Infectious coryza due to *Haemophilus paragallinarum* serovar B in China

P-J ZHANG
M MIAO
H SUN
Y GONG
Institute for Animal Husbandry and Veterinary Science, Beijing Academy of Agricultural and Forestry Sciences, Beijing, China

PJ BLACKALL
Agency for Food and Fibre Sciences, Queensland Department of Primary Industries, Animal Research Institute, Yeerongpilly, Queensland 4105

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Infectious coryza, a disease of the upper respiratory tract of chickens, is caused by *Haemophilus paragallinarum*.[1] The clinical signs of the disease include nasal discharge, facial swelling and a reduction in feed and water consumption.[1] Infectious coryza in poultry is a disease of economic significance in many parts of the world with the greatest economic losses resulting from an increased number of culls and marked reduction (10 to 40%) in egg production.[1] The most widely used serological classification scheme for *H paragallinarum* is the Page scheme, which recognises three different serovars, termed A, B and C.[2] The importance of the Page scheme is that inactivated vaccines protect only against those serovars present in the vaccine.[1]

There have been few studies performed in China on the serological characterisation of *H paragallinarum* isolates. All 29 Chinese isolates of *H paragallinarum* that have been examined to date have been shown to be serovar A.[3-5] We report the first isolation of *H paragallinarum* serovar B from an outbreak of infectious coryza in China.

A large 100,000 layer flock located in Liaoning Province had a history of outbreaks of a respiratory disease in successive flocks. The clinical signs of swollen sinuses, nasal and ocular discharge were suggestive of infectious coryza. A number of different, commercial infectious coryza vaccines were tried with no obvious effect on the disease. In 2001, the flock suffered an outbreak in which 50% of the 100,000 birds (130 to 300 days old) showed the typical clinical signs. The mortality associated with the outbreak was estimated to be between 2 to 5%. The outbreak caused an average 10% drop in egg production.

A satellitic non-haemolytic isolate was obtained in pure culture from the sinus of each of three chickens. The isolate required increased CO_2 for growth. The isolate was a Gram-negative short rod or coccobacillus that was catalase negative. The organism was confirmed as *H paragallinarum* by using a PCR test known to be specific for *H paragallinarum*.[6] The test was performed using a colony preparation as previously described.[6] Only one of the three isolates could be maintained in subculture. This isolate was transferred to the Animal Research Institute, Queensland and was shown to be Page serovar B using a haemagglutination–inhibition test.[7]

The pathogenicity of the isolate that was typed as serovar B was examined, in China, using 60-day-old commercial layer chickens from a farm with no history of infectious coryza, no use of infectious coryza vaccine and which was regularly tested as negative in the *H paragallinarum* serovar A and serovar C blocking ELISA.[8] Each of the four chickens used was inoculated, via the infra-orbital sinus, with 0.2 mL of an overnight broth culture corresponding to a dose of around 8 x 10^6 colony forming units per chicken. Two days after the challenge, all four chickens showed the typical clinical signs of infectious coryza. Three days after challenge, the nasal exudate from each chicken was tested using the species-specific *H paragallinarum* PCR test as previously described.[6] As well, the nasal exudate was cultured onto the medium described by Kume et al,[9] which was incubated in a candle jar for up to 48 h. Suspect *H paragallinarum*-like colonies from the Kume medium were confirmed by the species-specific *H paragallinarum* PCR. All four chickens were positive in the nasal swab examined directly by PCR and all four chickens yielded *H paragallinarum* colonies that were confirmed by PCR.

This isolate of *H paragallinarum* is the first reported occurrence of serovar B in China. The only previous serological characterisations of Chinese isolates of *H paragallinarum* reported the presence of serovar A only.[3-5] Our finding of serovar B has several important practical implications for the Chinese poultry industry. Page serovar B isolates show little cross-immunity with serovars A and C.[10] Hence, infectious coryza vaccines based on serovar A and/or C have little chance of providing protection against a Page serovar B challenge. A further complication is that, within Page serovar B, some isolates show limited cross-protection.[10] Hence, the presence of serovar B in a large commercial layer chicken farm in China indicates that there is a need for careful selection of the seed strains used to produce infectious coryza vaccines for use in China. In particular, unless there is specific knowledge of the serovars present in the target population, the use of bivalent vaccines that contain only Page serovars A and C cannot be recommended in China. We estimate that the majority of infectious coryza vaccines in use in China are based on serovar A alone or serovars A and C. As an example, our isolate was obtained from a farm with a repeated history of infectious coryza-like outbreaks despite regular use of inactivated infectious coryza vaccines based on serovars A and C. Clearly, the strains to be included in infectious coryza vaccines used on poultry farms in China need to be carefully considered.

There is a report that the reference strain *H paragallinarum* for serovar B, 0222, is non-pathogenic.[11] We have found that our field isolate of Page serovar B is fully pathogenic; a similar finding has been reported for other Page serovar B isolates.[12,13] Other than the single report of the lack of pathogenicity of strain 0222, a laboratory strain that has been extensively passaged in vitro, by Kume et al[11] there is no other evidence that serovar B isolates are non-pathogenic. Indeed, all the available evidence is the opposite; isolates of *H paragallinarum* serovar B are as pathogenic as any other serovar.[12,13] Hence, until there is further evidence, the isolates of *H paragallinarum* serovar B from clinical cases of infectious coryza should be accepted as pathogenic without need for pathogenicity testing.

While we were only able to serotype one isolate from this outbreak, there has never been a report of multiple serovars in an outbreak of infectious coryza. Absence of reports of multiple serovars in a single outbreak is not strong evidence because the ability to serotype *H paragallinarum* is not widely available. Hence, there are few reports on the serotyping of multiple isolates of *H paragallinarum* from a single outbreak. In one of the few such investigations, an Australian based study found no

evidence of multiple serovars within a series of epidemiologically connected outbreaks in which a total of 11 isolates from the single outbreak involving six farms were examined.[14] A further difficulty in serotyping multiple isolates of *H paragallinarum* from outbreaks of infectious coryza is that the isolation and maintenance of *H paragallinarum* is a challenging task for many diagnostic laboratories. Even in the best equipped laboratories there can be great difficulties in working with *H paragallinarum*. As an example, there has been a recent report of a very large outbreak of infectious coryza on a multi-age layer farm in California.[15] This outbreak was associated with nearly 50% mortality and almost 60% decrease in egg production.[15] Despite the best efforts of the diagnostic laboratory involved, no isolate of *H paragallinarum* could be maintained long enough for transfer to a laboratory that could perform serotyping.[15]

This report now means that serovar B has been reported in a number of Asian countries – China, Indonesia[16] and the Philippines.[17] To date, *H paragallinarum* serovar B has never been encountered in the studies of Australian *H paragallinarum* isolates.[18-21] Hence, as we have suggested earlier, *H paragallinarum* serovar B can be regarded as exotic to Australia.[16] As the currently available infectious coryza vaccines for use in Australia contain only serovars A and C, any entry of *H paragallinarum* serovar B would result in vaccine failures. The chicken is the only host for *H paragallinarum*,[1] meaning that the main entry point for serovar B into Australia would be via live chickens that are carriers of the organism. Since we have shown that *H paragallinarum* serovar B is present in village chickens in Indonesia,[16] the threat of entry of serovar B is via both any legal live chicken import as well as via illegal entries in the northern regions of Australia.

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The use and abuse of Aesculapian authority in veterinary medicine.

Aesculapian authority is the unique authority vested in those that society sees as healers and originated when medicine was inseparable from magic and religion. While implicitly recognised in human medicine, it has not been applied to veterinary medicine. The author of this article considers this situation may be changing with the increasing role of companion animal practice in the veterinary industry and as society begins placing more than market value on pets.

Increasingly owners consider these animals as family, and veterinarians are moving from the role of 'mechanic' (in treatment on a purely commercial basis) to that of the 'paediatrician'. With this change comes a growing relevance of the concept of Aesculapian authority to companion animal practice - and the resultant question of potential abuse of that authority.

The author compares the application of this authority between human and veterinary fields, covering the issues of informed consent, the conflict between personal gain and ethical treatment, and the problems associated with alternative medicine. Deliberations involving the consideration of euthanasia are scrutinised from the veterinary point of view.

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