Bordetella avium cross-reacts with *B. bronchiseptica* by ELISA but natural *B. avium* infection in rats is unlikely

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Summary

The specificity of a *Bordetella bronchiseptica* antibody ELISA for the monitoring of laboratory rodents was re-evaluated by studying the serological relationship of the *B. bronchiseptica* antigen and antigens of *B. avium, B. hinzii, B. holmesii* and an unclassified *Bordetella* sp. Immunization of rats with *B. avium* strains induced antibodies to the *B. bronchiseptica* antigen. *Bordetella* antibody free rats that were experimentally infected with a *B. avium* strain seroconverted to the bacterium but not to *B. bronchiseptica* and were *B. avium* culture positive at one week postinfection but not at 6 weeks. Pathogen free rats exposed to the *B. avium* infected rats remained free from any cultural or serological evidence of *B. avium* infection. Lung lesions were not seen in experimentally infected and exposed rats. Natural *B. avium* infection in rats therefore seems unlikely.

Introduction

Bordetella bronchiseptica is a pathogenic bacterium for most laboratory animal species *(Goodnow 1980)*. Infection by the bacterium may be detected by (selective) culture from the respiratory tract and by serology using for example an enzyme-linked immunosorbent assay (ELISA) *(Boot et al. 1993; Wullenweber & Boot 1994)*. Various close relatives of *B. bronchiseptica* have been described including *Bordetella avium* from birds, a *B. avium* - like bacterium, *B. hinzii, B. holmesii* and *B. trematum* from human patients *(Von Wintzigerode et al. 2001)*. As the host spectrum of these *Bordetella* species is not fully clear and their presence in laboratory animals therefore can not be ruled out, we explored (in 1995) the serological relationship between some of the 'new' species and our *B. bronchiseptica* antigen.

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As antisera to *B. avium* were found reactive with *B. bronchiseptica* we next studied the possibility of *B. avium* infection in rats.

Materials & Methods

Bacteria

The bacterial strains studied (Table 1) were obtained in freeze dried form and after reconstitution kept with our *B. bronchiseptica* strains at –70°C in brain heart infusion broth (SVM, Bilthoven NL) supplemented with 10 % (w/v) of glycerol. *B. bronchiseptica* strains were characterized by cultural and biochemical examination, cell-wall lipid profiling *(Boot et al. 1993)* and some by 16S rDNA sequencing.

Preparation of antigens and immunization

Antigens used for immunization and in the ELISA were prepared as described *(Boot et al. 1993)*. Four-week-old female random-bred Rivm:TOX rats were housed in filter-topped cages, handled under strict aseptic conditions, and cared for as decribed *(Boot et al. 1993)*. The rats were from our hysterectomy-derived barrier-maintained breeding colony which

Table 1: cross-reactivity among *Bordetella* species by ELISA

				reactivity with *B. bronchiseptica* antigen $				
Antibody to antigen nr.	bacterial species	collection nr.*	host	1	2	3	4	5
1	*B. bronchiseptica*	LAM Bbr1	Guinea pig	100 #	**46**	12	12	18
2	”	LAM Bbr65	Rat	**88**	100	5	5	9
3	“	LAM Bbr96	Rabbit	**111**	**38**	100	**43**	**68**
4	“	LMG 3521	Turkey	**108**	**32**	**55**	100	**83**
5	“	LAM Bbr175	Mouse	**140**	**56**	**49**	**74**	100
6	*B. avium*	LMG 10977	Chicken	**56**	**123**	**77**	**107**	**203**
7	“	LMG 1852 ^{T}	Turkey	24	25	**73**	25	**47**
8	“	Hinz 383-78	Turkey	26	**72**	**41**	**67**	**117**
9	“	Hinz 450-78	*Lonchura striata*	**57**	20	16	**30**	**35**
10	“	Hinz 700-78	Turkey	**133**	**69**	**66**	**59**	**109**
11	*Bordetella sp.*	LMG 13506	Human	**51**	24	26	20	**52**
12	*B. hinzii*	LMG 1872	Human	9	11	2	7	16
13	“	LMG 10979	Chicken	9	13	1	9	13
14	“	LMG 10980	Chicken	24	11	6	10	19
15	“	LMG 13497	Chicken	14	6	4	11	10
16	“	LMG 13501^{T}	Chicken	21	14	6	15	17
17	*B. holmesii*	ATCC 51541	Human	19	6	4	7	20

* ATCC: American Type Culture Collection; Hinz: Klinik für Geflügel, Tierärtzliche Hochschule, Hannover FRG (Dr. K-H. Hinz) , LMG: Laboratorium voor Microbiologie, Universiteit Gent Belgium; LAM: author’s collection; T: type strain.

autologous ODs considered to be 100 %; reactivity of antisera with other antigens calculated as % of autologous activity; ≥ 30 % regarded as positive (printed in bold).

$ antigens 6 - 17 were also tested with all antisera but results are not shown.

was free from *B. bronchiseptica* and other rodent pathogenic microorganisms as listed in the FELASA recommendations for the monitoring of rodent and rabbit colonies *(Nicklas et al. 2002)*. Two rats per bacterial antigen were immunized s.c. with 0.2 ml whole cell antigen 1 : 1 diluted in Stimune (Cedi Diagnostics BV, Lelystad NL) and s.c. booster-immunized after 3 weeks with 0.2 ml plain antigen. Rats were bled by cardiac puncture under KRA [Ketamine (Alfasan, Woerden NL) 90 mg/ kg i.p., Rompun (Bayer AG, Leverkusen Germany) 10 mg/ kg i.p, atropine (Vetinex Animal Health, Bladel NL) 0.05 mg/ kg i.p.] anesthesia one week after the booster immunization and all sera were stored at – 20°C until use.

ELISA and cross-reactivity studies

The ELISA was performed as described *(Boot et al. 1993)*. To determine cross-reactivity (CR) among the bacterial strains, microtitre plates were coated with the antigen adjusted to 7.5 mg / l protein in Na_2CO_3 of pH 9.6 and all antisera were two fold serially diluted from 1:50 to 1 : 12,800 in phosphate buffered saline pH 7.2 and tested with the autologous antigen. The serum dilutions (1 : 25 to 1 : 3200) yielding maximal optical densities (OD) at 450 nm in autologous assays were then used in assays with homo- and heterologous *Bordetella* antigens. Autologous ODs were considered 100 % and ODs obtained with other antisera were expressed as a percentage of the autologous activity. Percentages ≥ 30 were considered significant as

using this cut off level results of ELISA and culture in *B. bronchiseptica* naturally infected rats agree. Goat anti-rat IgG *(Cappel, Malvern, USA)* at a 1 : 10,000 dilution was used as the secondary antibody.

Observations in B. avium experimentally infected and exposed rats

Experimental infection was carried out in 8 female *Bordetella* antibody free Rivm:WU rats aged 5 weeks. The animals were blood-sampled through orbital puncture as indicated and intranasally (i.n.) dosed with 10^{5-6} cfu *B. avium* strain Hinz 800-78 (study nr. 10) and housed two per cage in type III macrolon cages in a Gustafsson type isolator. One rat per cage was exsanguinated by cardiac puncture under KRA anesthesia 1 week post infection (p.i.), inspected for gross lesions and cultured for *B. avium* from the nose, the pharynx, the trachea, the lung and the eyes on plain sheep blood agar made selective by the addition of 2 µg / ml clindamycin-HCl (Oxoid, Haarlem NL). Because cultural results were positive at 1 week p.i., from 2 weeks p.i. each of the remaining 4 infected rats was used as a source of infection to two uninfected Rivm:WU rats (totalling 8 exposed rats). Both infected and exposed rats were serum sampled at 2 and 4 weeks and killed at 6 weeks p.i. or post exposure respectively and tested for *Bordetella* infection by culture and ELISA using antigens of *B. avium* (study nr. 10) and *B. bronchiseptica* (study nr. 1) Lungs were instilled to inspiration size with, and were preserved in, 3.8% (w/v) phosphate buffered formaldehyde and embedded in paraffin wax. 4-5 μm thick sections were then prepared and routinely stained with haematoxylin and eosin. Sections were scored for histopathological abnormalities without knowledge of the treatment.

Results

Serological relationship

Autologous ELISAs showed optical densities above 1000 in serum dilutions ranging from 1 : 25 to 1 : 12,800 (data not shown). Homologous assays using optimal serum dilutions showed cross-reactivity (CR) among the *B. bronchiseptica* strains (Table 1), be it a one way CR in some cases. Antigen of *B. bronchiseptica* strain Bbr 1, which we use in the testing of sera from rodents and rabbits, detected antibodies to the four other *B. bronchiseptica* strains. Heterologous assays (Table 1) showed varying degrees of CR between *B. bronchiseptica* antigens and antisera to *B. avium* study nrs. 6 -10 and the unclassified *Bordetella* species nr. 11, but the reverse was not observed (data not shown). Notably antiserum to *B. avium* nr. 10 strongly reacted with antigen of *B. bronchiseptica* strain Bbr 1. No reactivity was found between *B. bronchiseptica* antigens and antisera to *B. hinzii* nrs. 12 - 16 and the *B. holmesii* strain nr. 17 and also reverse reactions did not show significant activity (data not shown).

Table 2: antibody activity to B. bronchiseptica and B. avium ELISA antigens in experimentally B. avium infected and exposed rats
day post infection or exposure

infection mode	tested n =	antigen	0	7	14	28	42
intranasal	8 (4 from Day 14)	*B. avium* (nr. 10)	2*	8.5	23	94.5	94
		B. bronchiseptica (nr. 1)	1.5	3.5	3	6.5	11.5
exposed to four experimentally infected rats, after four weeks.	8	*B. avium*	3.5	2.5	5	4	3.5
		B. bronchiseptica	4	3	4.5	5	4

* median of ELISA activities expressed as percentage of positive control.

Observations in B. avium experimentally infected and exposed rats

At 1 week p.i, *B. avium* was cultured from all four infected rats (the nose (3x) the trachea (1x) and the eye (1x)) but not from the four infected rats killed at 6 weeks p.i. In the latter group from 2 weeks p.i. antibodies to *B. avium*, but not to *B. bronchiseptica*, were detected (Table 2). Gross lesions were not observed in the respiratory tract of any of the infected animals. Histopathological examination revealed minimal interstitial pneumonia in one animal killed 1 week p.i. and moderate interstitial pneumonia in one animal killed 6 weeks p.i. Exposed rats remained seronegative in both ELISAs and *B. avium* was not detected by culture. Exposed rats had no histological lesions.

Discussion

When we established our *B. bronchiseptica* antibody ELISA to monitor rodents and rabbits for the infection, three other *Bordetella* species were known to occur in animals (Pitman 1984), namely the human pathogenic *B. pertussis* and *B. parapertussis* (also isolated from sheep) and an avian *B. bronchiseptica*-like group which was later described as *B. avium (Kersters et al. 1984)*. Currently the genus *Bordetella* comprises at least seven species *(von Wintzingerode et al. 2001)*. There is a close serological relationship between *B. bronchiseptica* and *B. (para)pertussis* and agglutinating antibodies to *B. avium* cross-react with whole cell antigens of *B. bronchiseptica* strains *(Hertle & Hinz 1984; Kersters et al. 1984)*. Natural infection by *B. (para)pertussis* in rodents and rabbits seems unlikely as it is difficult to establish experimental pertussis infection in these species *(Woods et al. 1989)*. Infection by the other *Bordetella* species can however not be ruled out and might lead to positive *B. bronchiseptica* ELISA results should they share major epitopes with the antigen. Indeed antibodies raised in rats to *B. avium* strains were clearly reactive with our *B. bronchiseptica* antigen (Table 1) and therefore this bacterium appeared a candidate for raising antibodies to *B. bronchiseptica* in natural infection.

The fact that *B. avium* was cultured from experimentally infected rats at 1 week p.i. but not at 6 weeks p.i., indicates that *B. avium* can be transiently present after dosing relatively high numbers of the bacterium. The disappearance of *B. avium* from the mucous membranes might be due to the development of antibody activity (Table 2) or be the result of internalization in macrophages and epithelial cells. Internalization leading to persistent infection has been described for *B. bronchiseptica (Guzman et al. 1994)*, with enhancement of long term survival with high infectious doses *(Forde et al. 1998)*. Even if this would have been the case in *B. avium* there is no indication that the infection was subsequently transmitted to cage mates. The seroconversion to *B. avium* in the experimentally infected rats is no proof of (persistent) colonization as serocoversion will likely also result from immunological stimulation of the bronchus-associated lymphoid tissue by dosing high numbers of non-colonizing bacteria *(Czerkinsky & Holmgren 1995)*. Our histopathological observations do not support long term *B. avium* infection in rats.

This study indicates that antibodies to *B. bronchiseptica* raised by immunization with heterologous *Bordetella* spp. such as *B. avium* are not necessarily formed after intranasal infection. A difference in immune response between immunized and naturally infected animals may be due to differences in the route of antigen administration and to the use of adjuvant in raising antisera. Differences in CR among *B. bronchiseptica* strains were found earlier *(Boot et al. 1993; Wullenweber & Boot 1994)* and formed the basis for the selection of *B. bronchiseptica* study nr. 1 for preparation of ELISA antigen to be used for monitoring. We feel that rats are not a natural host of *B. avium* and conclude that the *B. bronchiseptica* antibody ELISA remains a good tool for the monitoring of rodents and rabbits for *B. bronchiseptica* infection.

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