Virulence of Avian Serotype *A1 Pasteurella multocida* for Chickens and Mice

Short Communication

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Summary

The virulence of *Pasteurella multocida* (*P.multocida*) serotype A1 for chickens and mice was determined. Groups of chickens and mice were exposed intramuscularly and intraperitoneally to various concentration of *P.multocida* broth culture, respectively. This strain was highly virulent for chickens so that those exposed to only 7c.f.u. of the organism died in less than 24 hours. Groups of mice exposed to the virulent strain died during 48 hours post inoculation. Microbiological examination resulted in the isolation of *P.multocida* from exposed chickens and mice. No isolation was made from unexposed control groups. The result indicates that the isolate is highly virulent for both chickens and mice.

Key words: fowl cholera, *Pasteurella multocida*, virulence, chickens, mice

Introduction

Pasteurella multocida (*P.multocida*) a gram-negative bacterium causes avian cholera in wide range of domestic and wild avian species (Rhoades & Rimler 1984). This important disease, which can cause economic losses in poultry industry, has been recognized in domestic poultry for more than two centuries (Rimler & Rhoades 1989). The virulence of *P.multocida* in relation to fowl cholera is a highly complex

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entity. Attempts to understand the determinants of virulence factors have met with limited success. However, various *P.multocida* constituents such as toxins, capsule, lipopolysacharide, outer membrane proteins and plasmid have been considered as virulence factors.

P.multocida belonging to somatic serotype 1 and capsular serotype A can cause avian cholera in domestic poultry and free-living waterfowl. Although, *P.multocida* belonging to capsular types, A, B, D, and F, have been isolated from avian origin (Rhoades & Rimler 1987, Rhoades *et al* 1982, Carter 1967, Chandrasekan *et al* 1985, Rhoades *et al* 1988) but capsular type A has been known to associate with more severe forms of avian cholera (Rimler & Rhoades 1989). The objective of this study was to determine the virulence of *P.multocida* serotype A1 for chickens and mice.

Materials and Methods

Bacteria. A lyophilized ampoule of *P.multocida* serotype A1 isolated from an outbreak of fowl cholera in birds in north of Iran (Tavasoli *et al* 1984) was used. The capsular group and somatic type was previously determined (Sotoodehnia *et al* 1986). The bacterial stock was spread onto blood agar plates and incubated aerobically at 37°C for 24h. Five colonies were subsequently inoculated into tryptose broth and incubated overnight at 37°C. 2ml of the overnight culture was transferred to 10ml tryptose broth and subsequently incubated at 37°C for 6h with moderate shaking. When an OD540 corresponding to 8×10^8c.f.u./ml was reached, a 1ml sample was diluted serially to make the desirable concentrations as shown in tables 1 and 2.

Animal. 55 eight-week-old healthy chickens (Leghorn) with no previous history of fowl cholera were obtained. Ten groups of five chickens were inoculated by

intramuscularly (IM) route with the various bacterial concentrations carried on ice. Control group was similarly inoculated by IM route with sterile tryptose broth. In another trial four groups of six healthy Balb/C mice in each, between 20-22g weights, were selected and inoculated by the same strain with different concentrations via intrapreitoneal (IP). Bacterial examination was carried out for the dead animals.

Results and Discussion

Table 1 shows the number of birds that died following IM exposure and the time at which death occurred.

Table1. *Lethality of serotype A1 of P.multocida for chickens exposed by intramuscular inoculation*

SerotypeA1 inocula (c.f.u./chicken)	Time of death (day after exposure)*		Survivor
	1	2	
100	5/5**	---***	---
75	5/5	---	---
50	5/5	---	---
30	5/5	---	---
10	5/5	---	---
7	5/5	---	---
5	4/5	1/5	---
3	1/5	4/5	---
2	3/5	2/5	---
1	3/5	1/5	1/5
Control	0/5	0/5	5/5

*No death occurred later than the second day of exposure, **Number on this day/number in group, ***No chickens on this day

Control birds appeared normal during this study. In autopsy, vascular congestion and small areas of haemorragic, distributed among visceral organs were observed.

Microbiologic examination of livers of dead chickens resulted in recovery of *P.multocida* from all of them. Control group did not reveal the presence of *P.multocida*.

Table 2 shows the mortality of mice following IP exposure of serotype A1 of *P.multocida* during 2-day post inoculation observation period. *P.multocida* was recovered from livers of dead mice whereas all control mice were healthy.

Table 2. *Lethality of avian serotype A1 of P.multocida for mice exposed by intraperitoneal inoculation*

Serotype A1, inocula (c.f.u.)	Mortality within 48h post inoculation	Survivor
5000	6/6	---
500	6/6	---
50	6/6	---
5	6/6	---
Control	---	6/6

Our results indicated that strain A1 was highly virulent for domestic chickens. Typical lesions due to fowl cholera were observed in dead chickens and mice. This serotype had already been isolated from an outbreak of fowl cholera in a group of household birds including ducks, turkeys, geese and chickens in Astara, a city in north of Iran. Serotype A1 was isolated from any of the species involved in this outbreak. High morbidity and nearly 70% mortality has been reported among different species of birds (Tavasoli *et al* 1984). Later, an inactivated fowl cholera vaccine was prepared with the mentioned local strain and used effectively and extensively in infected foci (Sotoodehnia *et al* 1984). In recent years, other serotypes 4, 3, and 3×4 of serogroup A was isolated and recognized to cause of fowl cholera (Sotoodehnia *et al* 1998, Banani *et al* 2000, Jabbari *et al* 2001). Moreover, a few reports of isolation of *P.multocida* belonging to capsular types B and D are existed

from cases of fowl cholera in Iran. Information on the virulence of these avian isolates is limited (Chandrasekan *et al* 1985, Sotoodehnia *et al* 1986).

Transmission of avian cholera within a flock is considered to be primarily through indirect contact particularly contaminated water (Rimler & Rhoades 1989). The way of transmission or inoculation and the type of isolate is known to influence the virulence of *P.multocida* (Rimler & Rhoades 1987). Intravenous inoculation of the bacterium raptorial origin caused mortality in chicken but ocular or oral routes did not cause mortality (Pyone *et al* 1999). Further investigations, however, will be needed to determine the virulence of the local serotype A1 in different ages of ducks, turkeys and geese as well as other sensitive birds.

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