20 (30.0)	6/ 6*

Note : () : Per cent

* : Reading could not be made on all the samples owing to swarming of *Proteus* sp. over the culture medium.

blood agar plates without diluting them. As regards lung samples, upper portion of the left lobe was minced thoroughly with sterile scissors and stamped on the blood agar plate followed by spreading over the agar plate with a platinum loop. The inoculated blood agar plates were incubated at 37°C for 2 days. *P. pneumotropica* was identified according to Brennan et al. [1] and Hoag et al. [7] : the organisms were Gram-negative rods, non-motile, indole positive but H₂S negative in SIM medium, urease positive, and fermented glucose and lactose without gas production. All strains showing the above properties agglutinated with rabbit antiserum against *P. pneumotropica* NM-8 strain isolated

from the pneumonic lesions of a mouse.

Isolation results of *P. pneumotropica* in mice are shown in Table 1 according to their ages and the organs examined. The organisms were detected in 37 (92.5%) of 5-week-old mice, in 40 (100%) of 10-week-old mice and in 35(87.5%) of retired mice, showing no significant difference by ages. Regardless of mouse ages, the organisms were most frequently isolated from pharyngolaryngeal specimens ; positive isolation was obtained from 36 (97.1%) of 37 carriers, 39 (97.5%) of 40 carriers and 30 (85.7 %) of 35 carriers in 5-week-old, 10-week-old and retired mice, respectively. In 5-week-old and 10-week-old mice, isolation rate was next high in the nasal cavity,

followed by the trachea, external nares and vagina. In retired mice, however, isolation rate in the vagina was considerably high in comparison with those of other organs such as the nasal cavity, trachea and external nares, in which isolation rates were significantly lower than those of the young aged mice. It was noteworthy that the organisms seemed to be distributed in the respiratory tract centering the pharyngolarynx in young mice, but retained mainly in the pharyngolaryngeal area in old mice. Only a few mice harbored the organisms in their lungs and conjunctivas.

In the case of rats, *P. pneumotropica* was isolated from all individuals examined, regardless of their ages, as shown in Table 2. The pharyngolarynx was also the prominent site harboring the organisms like in mice, showing 100% isolation rate in all age groups of rats. The organisms were frequently isolated from other sites of the respiratory tract such as trachea, nasal cavity and external nares, but no isolation of the organisms occurred in the lung. The organisms were also frequently isolated from the conjunctiva, showing 65%, 65% and 30% in 5-week-old, 10-week-old and retired rats, respectively. Although reading could not be made in many vaginal samples by swarming of *Proteus* sp. over the culture medium, isolation rates of the organisms in this site seemed to be very high. Comparing with results of mice, it was worthy to note that the organisms localized in rats more commonly and in wider distribution.

No pneumonic lesion and abscess formation were observed in any of mice and rats examined in this study.

From the results described above, it was apparent that the pharyngolarynx was the most preferred site of *P. pneumotropica* in both mice and rats, and therefore numbers of the organisms localized in this site were counted on 5 each of 4-week-old mice and rats. Immediately after the animals were autopsied, a piece of the

pharyngolaryngeal tissue was cut off and homogenized with sterile broth by use of a glass homogenizer, making a 1% suspension for mice and 10% suspension for rats. Ten-serial dilutions were made with sterile broth from the suspensions and 0.1 ml of the dilutions was spread over a 5% horse blood agar, followed by 48 hr-incubation at 37°C.

As the results, numbers of the organisms in 5 mice examined were 1×10^5 , 1×10^4 , 6×10^3 , 2×10^3 and 6×10^3 per 1g tissue of the pharyngolaryngeal specimens, and those of 5 rats were so many as 3×10^7 , 3×10^8 , 2×10^7 , 6×10^7 and 1×10^8 . Thus it was shown that numbers of the organisms localized on pharyngolaryngeal area were much more in rats than in mice.

It was reported by many investigators that *P. pneumotropica* was isolated frequently from the respiratory tract [5,7,9,10,14] and uterus [4-6] of not only diseased but also healthy mice and rats. Moore et al. [12] isolated *P. pneumotropica* from the feces of gnotobiotic and barrier-held rats and mice and also from the entire intestinal tracts of all germ free rats inoculated perorally with the organisms, and thus they considered the gut might be the normal habitat of the organisms. In our present study, however, isolation rates of the organisms from the pharyngolarynx were so high as 100 per cent in rats and 85.7 to 97.5 per cent in mice, being significantly higher than those from the organs mentioned above, except for the gut or feces which were not examined, because of no available selective medium for the organisms. In addition, localization of the organisms in the pharyngolaryngeal area was shown to occur already in almost all of such young animals as 4 to 5 weeks old, yielding so large numbers of bacteria as 10^{7-8} /g tissue in rats and 10^{4-5} /g tissue in mice. From these findings, the pharyngolarynx seemed to be a primary localization site of the organisms in these animals.

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マウス，ラットにおける *Pasteurella pneumotropica* の保菌状態

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マウス，ラットにおける *Pasteurella pneumotropica* の保菌状態を調べる目的で本菌汚染コロニーから採取した5週齢，10週齢，退役後の動物について鼻孔，鼻道，咽喉頭，気管，肺，結膜，腔の菌検索を行った。

その結果，両動物種ともに年齢を問わず咽喉頭部で最高の検出率を示し，マウスでは保菌動物の85～97.5%，ラットでは100%の個体が陽性であった。この部位にお

ける菌数は4週齢マウスで組織1g当り 10^{3-5} ，4週齢ラットでは 10^{7-8} であった。鼻道と気管からの本菌検出率は30～90%の間で変動したが，鼻孔からの17～50%よりは高かった。肺からの検出は希であった。若齢ラットの結膜からは本菌が高率に検出されたが，マウスでは約5%に過ぎなかった。腔では保菌マウスの約30%，ラットの50～100%が陽性であった。