Occupational exposure to animals and antibodies against *Pasteurella multocida*

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ABSTRACT The relation between occupational exposure to cattle and prevalence of antibodies against *Pasteurella multocida* was evaluated in 680 workers. Three groups of exposed workers in abattoirs and slaughterhouses (S), in industrial breeding (I), and in traditional breeding (T) were compared with control workers not exposed to cattle or chicken (C). The prevalence of antibodies against capsular antigen A determined by indirect haemagglutination was significantly higher in the exposed groups (S: 26.2%; I: 29.0%; T: 32.1%) than in the control group (C: 14.0%). The prevalence of antibodies against capsular antigen D did not differ significantly between the groups. The prevalence of antibodies against one or more somatic antigens 1,2,3,7,8, or 9 was higher in the exposed groups with a significant difference only for group T versus group C ($p < 0.05$). There was also a significant relation between antibodies against capsular antigen A and the contacts with pets. This high prevalence of antibodies against *P. multocida* suggests that the infection is frequently subclinical and not only a disease associated with pets but also an occupationally related infection.

Pasteurella multocida is a Gram-negative coccobacillus commonly found in the oral and nasal cavities of many animals. Human infections typically follow animal bites or scratches but in some cases the infection is not confined to soft tissues.¹

Proof of *P. multocida* as the cause of infection requires culture of the organism but isolation of the bacteria may be difficult. Serological diagnosis is routinely used by veterinarians and has been assayed in a few cases of proved infection in man. High titres of antibodies against *P. multocida* are found in recent infections.^{2,3} An epidemiological survey in an urban population of 245 subjects showed a high prevalence of antibodies against *P. multocida* (16%) with a relation with pets⁴; no seroepidemiological study was performed among workers exposed to animals.

The purpose of the present study was to determine the relation between occupational exposure to cattle and the prevalence of antibodies against *P. multocida*.

Materials and methods

STUDY POPULATION

Between January and June 1985, 680 workers had a blood sample taken and were given a questionnaire. All resided and worked in the same geographical area in four districts in west France. We compared 157 control subjects with 523 subjects occupationally exposed to animals: four types of workers were selected according to their exposure to cattle or chickens:

Group S—Workers in abattoirs and in chicken slaughtering plants.

Group I—Farmers in industrial breeding.

Group T—Farmers in traditional breeding.

Group C—Control subjects not exposed to cattle or chickens.

QUESTIONNAIRE

Each subject completed the questionnaire. It contained questions on individual characteristics such as age, race, type and duration of work, contacts with pets or cattle, and recent bites or scratches.

SEROLOGICAL TESTS

The strains used for the tests were classified as the capsular serotypes defined by Carter⁵⁻⁷ and as the somatic serotypes defined by Namioka.⁸⁻¹⁰

Purified capsular antigens A and D were obtained by the technique of Carter⁶ or of Westphal *et al*¹¹ from 18 hour cultures of *P. multocida*. The pellets were resuspended in phosphate buffered saline (PBS) and heated at 56°C for 30 minutes. After centrifugation each supernatant was treated by hyaluronidase (50 U) for two hours at 37°C. Then the bacterial extract was fixed on sheep red blood cells. One millilitre of the antigen solution was incubated with 0.1 ml of sheep red blood cells in PBS for two hours at 37°C. The sensitised blood cells were centrifuged, washed, and diluted to 1% in PBS.

One millilitre of decomplexed serum of each patient was mixed with 0.1 ml of sheep red blood cells. After two hours at 37°C or 12 hours at 4°C, the cells were removed by centrifugation and the adsorbed serum was diluted by twofold serial dilutions in ten tubes (0.4 ml per tube) or dispensed into a microtitre plate (0.05 ml per well). The same volume of sensitised red blood cells was added to the tubes or to the wells. The rack of tubes was shaken and then kept at room temperature for two hours, at which time a reading was taken.

The somatic antigens were obtained by the technique of Namioka and Murata⁸ or by a technique derived from Carter⁶: the 18 hour growth from a tryptose serum agar plate was removed and collected by centrifugation. The bacteria were suspended in PBS and treated by hyaluronidase (50 U) for two hours at 37°C. After washing, they were treated by sodium dodecyl sulphate (0.02%) for 30 minutes at room temperature. The bacteria were washed three times after which sufficient PBS (formol 0.3%) was added to obtain an opacity equivalent to tube 50 of Brown. On a glass plate, 50 µl of each suspension of somatic antigen and 50 µl of the serum were mixed and mechanically shaken for two minutes. To facilitate reading, the reactions were observed using a lens. The sera were examined without dilution. If the reac-

tion was positive with pure serum, twofold dilution from 1/5 were tested.

Appropriate controls included positive serum from a known hyperimmunised rabbit and negative serum which served as a reference standard for each batch of test serum.

STATISTICAL ANALYSIS

Group C was compared with the three exposed groups to take into account contacts with pets and occupational exposure to animals. Standard descriptive statistics were used to represent response as a frequency distribution and to calculate group means and variances. Chi square tests were used with 2 × 2 contingency tables to determine whether the relation shown between the variables were statistically significant. Comparisons of quantitative data were made using the standard unpaired *t* test.

Results

Table 1 shows the characteristics of the populations. The comparison between the control group (C) and the groups occupationally exposed to animals showed a higher frequency of pets in group I ($\chi^2 = 6.45$, $p < 0.02$) and in group T ($\chi^2 = 44.8$, $p < 0.0001$) and of recent bite or scratches in group I ($\chi^2 = 22.3$, $p < 0.0001$).

Among the whole population, antibodies against capsular antigen A were found in 26.3% with titres from 1/20 to 1/640 and against capsular antigen D in 6.7% with titres from 1/20 to 1/80. The antibodies against capsular antigen D were observed only in association with antibodies against capsular antigen A.

The prevalence of antibodies against somatic antigens 1,2,3,7,8, and 9 was 8.9% of the whole population with titres from pure serum to 1/80.

There was a significant relation between the presence of antibodies against capsular antigens and the presence of antibodies against somatic antigens ($\chi^2 = 12.55$, $p < 0.0005$): the presence of one type of antibody increased the frequency of observing the other type in association (table 2).

Table 1 Characteristics of control group (C) and three exposed groups: S workers in slaughterhouse, I intensive breeding, T traditional breeding

	Group C	Group S	Group I	Group T
Sample size	157	107	145	271
Sex ratio (%)	64.3	53.8	71.0	54.6
Age (mean ± SD, yr)	40.3 ± 12.3	35.8 ± 10.2*	36.1 ± 11.7*	47.8 ± 12.7†
Duration of exposure (mean ± SD, yr)	12.4 ± 8.9	10.4 ± 7.0	12.4 ± 10.7	25.5 ± 14.8†
Pet owner (%)	59.2	55.1	73.1	87.5†
Recent bite or scratches (%)	1.9	6.5	17.9†	1.8

* $p < 0.01$; † $p < 0.001$.

Table 2 Significant relation between antibodies against capsular antigen A and antibodies against somatic antigens (χ^2 : 12.55, $p < 0.0005$)

	Antibodies against capsular antigen A		Total
	Present	Absent	
Antibodies against somatic antigens:			
Present	28	33	61
Absent	151	468	619
	179	501	680

The distribution of the titres of the different types of antibodies against *P multocida* antigens according to the groups are shown in tables 3, 4, and 5. The presence of antibodies against capsular antigen A differed significantly between groups (table 3): the prevalence was 14.0% in the control group, 26.2% in group S ($p < 0.02$), 29.0% in group I ($p < 0.01$), and 32.1% in group T ($p < 0.001$). Significant relations between group C and the exposed groups were not found for antibodies against capsular antigen D (table 4), or for each antibody against somatic antigens 1,2,3,7,8, or 9 (table 5). The prevalence of antibodies against one or more somatic antigen was higher in the exposed groups (figure), however, with a significant difference only for group T versus group C ($p < 0.05$).

In the whole population the prevalence of antibodies against capsular antigens A increased significantly with contact with pets and cattle (table 6). The prevalence of antibodies against somatic antigens increased only for the subgroup with simultaneous contact with pets and cattle. The mean age and the mean duration of exposure were higher for the subjects with antibodies against capsular antigens than for the subjects without antibodies (table 7).

Table 3 Distribution of titres of antibodies against capsular antigen A by: C control, S slaughterhouse, I intensive breeding, T traditional breeding

Type of exposure	Titres of antibodies against capsular antigen A						
	0	1/20	1/40	1/80	1/160	1/320	1/640
Group C:							
No	135	14	6	1	1		
%	86.0	8.9	3.8	0.6	0.6		
Group S:							
No	79	14	9	4	1		
%	73.8	13.1	8.4	3.7	0.9		
Group I:							
No	103	17	17	3	1	3	1
%	71.0	11.7	11.7	2.1	0.7	2.1	0.7
Group T:							
No	184	48	23	7	6	3	
%	67.9	17.7	8.5	2.6	2.2	1.1	

Table 4 Distribution of titres of antibodies against capsular antigen D by group

Type of exposure	Titres of antibodies against capsular antigen D			
	0	1/20	1/40	1/80
Group C:				
No	145	6	5	1
%	92.4	3.8	3.2	0.6
Group S:				
No	94	10	3	
%	87.9	9.3	2.8	
Group I:				
No	135	8	2	
%	93.1	5.5	1.4	
Group T:				
No	260	9	1	1
%	95.9	3.3	0.4	0.4

Discussion

A particularly noteworthy observation was that the prevalence of healthy subjects with antibodies against capsular antigen A of *P multocida* is high, even in the control group (C:14%); this prevalence is similar to that previously observed in an urban population (16%).⁴ The prevalence of antibodies against capsular antigen A is higher in occupationally exposed groups (S: 26.2%; I: 29.0%; T: 32.1%) (table 3). This high prevalence of antibodies against *P multocida* may reflect underdiagnosed cases of infection or asymptomatic immunisation.

The prevalence of both human brucellosis and

Table 5 Distribution of titres of antibodies against somatic antigens 1,2,3,7,8, and 9 by group

Somatic antigen	Type of exposure	Titres of antibodies against somatic antigens						
		0	1/1	1/5	1/10	1/20	1/40	1/80
0:1	C	157						
	S	106		1				
	I	143	1	1				
	T	267	1	1	2			
0:2	C	154	3					
	S	105	1	1				
	I	143	2					
	T	267	3		1			
0:3	C	155	1	1				
	S	107						
	I	144		1				
	T	268	2		1			
0:7	C	155	1		1			
	S	102	2	2	1			
	I	140	2	3				
	T	249	10	8	2	1		1
0:8	C	155	1	1				
	S	104	3					
	I	142	2		1			
	T	257	8	3	2	1		
0:9	C	156	1					
	S	107						
	I	143	1			1		
	T	268	2	1

Prevalence of antibodies against capsular antigens (solid columns) and against somatic antigens (shaded columns) by group (+ $p < 0.05$; * $p < 0.02$; ** $p < 0.01$; *** $p < 0.001$).

pasteurellosis seems similar: in slaughterhouses the frequency of antibodies against *Brucella* is about 8.8%¹² to 24%¹³ according to the titres and the working conditions.

We have not found a significant relation between a history of recent bites or scratches and the presence of antibodies but the subjects with antibodies had a higher mean duration of exposure than the subjects without antibodies (table 7): the immunological memory is perhaps better than recall explored by questionnaire.

We have studied only capsular antigens A and D because the two others (B and E) are not found in

Europe. The higher prevalence of antibodies against capsular antigen A than antigen D is explained by the preponderance of this serotype in the animal and human infections. This result supports other findings in an urban population.⁴ The somatic antigens 0:7 and 0:8 were also the most frequent (table 5).

A few subjects only had titres as high as those found in acute infections in animals or man.³ The low titres observed may be declining after infection or they may follow weak antigen stimulation by scratches or mucous carriage.

In conclusion, despite its high prevalence among animals, pasteurellosis is rarely diagnosed in man. This serological survey, however, suggests that the infection is frequently subclinical. The high prevalence of antibodies and the significant relation with contacts with pets, cattle, and chickens suggest that it is not only a disease associated with pets but also an occupationally related infection.

We are grateful to Drs G Benesy, H Blanchet, M F Demange, and J Y Saillard, mutualité sociale agricole of Laval, Angers, Niort, and Nantes, for carrying out the data. We are also grateful to Drs F Neukirch and T Berriau, Miss D Deschamps, and Mr D Lafon for their help; to Dr J L Kearns for reading this paper; and to Mrs B Najac for preparing the manuscript.

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Table 6 Prevalence of antibodies against *P. multocida* antigens and exposure to pets or cattle. The differences were significant between subjects not exposed to animals (first column) and subjects exposed to pets or cattle or both

	Without occupational exposure		With occupational exposure	
	Without pet	Pet owner	Without pet	Pet owner
No of subjects	64	93	121	402
Antibodies against capsular antigen A:				
Present	5	17	30	127
Prevalence (%)	7.8	18.3	24.8*	31.6†
Antibodies against somatic antigens:				
Present	5	4	10	42
Prevalence (%)	7.8	4.3	8.3	10.5

* χ^2 : 7.87, $p < 0.01$; † χ^2 : 15.4, $p < 0.001$.

Table 7 Comparison of mean age and duration of exposure for subjects with and without antibody against capsular antigens (Student's *t* test)

	Age		Duration of exposure	
	Without antibody	With antibody	Without antibody	With antibody
Mean (years)	41.6	42.4	16.6	19.7
Standard deviation	13.5	12.0	13.6	13.1
No	489	175	416	158
Significance	NS		0.015	

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