The pathogenicity and prophylaxis of serotype CR88 in chickens

Layer farms may regularly detect the presence of the CR88 serotype of the infectious bronchitis virus. What is the pathotype of this virus and what is the level of protection provided by conventional Massachusetts vaccination?

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The infectious bronchitis virus (IBV) Serotype CR88 (also known as 793/B and 4/91) was officially discovered in 1988 but has been present since at least 1985. It crops up regularly in poultry farms during the rearing period as well as the laying period. The major outbreaks in France in 1988, 1994 and 1996 were attributed to it. At first sight, this is surprising since there are a large number of IBV serotypes in the world (more than 30) and at least four have been identified in France over the last twenty years (Massachusetts, PL84, CR88 and CR84 which is nephropathogenic). The obvious conclusion is that only certain strains, like CR88 or CR84, are capable of persisting in the field. Typically they are highly pathogenic, affecting the respiratory or genital systems, or the kidneys.

In France, only the CR84 nephropathogenic strain can lead to high mortality in chickens (30% on average), whereas the other serotypes isolated are revealed above all in respiratory and genital pathologies. However, from 1993, field observers have several times noted a slight increase in mortality and the presence of muscular congestion considered to be unusual during outbreaks of infectious bronchitis attributed to the CR88 virus in broilers. It therefore seemed interesting to us to compare the pathotype of this virus with that of the standard reference infectious bronchitis virus of the fowl, the Mass 41 strain and the level of protection provided by conventional Massachusetts vaccination.

Study of the pathotype

To know more about the pathotype of the CR88 virus an experimental study was carried out on specific pathogen free (SPF) chickens kept in protected housing on the AFSSA site in Ploufragan. An initial group of 48 chickens aged 23 days had been inoculated intranasally with 10^6 EID50 (30% embryonic infecting dose) of the reference virus from the CR88 antigenic group, i.e. the CR88121 strain. At the same time, 48 other SPF chickens kept in different housing were inoculated with an identical dose of the Mass 41 virus. The observations and samples defined below were carried out during the week following these viral challenges.

Infection with these viruses caused no deaths. Respiratory symptoms were noted on the 3rd, 5th, 7th and 10th days post-challenge (PC) by using an individual scoring system whose reproducibility has been verified several times:

A) The head of the bird being maintained against the ear of the observer:
- no respiratory sign when listening during one minute Æ Score 0
- rare and/or slight respiratory signs when listening during one minute Æ Score 1
- frequent and/or intermediate respiratory signs when listening during one minute Æ Score 2
B) The bird being held with outstretched arms:
- intermediate to strong respiratory signs when observing during one minute Æ Score 3
C) The bird being at 1.5 metres or more from the observer:
- strong respiratory signs when observing during one minute Æ Score 4.

As expected, from the third day PC respiratory signs were already clearly present in the chickens inoculated with the Mass 41 virus, in the form of coughs and rales (see Figure 1). At the beginning of the clinical change, about half the birds in this group were already affected, as against only 13% of the chickens inoculated with the CR88121 virus. On the 5th day PC three-quarters of the chickens in both groups had as many respiratory symptoms, but those inoculated with CR88121 were all more apathetic and some were even prostrate. On the 7th day PC only the respiratory signs remained, and these were noticeably more intense and frequent in the birds inoculated with Mass 41 (82% of the birds were ill, average intensity 2.3 out of 4 in those affected) than in those inoculated with CR88121 (57% of birds were ill, average intensity 1.3 out of 4 in those affected). This trend was clearly confirmed on the 10th day PC, since half the chickens infected with Mass 41 still showed respiratory problems, against none of those infected with CR88121.

Persistence

Persistence of the challenge virus in the trachea was checked for in ten chickens per group at 3, 5 and 7 days PC. To do this, the upper part of the trachea was sampled, the lower part being kept so that an evaluation could be made of ciliary activity. Scrapings of tracheal mucous membrane were cultured on fertilised eggs after which deaths and embryonic lesions were recorded that...
axy of infectious bronchitis

On the 3rd day after inoculation, the virus was present in the trachea of all the birds inoculated with CR88121 or Mass 41 (Figure 1). At the end of the observation period, or seven days PC, the Mass 41 virus was only found in five chickens out of 10, whereas CR88121 virus, to the extent that the initial attack on these tracheal cells seems to have been less severe or slower to develop, as suggested by the ciliary activity in the trachea.

This ciliary activity was evaluated from six tracheal sections (0.5 mm thick) on 10 chickens per group. These sections were observed under the microscope to establish whether or not there was ciliary beating along the entire length of the epithelial mucous membrane inside the trachea. A bird was considered affected when at least 3 sections of trachea out of 6 proved to be ciliostatic. All the chickens of the group inoculated with CR88121 (although in this group some birds did not show tracheal ciliostasis), Contrary to all expectations, some severe histological lesions were observed in the kidneys of 2 of the 8 birds inoculated with CR88121. These inflammatory lesions in the medullary area of the lobes were qualitatively judged to be similar to those observed in severe coronavirus-induced nephropathy. This could explain the apathy, and even the prostration, observed in this group on the 5th day PC. Such lesions were not found in the birds inoculated with Mass 41. Finally, the Harderian glands and the lungs of the chickens in the two groups only showed slight lesions with no real pathological significance.

Protection against CR88

The clear pathogenic nature of the isolates of the CR88 virus and its prevalence for nearly 15 years needs to turn to medical prophylaxis (vaccination). Different vaccination schedules have been proposed including, of course, live or inactivated vaccines aimed specifically at the CR88 serotype, but also at other serotypes. We are going to focus exclusively on the vaccination of the chick. It has already been clearly demonstrated that vaccination with live CR88 virus effectively protects the chicken against viral strains belonging to this serotype. However, initial vaccination of young birds, when performed (as it most often is) in the hatchery, is always carried out with attenuated live vaccine of the standard Massachusetts serotype such as MM and H120 viruses, the last being the most frequently used. It was therefore interesting to find out the degree of cross or “heterologous” protection given by this type of vaccine against CR88.

The methodology was as follows: 96-day-old SPF chicks kept in protected housing were eye drop vaccinated with 10^5 EID50 of H120 strain (Cevac® Bron 120L). Another group of 96 chicks was not vaccinated. At 23 days of age, a sample of blood was taken from 20 birds in each group and tested for antibodies. Half of the birds in each group...
were then challenged with the CR88121 virus and the other half with the Mass 41 virus. These two pathogenic strains were administered nasally at the same dose of 10^5EID50 per bird. The observations and samples taken after the viral challenge were the same as those described in chapter 1. The symptom and lesion scores of both vaccinated and non-vaccinated birds were transformed mathematically, to reveal only the percentage of protection of the vaccinated birds compared with the non-vaccinated controls. This was called the “percentage of relative protection” (PRP):

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\text{PRP} = \frac{\% \text{ of Control affected} - \% \text{ of Vaccinated affected}}{\% \text{ of Control affected}} \times 100
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Results are given in Figures 1, 2 and 3.

Clinically, as expected, the relative protection against respiratory symptoms was total after the homologous challenge with Mass 41 (100% at 3, 5, 7 and 10 days post-challenge (PC)). Less expectedly this time, this protection against respiratory symptoms from the heterologous CR88121 challenge reached a very respectable score of 100% at 3 days PC, 86 at 5 days PC and 88 at 7 days PC (it could not be calculated at 10 days PC as no control challenged birds showed any sign) (Figure 1 and Table 1). None of the H120 vaccinated chickens infected with CR88121 showed any sign of apathy nor prostration, unlike the non-vaccinated ones.

Concerning lesions, the results differed according to the types of analysis and/or the organs examined. Taking ciliostasis and histological lesions in the trachea as criteria, the protection given by the H120 vaccine against the homologous challenge was almost complete. Relative protection against the heterologous challenge was good to intermediate when judged on ciliostasis (Figure 2): 66% 3 days PC, 100% 5 days PC and 50% 7 days PC. Strangely, the protection was nil when judged on tracheal histological lesions. We have no explanation of this discrepancy in results obtained using these two presumably related criteria.

Histological lesions were found in the kidney, only in unvaccinated birds challenged with the CR88 virus: two of the eight birds observed in this group presented severe microscopic lesions in this organ, as against no bird in the group vaccinated with H120. With regard to virus persistence (Figure 3) the percentage of protection in terms of the re-isolation of the respective challenge viruses from the trachea was over the observation period 47% and 43%, respectively, under homologous and heterologous challenges. This criterion must however be considered with caution, as only one bird carrying the virus may easily excreted viral particles that are immediately breathed in by its neighbours, and these few particles will show up after 3 passages on embryonated eggs.

To summarise this research into cross-protection, we conclude that 88% of the SPF chickens vaccinated with H120 were clinically protected against heterologous challenge with the CR88121 virus. This protection is not as complete as that given by homologous vaccination with live CR88 vaccine, but it is substantial.

The mechanism of protection

Antibodies directed against the infectious bronchitis coronavirus were checked at time of challenge on a sample of 20 vaccinated birds, using three different ELISA kits. At most 3 birds out of 20 were then found serologically positive by ELISA, and the titres obtained were not very high. We also looked for serum neutralising antibodies corresponding to the viral strain, and only one bird out of 20 was slightly positive. The circulating antibodies (detectable by ELISA or viral neutralisation) were not therefore the cause of the high levels of homologous and heterologous protection obtained.

Finally, the antigenic difference between the CR88 and Massachusetts serotypes at 1 day old has been proved to be substantial. This result was obtained in SPF chickens kept in protected housing and is not certain that it would be the same in a more conventional microbial environment. Furthermore, this protection, whether homologous or heterologous, does not seem to need the action of serum antibodies.

Conclusion

The experimental study revealed the renal tropism of coronavirus CR88121 which is in addition to its respiratory and genital tropism. Although the various criteria used gave sometimes conflicting results, the clinical protection given against the CR88 virus by the standard vaccination with Massachusetts serotype at 1 day old has been proved to be substantial. This result was obtained in SPF chickens kept in protected housing and is not certain that it would be the same in a more conventional microbial environment. Furthermore, this protection, whether homologous or heterologous, does not seem to need the action of serum antibodies.

Finally, the antigenic difference between the CR88 and Massachusetts serotypes (the latter being usually used as antigen in the commercial ELISA kits), might lead to an underestimation of the frequency of infection in young birds by CR88 when ELISA is used for checking seroconversion.