Characterization of *Pasteurella multocida* Isolated from Rabbits in Canada

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ABSTRACT

In a survey for the somatic and capsular serotypes of *Pasteurella multocida* present in domestic rabbits in Canada, but mainly in Ontario, samples were obtained from research facilities, commercial rabbitries and from abattoir and necropsy specimens. Sources of isolates were upper respiratory tract infections, localized bronchopneumonias, acute fibrinous pneumonias, abscesses and *otitis media*. Of 59 isolates obtained, 47.0% were type 12:A, 30.5% 3:D and 12.0% were 3:A. Less common types were 12(4):A, 12:D, 4(12):A and 3:untypable. Somatic group 3 was most commonly isolated from acute pneumonic disease, while serogroup 12:A was most commonly found in upper respiratory tract infections and in localized chronic bronchopneumonia. Two serotypes of *P. multocida* were isolated from four pneumonic lungs collected from abattoir specimens. Most isolates were susceptible to the commonly used antibiotics.

Key Words: *Pasteurella multocida*, serotype, rabbit, respiratory disease.

RÉSUMÉ

Les échantillons qui servirent à effectuer un relevé des sérotypes somatiques et capsulaires des *Pasteurella multocida* isolées de lapins du Canada, surtout d'Ontario, provenaient de laboratoires de recherche, de clapiers commerciaux, d'abattoirs et d'une salle de nécropsies. Les souches isolées originaient d'infections des voies respiratoires supérieures, de pneumonies fibrineuses aiguës, d'abcès et d'otites moyennes. Des 59 souches ainsi obtenues, 47% appartenaient au sérotype 12:A; 30,5%, au sérotype 3:D, et 12%, au sérotype 3:A. Les sérotypes suivants s'avérèrent les moins fréquents: 12(4):A, 12:D, 4(12):A et 3: non typable. Le sérotype somatique #3 s'avéra responsable de la plupart des cas de pneumonie aiguë, tandis qu'on isola le sérotype 12A de la majorité des infections des voies respiratoires supérieures et des broncho-pneumonies chroniques localisées. On isola aussi deux sérotypes de *P. multocida*, à partir de quatre poumons qui présentaient des lésions de pneumonie détectées à l'abattoir. La plupart des souches précitées s'avérèrent sensibles aux antibiotiques usuels.

Mots clés: *P. multocida*, sérotype, lapin, maladie respiratoire.

INTRODUCTION

Pasteurellosis due to *Pasteurella multocida* is an important disease in both commercial rabbitries and in laboratories using rabbits for research purposes. A variety of clinical signs and lesions have been associated with *P. multocida* infections in the domestic rabbit. Snuffles due to rhinitis, chronic bronchopneumonia and acute fibrinous bronchopneumonia are patterns of disease seen in the respiratory tract. Conjunctivitis, purulent *otitis media*, localized abscessation, genital tract infections and acute septicemic pasteurellosis may also occur with this disease (1). *Bordetella bronchiseptica* infections have also been associated with the respiratory disease complex in rabbits. Pneumonic lesions have been reproduced in immunosuppressed rabbits inoculated intratracheally with *B. bronchiseptica* (2). However, this organism is generally considered to be a relatively nonpathogenic resident of the respiratory tract in the domestic rabbit (1). Estimates of the number of animals which harbour *P. multocida*, as either apparent or inapparent carriers, have varied from 20 to 70% (1). A variety of procedures have been used to reduce or eliminate pasteurellosis in rabbitries, including the establishment of *Pasteurella*-free breeding colonies using caesarian-derived stock (3), the reduction of carriers by antibiotic or sulfonamide therapy (4), and the elimination of carriers by repeated bacterial examination of nasal samplings (1). Recently, there have been reports of effective immunization of rabbits against pasteurellosis using live *P. multocida* vaccines (5,6). However, prior to the implementation of a vaccination program, it is essential that the appropriate strain(s) of *P. multocida* be selected for immunization. In recent surveys, serotype 12:A was the prevalent isolate from domestic rabbits (7,8). However, there is some evidence that isolates of serotype 3:A may be more pathogenic for rabbits than are strains of 12:A (6). This survey was designed to investigate 1) the serotypes of *P. multocida* prevalent in Canada, 2) the correlation, if any, between capsular and somatic types and patterns of disease and 3) the sensitivity of *P. multocida* isolates to commonly used antibiotics.

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MATERIALS AND METHODS

A total of 59 *Pasteurella multocida* isolates were obtained from the following sources: Eight research facilities including Ontario, Quebec, Manitoba, Saskatchewan and Alberta, six commercial rabbitries in Ontario and two licensed abattoirs in Ontario. In samples obtained from research facilities, all had a history of sporadic cases of snuffles and occasionally *otitis media* and lower respiratory tract disease. In samples collected from live animals, nasal swabs were collected from rabbits with clinical evidence of snuffles. Bacteriology samples were also obtained from animals examined at necropsy from Research Facility A (University of Guelph). In samples obtained from research facilities other than the University of Guelph, swabs were streaked on blood agar plates and incubated at 37° C prior to submission, or swabs were placed directly in Cary-Blair transport medium. Samples were sent to the University of Guelph by mail or by courier service. One to six specimens were received from each research facility. Of samples collected from commercial rabbitries, three of the six rabbitries surveyed had a history of sporadic deaths due to pasteurellosis. In live rabbits sampled from commercial rabbitries, animals were selected which had clinical evidence of snuffles. If insufficient animals of this type were identified, asymptomatic animals were also used. Four to six samples were collected per rabbitry. Swabs were placed in transport medium and brought directly to the Diagnostic Laboratory. In two rabbitries, only samples collected from animals submitted for necropsy were available for serotyping. The sources of isolates are shown in Table I. The isolates were obtained from nasal swabs from apparently normal live rabbits and those affected with "snuffles". Bacterial cultures made at the abattoir or at routine necropsy from rabbits which had *Pasteurella*-associated lesions. In the abattoir survey, samples were collected from a total of 60 rabbits from two abattoirs. Swabs were collected from diseased tissues including consolidated portions of lung and areas of abscessation. Bacteriology swabs were transported to the diagnostic laboratory in Cary-Blair transport medium. The majority of specimens collected at the abattoir were from rabbits eight to ten weeks of age.

All bacteriology samples were cultured and isolates were identified in the Diagnostic Laboratory, Department of Veterinary Microbiology and Immunology, Ontario Veterinary College. Of the specimens submitted, 45 cultures were evaluated for the presence of *B. bronchiseptica* in addition to *P. multocida*. Swabs were streaked onto blood agar plates and incubated aerobically at 37° C for 24-48 hours. Colonies were identified as *P. multocida* or *B. bronchiseptica* using established morphological and biochemical criteria (9,10) and antibiotic sensitivity tests were performed using the Kirby-Bauer standard disc diffusion method (10). Both the capsular antigens A and D were determined by nonserological procedures described by Carter and Rundell (11) and Carter and Subronto (12) respectively. Somatic antigens were determined by the gel diffusion precipitin test as described previously (13,14,15). Selected tissues collected for histopathological evaluation were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 6 μm and stained with haematoxylin and eosin.

RESULTS

In the nasal swab survey from research facilities, results are summarized in Table I. In the cultures collected from the lungs of rabbits which died during two enzootics of acute pasteurellosis in facility A, two different serotypes were isolated (Table I). In the survey of commercial rabbitries, two of the 16 nasal swabs collected (12.5%) were positive for *P. multocida* on culture (Table I). In the two rabbits necropsied from rabbitry E, both had acute fibrinous pneumonia. A different serotype of *P. multocida* was isolated from each of these animals (Table I). In one sample collected from a case of acute fibrinous pneumonia in the one animal available from rabbitry F, serotype 3:A was isolated (Table I).

A correlation of the disease process

TABLE I. Isolates of *P. multocida* and *B. bronchiseptica* From Rabbits in Canada

Source	Site of Sampling	Isolates of *P. multocida*	Isolates of *B. bronchiseptica*
(R) A	Nasal	1/6[a] (3:A)[b]	ND
(R) A	Lung	1/1 (3:D)	ND
(R) A	Lung	3/3 (3:A)	1/3
(R) B	Nasal	3/3 (12:A)	ND
(R) C	Nasal	5/6 (12:A, 3:D)	ND
(R) D	Nasal	0/3	0/3
(R) E	Nasal	1/1 (12:A)	ND
(R) F	Nasal	1/6 (3)[c]	5/6
(R) G	Nasal	2/2 (3:D)	ND
(R) H	Nasal	1/2 (12:A)	2/2
(C) A	Middle Ear	1/1 (12:A)	ND
(C) A	Nasal	0/5	ND
(C) B	Nasal	0/4	ND
(C) C	Nasal	1/3 (12:A)	ND
(C) C	Abscess	1/1 (12:A)	ND
(C) D	Nasal	1/4 (12:A)	ND
(C) E	Lung	2/2 (12:A, 3:D)	0/2
(C) F	Lung	1/1 (3:A)	0/1
(A) A	Lung	5/26	ND
(A) A	Abscess	2/2	ND
(A) B	Lung	20-25	10/25
(A) B	Abscess	7/8	0/3
		59/115	18/45

[a]No. of positive isolates/No. sampled
[b]Serotypes of isolates from research and commercial rabbitry survey
[c]Untypable capsule
(R) = Research facility
(C) = Commercial rabbitry
(A) = Abattoir
ND = Not determined

and the capsular and somatic typing of isolates of *P. multocida* are presented in Table II. In this survey, serotype 12:A was the most frequent isolate recovered from the upper respiratory tract. Type 12:A was also the prevalent isolate from the lower respiratory tract of clinically normal rabbits submitted for slaughter (Table II). In this study, serotypes 3:A or 3:D (77%) were most frequently associated with the acute fatal form of pasteurellosis characterized by fibrinous pneumonia (Table II). In two epizootics of acute fatal pasteurellosis, foci of hepatic necrosis were observed at necropsy.

In four abattoir specimens cultured, more than one serotype of *P. multocida* was isolated from single samplings of consolidated lung from individual animals. Combinations were as follows: 12:A and 3:D; (three animals) and 12 (4):A and 3:D (one animal). Of 45 specimens cultured for both *P. multocida* and *Bordetella bronchiseptica*, the latter was isolated from 18 samples (Table I). Mixed infections occurred in nine, including one case of acute fibrinous pneumonia. In five cases of clinical snuffles and in four cases of enzootic bronchopneumonia, *B. bronchiseptica*, but not *P. multocida*, was isolated.

ANTIMICROBIAL RESISTANCE

Of the 59 isolates of *P. multocida* tested, patterns of resistance to sulfonamides and antibiotics were as follows:

Resistance to triple sulfas (250 μg): 13/59 (22%); resistant to penicillin: 2 (3.4%); resistant to neomycin: 5 (8.5%); resistant to tetracycline 1 (1.7%); resistant to gentamicin 2 (3.4%); resistant to chloramphenical 0 (0%).

PATHOLOGICAL FINDINGS

In abattoir specimens examined, lesions were present most frequently in the lung. Localized areas of consolidation occurred in the anteroventral portions of one or both lungs and mucopurulent exudate was present in the bronchi in approximately 25% of the affected specimens. Pulmonary abscessation was present in seven specimens and acute fibrinous bronchopneumonia was observed in one of the abattoir specimens. However, pulmonary lesions frequently were minimal and consisted of foci of tan to red discoloration involving scattered lobules. In spontaneous deaths associated with *P. multocida* in commercial rabbitries and research facilities, lesions varied and included acute fibrinous bronchopneumonia, fibrinous pleuritis, pericarditis and abscessation (Table II). Histologically, in those slaughterhouse specimens with pneumonic lesions, changes varied from peribronchial cuffing with minimal changes in alveolar septa to chronic bronchitis and focal pneumonia, characterized by peribronchial lymphocytic infiltration, proliferation of bronchial epithelial cells and leukocytic infiltration.

DISCUSSION

Based on our findings, serotype 12:A is the predominant serotype associated with upper respiratory tract infections and localized bronchopneumonia, while serotypes 3:A and 3:D were more often associated with the acute fulminating form of the disease than other serotypes. In other reports, type 12:A was the predominant serotype (7,8), and in other studies, capsular type A (16) and somatic type 12 (17) were the most prevalent types of *P. multocida* isolated from rabbits. In general, in this survey, a single serotype was isolated from rabbits in most of the individual commercial and research facilities sampled. However, it is possible that multiple serotypes would have been identified, had the survey included larger numbers of animals sampled over a period of several weeks. The factors responsible for the virulence of individual serotypes of *P. multocida* have not been determined. Glorioso *et al* (18) have demonstrated that the ability of serotype A to adhere to rabbit nasopharyngeal epithelium was greater than that of capsular types B, D or E. They suggested that bacterial attachment plays an important role in the colonization of the upper respiratory tract. However, capsular type D was isolated from three of nine cases of acute pasteurellosis in our study (Table II). This finding indicates that virulence factors of *P. multocida* are not necessarily confined to the capsule of the organism. The more frequent isolation of serotype 3 (77%), than type 12 from acute pasteurellosis suggests that the lipopolysaccharide antigen may be an important virulence factor. Lu and Pakes also suggested that serotype 3 was more virulent for rabbits (6). It is evident from this study, and from previous reports (4,16), that antibiotic resistance is not a major problem with *P. multocida* infections in rabbits. Antibiotic therapy may be useful during clinical outbreaks of lapine pasteurellosis. However, although antibiotics have been used as a means of controlling the disease in specific pathogen-free colonies (3), it is not an effective means of eliminating the carrier state of *P. multocida* (1).

TABLE II. Capsular and Somatic Types of *Pasteurella multocida* Isolated from Rabbits

		Source of Bacterial Isolates						
Somatic Type	Capsular Type	Upper Respiratory Tract	Localized Bronchopneumonia	Acute Fibrinous Pneumonia	Abscess	*Otitis Media*	Total	Percentage
3	A	1	1	4	1	0	7	12
3	D	4	9	3	2	0	18	30.5
3	UT[a]	1	0	1	0	0	2	3.4
12	A	9	10	1	7	1	28	47
12	D	0	1	0	0	0	1	1.7
4 (12)	A	0	1	0	0	0	1	1.7
12 (4)	A	1	1	0	0	0	2	3.4
Totals		16	23	9	10	1	59	

[a]UT = Untypable

The spectrum of lesions seen in our abattoir samples is similar to those observed in a survey of slaughterhouse specimens performed in the southern United States (19). Unlike lower respiratory tract infections in most species, there currently is no evidence that viral infections play a role in bronchopneumonias seen in the domestic rabbit. However, mixed bacterial infections do occur. *Bordetella bronchiseptica* has been isolated from pneumonic lesions and from grossly normal lungs (20,21), but the significance of this organism as a pathogen remains contentious. Flatt and Dungworth (21) isolated this organism from 97% of macroscopically normal lungs sampled and concluded that *B. bronchiseptica* did not play an important role in the pathogenesis of enzootic pneumonia in this species. However, rhinitis and focal bronchopneumonia have been reproduced as readily with *B. bronchiseptica* as with *P. multocida* in inoculated, immunosuppressed, specific pathogen-free rabbits. Lesions of the respiratory tract were similar, regardless of which organism was used (2). Our findings support the suggestion that *B. bronchiseptica* is also a relatively important respiratory pathogen in the rabbit. However, there is no evidence that the organism alone can produce the acute systemic disease or fibrinous pneumonia of the type associated with peracute *Pasteurella* infections.

The simultaneous recovery of two serotypes of *P. multocida* with different capsular and somatic antigens from the lungs of four abattoir specimens was an unexpected finding. The colonial appearance of the two capsular serotypes was different, the type D strains lacking the mucoid character of the type A. In each of these cases, the organisms were recovered from one swab from a single bronchial sampling collected from an area of localized bronchopneumonia. Thus, the simultaneous colonization of the lower respiratory tract by two or more serotypes of *Pasteurella* may occur without necessarily producing clinical disease.

The variety of lesions observed in *Pasteurella*-infected rabbits seen in this study emphasizes the wide spectrum of tissue tropisms of the organism in this species. In addition, the variations in type and distribution of pulmonary lesions, from localized bronchopneumonia to acute fibrinous pneumonia may be due to factors such as body condition, age at exposure and environmental conditions, but is also suggestive of variations in virulence in individual strains. In view of the prevalence of serotype 12:A and 3:A in the United States, isolates of these strains have been used as the serotype for recent immunization trials (5,6). However, in our study, three of the nine isolates of *P. multocida* from fatal cases of pasteurellosis were somatic serotype 3:D. Thus, for an effective immunization program in this species, serotype 3:D should also be considered as a possible component of a polyvalent commercial vaccine.

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