A study of lung lesions in asymptomatic rabbits naturally infected with *B. bronchiseptica*

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INTRODUCTION

A health monitoring study of apparently clinically healthy, purpose bred, laboratory rabbits in Sweden revealed a high frequency of chronic inflammatory lung lesions (*Feinstein & Rehbinder* 1988). *Bordetella bronchiseptica* was isolated from the respiratory tract of many of the rabbits, which suggested an association between *B. bronchiseptica* and the occurrence of pneumonic changes. In rabbits, however, the role of *B. bronchiseptica* regarding respiratory lesions is controversial. *B. bronchiseptica* has been considered from an opportunistic agent carried by healthy rabbits to a frank respiratory pathogen (*Oldenburg et al.* 1972, *Flatt* 1974). In addition, descriptions of naturally occuring lung lesions in rabbits are scanty and they are more concerned with *P. multocida* than with other respiratory pathogens (*Flatt & Dungworth,* 1971 a and b). For these reasons, we have performed a histological study of lung lesions observed in asymptomatic rabbits suffering of a natural *B. bronchiseptica* infection.

MATERIALS AND METHODS

Animals: We have examined material obtained during a previous study (*Feinstein & Rehbinder* 1988). The present investigation comprised 40 purpose bred rabbits of varying age, both young and adult, and of both sexes from 8 different colonies. Most of the rabbits were of the New Zealand White breed, born and maintained under various conventional conditions, but for colony no 4 which was provided with strict barriers and submitted rabbits of the NZW/Swiss hare breed. Housing conditions and sanitary aspects of each colony are described elsewhere (*Feinstein & Rehbinder* 1988). As depicted in Table 1 the number of *B. bronchiseptica* infected rabbits found in each colony was variable. The rabbits did not show any clinical signs and were naturally infected by *B. bronchiseptica.*

Procedures: The rabbits were sacrificed by intramuscular injection of 0.5 ml/kg Fluanison-Fentanil (Hypnorm, Jansen Pharmaceutica) followed by intracardial injection of 60 mg/kg pentobarbitone (Mebumal, ACO läkemedel). Intermediately after killing a necropsy was performed and samples were obtained for diverse studies, as described elsewhere (*Feinstein & Rehbinder* 1988).

Bacteriology: The bacteriological procedures were performed at and following the standard operational procedures of Department of Bacteriology, The National Veterinary Institute, Uppsala, Sweden, which could be consulted for further information. For routine bacteriology, samples were obtained by passing a swabb over conjunctiva, nasal cavities, middle ear, and tonsils. A portion of lung tissues was also cultured, and except for conjunctiva, samples from these locations were also cultured for Mycoplasma spp.

Microscopical studies: Portions of lung tissues were fixed by immersion in 10 % buffered formalin pH 7.4 during 24 hs. After routine processing and embedding in paraffin wax 4 μm thick sections were cut and stained with hematoxilin and eosin. Selected sections were also stained with Giemsa, Gram, PAS and van Gieson.

Table 1. Distribution of *B. bronchoseptica* infected rabbits in 8 colonies.

Colony	Body weight (kg)	Age*	Sex**
1	1.04	Y	F
	1.19	Y	F
	1.35	A	M
	1.36	A	F
	1.45	A	M
	1.46	A	M
	1.55	A	F
2	2.55	A	F
	2.56	A	M
3	0.97	Y	M
	1.01	Y	M
	1.60	A	F
	2.73	A	M
	3.01	A	M
	3.12	A	M
4***	2.55	A	M
	2.72	A	M
	2.85	A	M
5	0.90	Y	M
	0.93	Y	F
	0.97	Y	F
6	0.70	Y	M
	0.85	Y	M
	2.14	A	M
	2.25	A	M
	2.62	A	F
	2.72	A	F
7	0.69	Y	F
	0.83	Y	M
	1.23	Y	M
	1.26	Y	F
	1.46	A	F
8	0.77	Y	F
	0.78	Y	M
	0.93	Y	F
	0.93	Y	F
	1.39	A	F
	1.40	A	F
	1.63	A	F
	1.65	A	F

* Y: young A: adult.
** F: female M: male.
*** Barriered colony. (This rabbitry appears as colony No 5 in *Feinstein & Rehbinder* 1988).

RESULTS

Bacteriology

B. bronchiseptica was the only agent consistently isolated from lungs.

B. bronchiseptica was isolated from 36 rabbits affected by pulmonary changes and from 4 rabbits which were free of lung lesions. Fig. 1 displays the number of rabbits with and without lung lesions, as related to the organ from which *B. bronchiseptica* was isolated. *B. bronchiseptica* was most frequently isolated from the nasal mucosa (38 isolates), followed by lungs (20 isolates), tonsils (8 isolates) and conjunctiva (4 isolates) and middle ear (4 isolates), respectively. Not urusually, *B. bronchiseptica* was cultured from various organs of one and the same rabbit. *B. bronchiseptica* was isolated from young and adult rabbits, and there was no significant difference between sex or age of rabbits and recovery of *B. bronchiseptica* from a certain organ. Diverse Streptococci spp and Staphylococci spp were cultured from upper respiratory organs but not from lungs. A mycoplasma spp (that was not *M. pulmonis*) was isolated from nasal mucosa of a rabbit that yielded also *B. bronchiseptica* and displayed lesions of focal chronic interstitial pneumonia (FCIP). In the present study neither Pasteurella spp and Klebsiella spp nor other microorganisms were isolated from the lungs.

Macroscopical examination

Macroscopical changes were very rare. They appeared as a variable number of irregularly distributed white or greyish rounded areas that measured up to 5 mm in diameter.

Microscopical examination

Chronic inflammatory lung lesions were noticed in 38 out of 40 rabbits. The majority of *B. bronchiseptica* infected rabbits exhibited complex pulmonary lesions characterized by a simultaneous occurrence of FCIP, vascular changes and inflammation of the bronchial tree. The frequency of lesions in young and adult rabbits is displayed in Fig. 2. FCIP

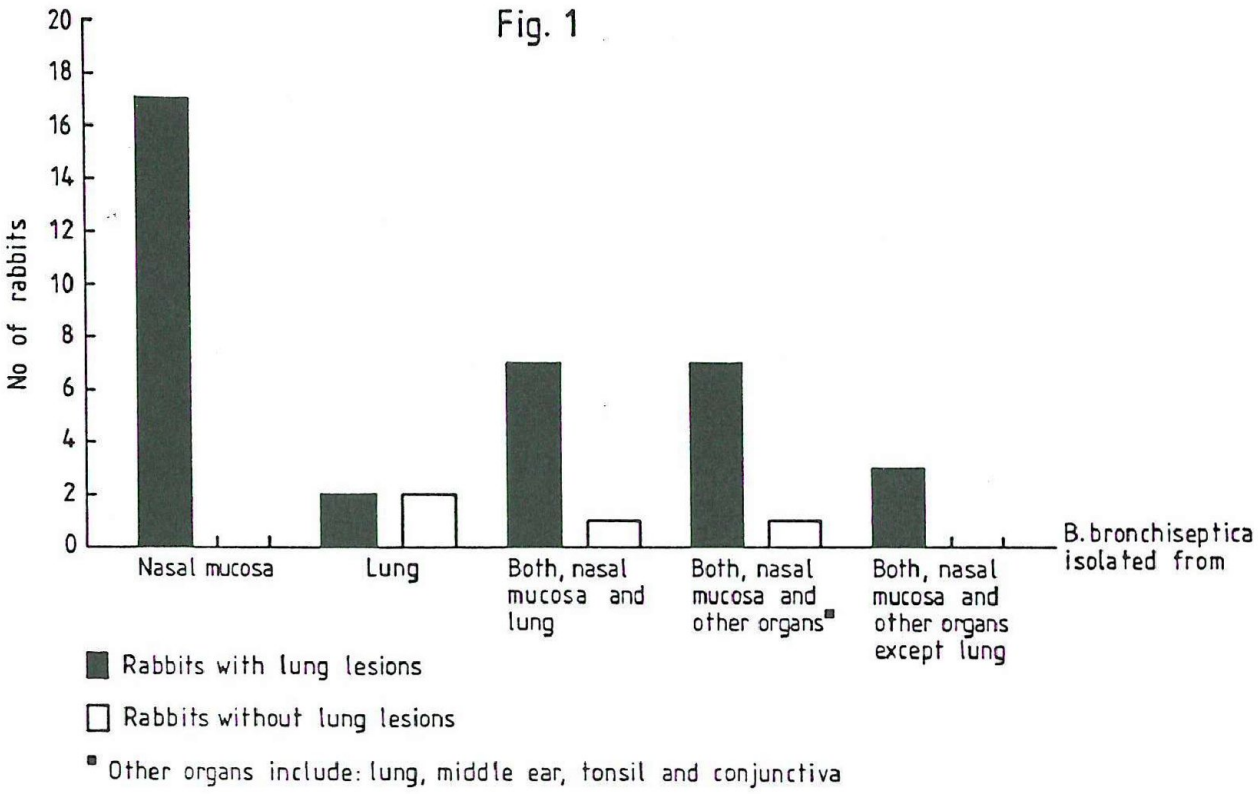

Fig. 1. Number of rabbits with and without lung lesions, as related to the organ from which *B. bronchiseptica* was isolated.

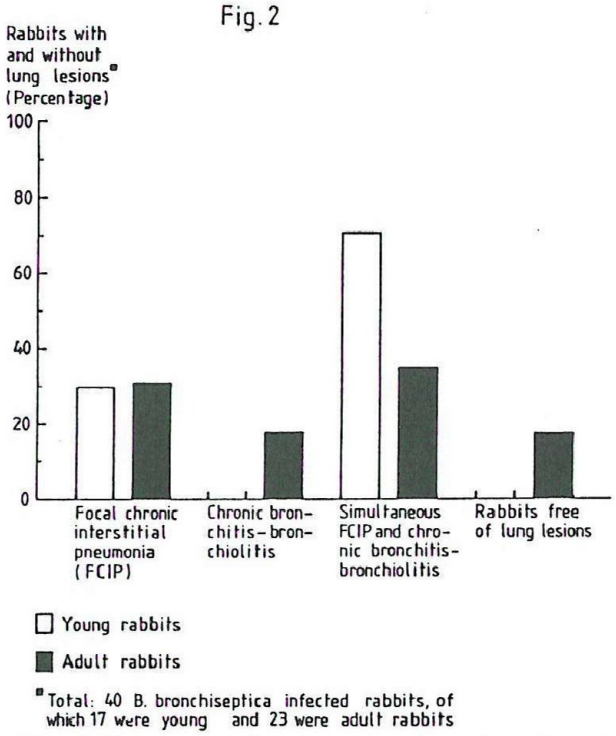

Fig. 2. Frequency of pulmonary alterations found in young and in adult rabbits.

appeared as clearly demarcated areas characterized by infiltrations of monocytes, macrophages and mononuclear leukocytes irregularly distributed throughout the lung tissues (Fig. 3). Most rabbits also displayed a discrete number of eosinophils in the exudate. The size and number of pneumonic foci was quite variable. Occasionally, confluence of microscopic zones of consolidation produced pneumonic areas that reached a few mm in diameter. Thickened alveolar septae appeared as highly cellular sinuous cords that contrasted against the more pale, eosinophilic background produced by large macrophages filling the alveoli. Frequently, macrophages displayed a vacuolated cytoplasm or contained a variable number of small cytoplasmatic granules (up to 2 μm in diameter) which appeared basophilic or eosinophilic and were stained dark blue by Giemsa. Macrophage nuclei were large, pale and oval with one or two prominent nucle-

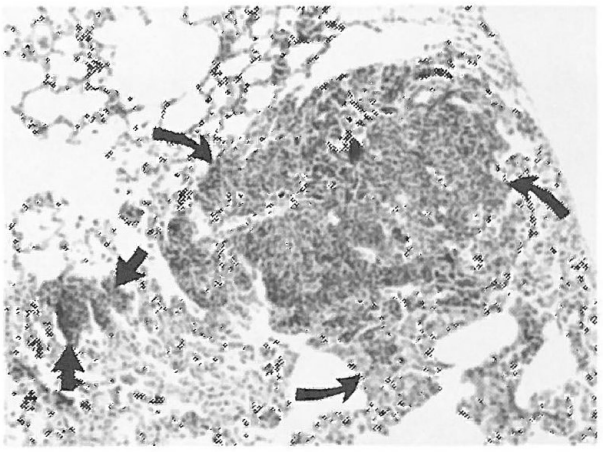

Fig. 3. Focal chronic interstitial pneumonia. Observe marked infiltration of lymphoid cells, monocytes and macrophages (long arrows) and thickened alveolar septae mainly due to infiltration of mononuclear cells (short arrows). Giemsa, × 40.

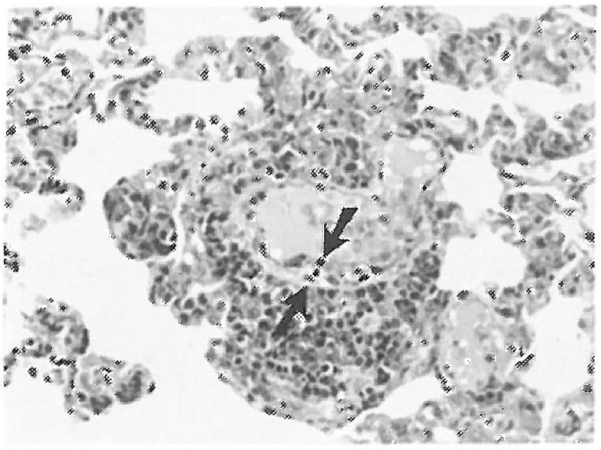

Fig. 5. Vascular changes. A small vessel surrounded by a moderate infiltration of lymphoid cells, monocytes and macrophages. Notice disruption of the vascular wall, which is thickened and also infiltrated by a low number of leukocytes (arrows). Giemsa, × 400.

oli. In numerous rabbits we noticed large bi- or multinucleated macrophages that were usually located in exudate filled alveoli (Fig. 4). Such syncytial cells could display up to 8 nuclei that were positioned in the cell periphery or, less frequently, were irregularly distributed throughout the cytoplasm.

We observed infiltrations of mononuclear leukocytes, monocytes, and a few eosinophils surrounding small and middle sized arteries and veins (Fig. 5). Usually, perivascular infiltrations were placed around a few vessels only, but in lungs displaying prominent inflammatory changes most vessels were involved. The magnitude of perivascular infiltrations varied markedly between different areas of the same lung, ranging from a few cells to several layers of inflammatory cells placed around the vessel. Severe perivascular cuffing produced compression of adjacent pulmonary tissues. Only rarely perivascular infiltrations occurred in the absence of other inflammatory lung changes. In addition, in a few rabbits a moderate number of vessels also exhibited infiltrations of the vascular wall with mononuclears and a low number of monocytes, macrophages and granulocytes (vasculitis) (Fig. 5). In cases of severe vasculitis the vessel wall was markedly disrupted by infiltrating cells and also by oedema and degenerative and necrotic changes with moderate deposition of a acidophilic amorphus or fibrilar material. The endothelium was usually preserved, but sporadically we noticed swelling of endothelial surface. The general appearance of the lung lesions suggested a chronic stage, but fibroblastic proliferation was generally mild. However, in areas of pronounced infiltration we noticed a moderate fibrious deposition. Mitotic figures occurred regu-

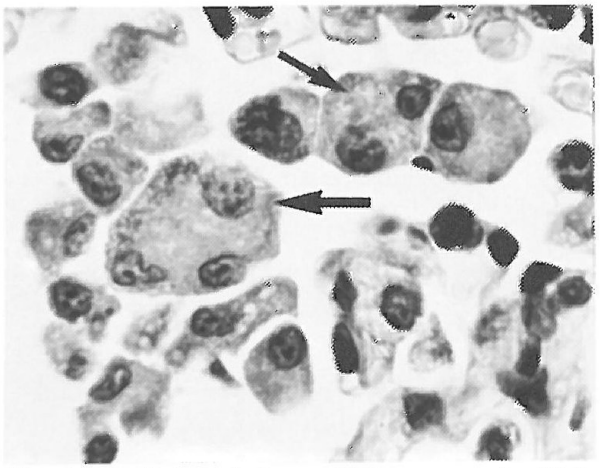

Fig. 4. Focal chronic interstitial pneumonia. Multinucleated macrophages (arrows) located in an alveolar space. Giemsa, × 400.

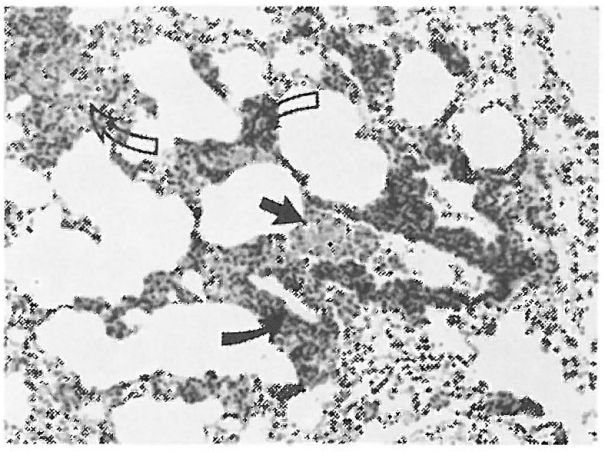

Fig. 6. Chronic bronchiolitis. Exudated inflammatory cells are located in a terminal bronchiolus (short arrow). Notice moderate perivascular cuffing of mononuclear cells around a neighbouring vessel (long full arrow) and thickened alveolar septae and consolidation (hollow arrows). Giemsa, × 100.

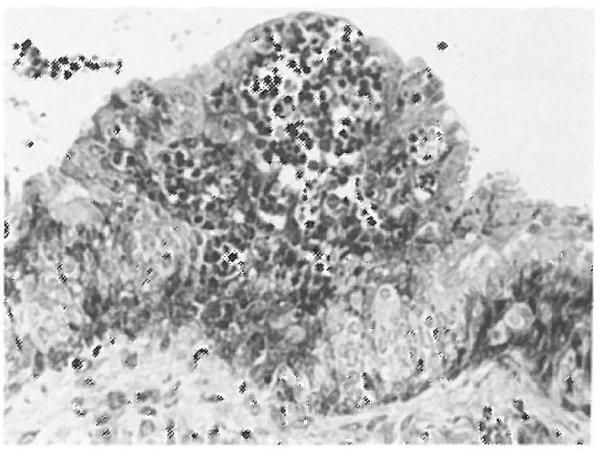

Fig. 7. Focal chronic purulent bronchitis. Bronchial mucosa displaying a focus of mixed leukocytic infiltration and focal degenerative and necrotic changes of the epithelium. H & E, × 100.

larly in consolidated zones, in perivascular infiltrates and also in alveoli and septae. Generally, we could not determine which type of cell was undergoing mitosis, but cytoplasmic features of certain dividing cells resembled macrophages.

Pulmonary lesions occurred simultaneously with moderate focal chronic bronchitis and bronchiolitis. As displayed in Fig. 6, focal chronic bronchiolitis was frequently located at the bronchioloalveolar junction. In a few rabbits we observed chronic inflammation of airways in lungs lacking pneumonic changes. In focal chronic bronchitis and bronchiolitis the bronchial mucosa appeared to be, from mild to severely, infiltrated by mononuclears and granulocytes (mainly eosinophils). In the bronchial epithelium we frequently noticed loss of ciliae, cell swelling, vacuolar degeneration, necrotic changes and a moderate number of mitotic figures. Degenerate and necrotic epithelial cells and desquamated leukocytes were present in the bronchial lumen, mixed with respiratory secretions and cell debris. In severe bronchitis the bronchial associated lymphoid tissue (BALT) was apparently enlarged and exhibited numerous mitotic figures and pronounced lymphocytolysis. A markedly enlarged BALT produced compression of adjacent lung tissue. Focal chronic purulent bronchitis and purulent bronchiolitis was diagnosed in 5 rabbits in which a pronounced neutrophilic infiltration and extensive damage to the bronchial mucosa were dominant features (Fig. 7). Giemsa and Gram stained lung sections did not reveal bacteria.

DISCUSSION

Several studies have demonstrated the presence of *B. bronchiseptica* in the respiratory tract of rabbits (*Webster* 1924, *Flatt & Dungworth* 1971 b, *Oldenburg et al.* 1972). In addition, our investigation revealed a high frequency of lung lesions in *B. bronchiseptica* infected rabbits, showing no clinical signs of respiratory disease.

In other animal species, such as guinea pigs, *B. bronchiseptica* is regarded as a pathogen capable of producing respiratory disease (*Ganaway* 1976, *Baskerville et al.* 1982, *Manning et al.* 1985). In rabbits, however, *B. bronchiseptica* has been considered from an opportunistic organism found in healthy animals to a frank respiratory pathogen (*Winsser* 1960, *Oldenburg et al.* 1972, *Goodnow* 1980). To determine whether *B. bronchiseptica* was the cause of the pul-

monary lesions found was out of the scope of the present study. Nevertheless, we suspect a *B. bronchiseptica* involvement because this agent was repeatedly isolated from respiratory organs and lungs of rabbits displaying pulmonary changes and we observed very few *B. bronchiseptica* infected rabbits that were free of histological lung lesions. In addition, after our study was completed an antibiotic treatment against *B. bronchiseptica* was conducted at the only barriered colony here considered and rabbits from this place, thenceforth submitted for health monotoring, each sixth month, have been negative both for *B. bronchiseptica* as well as for lung lesions (Up to the present time, from this rabbitry 26 NZW/Swiss hare rabbits, both young and adult, and of both sexes have been investigated. Unpublished). In the present study, *B. bronchiseptica* was isolated from nasal cavities more frequently than from pneumonic lungs. Partly, this could reflect differences in the sampling methods for bacteriology: A swabb was rubbed over large areas of nasal mucosa while lung samples consisted of randomly collected portions of tissues, perhaps obtained from a bacteria-free zone, as many lungs did not reveal any macroscopical changes. *Watson et al.* (1975) noticed pulmonary changes in rabbits experimentally infected by *P. multocida* or by *B. bronchiseptica,* though the latter induced the most severe lesions. In addition, they observed a low recovery rate of bacteria from lungs which was attributed to a patchy distribution of lesions or to the amount of tissues submitted for analysis.

Nevertheless, in the present study samples from other locations such as tonsils were collected also by swabbing, but the number of *B. bronchiseptica* isolates from these sites was even lower than for lungs, which suggests a role of factors others than sampling. In this respect, the existence of a carrier state has been debated. Rabbits experimentally infected by *B. bronchiseptica* cleared this agent from trachea and lungs but not from the nasal mucosa, and rarely recovered from the infection (Yoda et al. 1982). Unfortunately, in the refered study a histological analysis of lungs from *B. bronchiseptica* carriers was not performed. *Bemis* (1986), opposing the existence of a true carrier state considered a recovery possible in animals not exposed to sources of reinfection. In guinea pigs a carrier state is recognized, since *B. bronchiseptica* is frequently isolated from the upper respiratory portions of clinically healthy guinea pigs (*Baskerville et al.* 1982, *Manning et al.* 1985). In the present study, it appears likely that many of the rabbits suffered from a chronic *B. bronchiseptica* infection. They had cleared this agent from the lungs but not from the nasal mucosa, and still they exhibited pulmonary sequelae, which is also observed in swine bordetellosis (*Bemis* 1986). *Maeda & Shimizu* (1975) described severe respiratory lesions in young rabbits experimentally infected by *B. bronchiseptica* strains of rabbit and pig origin, but they did not mention vascular changes, which was a striking feature in our material. Pneumonia and vascular changes was described in swine and rabbits naturally and experimentally infected by *B. bronchiseptica* (*Duncan* 1966, *Oldenburg et al.* 1972, *Watson et al.* 1975), and *Duncan et al.* (1966) postulated a diffusible bacterial product as responsible for the damage to blood vessels. Bordetella species synthetize at least one toxin which is similar to the lipopolysaccharides of other gramnegative bacteria (*Goodnow* 1980). In addition, vascular (and lung) changes could arise by other mechanisms, and it is likely that a *B. bronchiseptica* infection, even if localized at the level of the nasal mucosa, will generate antibodies which could be involved in a type III hypersensitivity reaction with development of pulmonary changes secondary to the deposition of immune complexes in lung tissues and small vessels (*Benfer Kaltreider* 1976). In order to corroborate this possibility further investigations are required.

In the present study Pasteurella spp, agents repeatedly associated with respiratory dise-

ase in rabbits (*Flatt* 1974), were not observed. It is worth mentioning, that at our Institute, Pasteurella spp was isolated at other occasions, usually from rabbits submitted for diagnosis due to respiratory disturbances or sudden death. In the present investigation the lesions were different from what was described by others in pasteurella infected rabbits (*Flatt & Dungworth* 1971 a and b, *Flatt* 1974). Our results thus do not contradict the role of Pasteurella spp concerning rabbit respiratory diseases. Instead they point at *B. bronchiseptica* as an important factor associated with lung lesions in asymptomatic rabbits. This, however, does not prove that *B. bronchiseptica* was the sole cause of the alterations. *Ostler* (1961), refering to pneumonia of rabbits, mentioned that the bacteria isolated could constitute a secondary infection following a viral or a protozoan disease, and *Feinstein et al.* (1989) described focal chronic interstitial pneumonia in numerous rabbits that were negative for known respiratory pathogens. It is apparent that lung lesions, a problem occurring most frequently among laboratory rabbits (*Feinstein & Rehbinder* 1988), require further studies.

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Summary
A histological study of lungs performed in 40 rabbits carrying a naturally acquired subclinical *B. bronchiseptica* infection revealed chronic inflammatory pulmonary lesions in 38 rabbits. *B. bronchiseptica* was the only agent consistently isolated from lungs. Pasteurella spp. was not isolated from any animal. *B. bronchiseptica* infected rabbits frequently displayed changes characterized by a simultaneous occurrence of focal chronic interstitial pneumonia, vascular and perivascular infiltration of monocytes and lymphoid cells, and inflammation of bronchi and bronchioli.

Sammanfattning
I en histologisk studie av lungor från 40 kliniskt friska kaniner, men bärande en naturligt erhållen subklinisk *B. bronchiseptica* infektion, påvisades kroniska inflammatoriska lungförändringar hos 38 av djuren. *B. bronchiseptica* var det enda agens som regelbundet kunde isoleras från lungorna. Pasteurella spp. kunde ej påvisas hos något av djuren. De *B. bronchiseptica* infekterade kaninerna uppvisade frekventa förändringar karaktäriserande av samtidigt uppträdande fokal kronisk interstitiell pneumoni, vaskulära och perivaskulära infiltrat av monocyter och lymfoida celler samt inflammatoriska förändringar i bronker och bronkioli.

Yhteenveto / K. Pelkonen
Tutkimuksessa tehtiin histologinen tutkimus 40 kanista, joilla oli luonnollisesti saatu subkliininen *B. bronchiseptica*-infektio. Havaittiin, että näistä 38:lla oli kroonisia tulehdusmuutoksia keuhkoissa. Ainoa kaikista keuhkoista eristettävissä oleva taudinaiheuttaja oli *B. bronchiseptica.* Yhdestäkään eläimestä ei löytynyt Pasteurella spp. *B. bronchiseptican* kantajista löytyi yleisesti muutoksia, joita luonnehtivat yhtaikainen fokaalinen krooninen interstitiaalipneumonia, monosyyttien ja imusolujen vaskulaarinen ja perivaskulaarine infiltraatio, ja keuhkoputkien ja bronkiolien tulehdus.

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