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4 Short Communication.

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6

7 **Onset of immunity following in ovo delivery of avian Metapneumovirus vaccines.**

8

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26

27 **Abstract**

28 Avian metapneumovirus (aMPV) is an important cause of disease in chickens and
29 turkeys. As infection can occur early in life and spread of the virus throughout a flock
30 is rapid, an early onset of immunity post vaccination would be advantageous. We
31 have studied the serological immune response and the onset of protective immunity of
32 an aMPV vaccine delivered to chickens via the *in ovo* route compared to oculonasal
33 delivery at day old. A one thousand fold lower dose delivered *in ovo* to chicken
34 specific pathogen free (SPF) embryos, than vaccination at day old, provided a
35 significantly higher antibody response. In the presence of maternally derived
36 antibody (MDA), there was no significant difference in antibody response between
37 the vaccination routes. However, the onset of immunity (OOI) for the vaccine
38 delivered to MDA positive chicken embryos was 5 days post hatch in comparison to 8
39 days post hatch for the same dose of vaccine given at day old indicating that chicks
40 would be protected against disease earlier in the field if vaccinated by the *in ovo*
41 route. In further experiments the OOI for a turkey vaccine delivered to MDA positive
42 turkey embryos was shown to be 8 days post hatch.

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53 **Introduction.**

54 aMPV is a significant disease of turkeys and chickens and has an almost worldwide
55 prevalence. Four subtypes have been recognised; A and B, mostly found in Europe,
56 Africa and Asia; C, found in the USA; and D (or non A non B) which was recognised
57 in France in the 1980s. Vaccines based on the A and B subgroups can cross protect
58 against all subtypes though reciprocal cross protection has not been demonstrated
59 (Cook *et al.*, 1995, Cook *et al.*, 1999 and Toquin *et al.*, 1999). The disease in turkeys
60 is characterized by tracheal rales, sneezing, swollen sinuses plus nasal and ocular
61 discharge whilst the disease in chickens is less severe and aMPV is involved as part of
62 a disease complex associated with swollen head syndrome. A drop in egg production
63 and/or an increase in egg abnormalities is found in both species. In both turkeys and
64 chickens the onset of clinical signs occurs soon after infection and spread of the
65 disease through the flock is rapid. For this reason it would be advantageous to
66 prepare vaccines with a rapid onset of protection.

67

68 *In ovo* vaccination against Marek's disease is in widespread use in the USA and South
69 America with vaccination against infectious bursal disease and fowlpox used to a
70 lesser extent. This technique has the advantage of giving a uniform delivery of
71 vaccine which may be an important factor in the potential reversion to virulence of
72 some aMPV vaccines (Naylor and Jones, 1994 and Catelli *et al.*, 2006). In addition,
73 in some cases *in ovo* vaccination provides a more rapid onset of immunity than day
74 old vaccination (Sharma and Burmester, 1982). Previous work has demonstrated that
75 *in ovo* vaccination can induce aMPV-specific antibody responses and protection
76 against challenge in maternally derived antibody (MDA) positive turkeys

77 (Worthington *et al*, 2003). In this report we compare the response to chicken aMPV
78 vaccines delivered *in ovo* to day old vaccination and determine the onset of immunity
79 for both chicken and turkey aMPV vaccines given *in ovo* in the face of MDA.

80

81 MATERIALS AND METHODS

82

83 Chickens.

84 Embryonated eggs from specified Pathogen Free (SPF) White Leghorn chickens
85 (Lohmann, Germany) or commercial Cobb broiler breeders (Cobb, Norfolk, UK) were
86 used. All chicks were hatched in a contained environment and transferred to negative
87 pressure isolators for the remainder of the experiments. Chick starter crumbs and
88 water were provided *ad libitum* during the course of the experiments.

89

90 Turkeys.

91 Embryonated eggs from commercial turkey breeders (British United Turkeys, UK)
92 were used. Hatched poults were housed in negative pressure isolators and fed turkey
93 starter crumbs and water *ad libitum*.

94

95 Virus strains.

96 Vaccines

97 For the chicken experiments a vaccine based on the aMPV 11/94 strain, a subtype B
98 aMPV (Nobilis Rhino CV®, Intervet) was used. For the turkey experiments a vaccine
99 based on the 8544 strain, a subtype A aMPV (Nobilis TRT ®, Intervet) was used.
100 Both vaccines are based on live viruses that have been attenuated by passage on chick
101 embryo fibroblasts (CEF). The titre of the vaccine viruses was established in CEFs,

102 calculated using the method of Reed & Muench (1938) and expressed as 50% tissue
103 culture infectious doses (TCID₅₀). The chicken vaccine was diluted in Marek's
104 disease vaccine diluent (Nobilis Marek CA diluent, Intervet) and the turkey vaccine in
105 phosphate buffered saline.

106

107 *Virulent viruses.*

108 For aMPV challenge of chickens 0.1ml of subtype B aMPV strain 2/95 was given via
109 the oculonasal route ($10^{3.5}$ CD₅₀/bird) . For the challenge of turkeys 0.1ml of subtype
110 A aMPV strain 3B was given via the oculonasal route ($10^{4.0}$ CD₅₀/bird). The titre of
111 the virulent viruses was established in tracheal organ cultures (Cook *et al.*, 1976),
112 calculated using the method of Reed & Muench (1938) and expressed as 50%
113 ciliostatic doses (CD₅₀).

114

115 **Vaccinations**

116 For embryo vaccinations, chicken eggs on the 18th and turkey eggs on the 24th day of
117 embryo development were inoculated into the amniotic fluid using a 20 gauge, 2.5cm
118 long needle. 0.1ml of the virus dilution was inoculated. Placebos received 0.1ml of
119 the respective diluent. Eggs were hatched in separate incubators. Hatch was assessed
120 after 21.5 days of incubation for chickens and 28.5 days for turkeys.

121

122 **Serology**

123 Serological responses to aMPV were studied using an ELISA based on a subtype A
124 antigen (Cook *et al.*, 1999). A titre of $\geq 4.64 \log_2$ is considered positive for chickens.
125 For poults a titre of $\geq 6.64 \log_2$ is considered positive.

126

127 **Assessment of clinical signs post challenge**

128

129 Following aMPV challenge chicks and poults were assessed daily for 10 days and

130 clinical signs of aMPV infection scored for chicks as follows; no clinical signs=0,

131 slight nasal discharge = 1, moderate nasal discharge = 2, copious nasal discharge = 3,

132 turbid nasal discharge = 4, ocular discharge = 5, swollen sinus = 6 and for poults as

133 follows; no clinical signs=0, slight nasal discharge = 1, turbid nasal discharge = 2,

134 copious nasal discharge = 3, copious turbid nasal discharge = 4, ocular discharge = 5,

135 slight swollen sinus = 5, moderate swollen sinus = 6, severe swollen sinus = 7.

136 Clinical signs per bird are scored cumulatively and total clinical scores per group are

137 presented. Protection is considered to be a total cumulative group score of less than

138 10 over the clinical monitoring period.

139

140

141

142 **Experimental design.**

143 The serological immune response induced by in ovo vaccination.

144

145 Groups of twenty SPF chicken eggs were inoculated with graded doses of the 11/94

146 vaccine ($10^{4.5}$, $10^{3.5}$, $10^{2.5}$ or $10^{1.5}$ TCID₅₀) or with diluent via the amniotic route on day

147 18 of incubation. The chicks were allowed to hatch in separate hatchers and live

148 chicks counted at 21.5 days of incubation. A further group of 20 non-vaccinated

149 chicks were hatched at the same time and vaccinated via the oculonasal route with

150 $10^{4.5}$ TCID₅₀ on the day of hatch. All chicks were monitored for clinical signs of

151 aMPV for 10 days. Twenty two days post hatch all chicks were bled, the serum

152 collected and tested for aMPV responses.

153

154 Comparison of onset of immunity in MDA positive chickens.

155

156 Groups of 60 aMPV MDA positive chicken eggs taken from breeders vaccinated with
157 inactivated aMPV vaccine, were inoculated with graded doses of 11/94 vaccine ($10^{3.5}$,
158 $10^{2.5}$ or $10^{1.5}$ TCID₅₀) or with diluent via the amniotic route. The chicks were allowed
159 to hatch in separate hatchers and live chicks counted at 21.5 days of incubation.

160 Clinical signs of aMPV were monitored post hatch. A further group of 60 non-
161 vaccinated chicks were hatched and vaccinated via the oculonasal route with
162 $10^{1.5}$ TCID₅₀. At 5, 8, 12, 15 and 43 days post hatch groups of 8 to 10 vaccinated or
163 control birds were bled for serum then challenged with virulent aMPV via the
164 oculonasal route. Clinical signs of aMPV were monitored for 10 days post challenge.

165

166 Onset of immunity in MDA positive turkey poults.

167

168 Groups of 30 aMPV MDA positive turkey eggs were inoculated with graded doses of
169 8544 vaccine ($10^{3.5}$, $10^{2.5}$ or $10^{1.5}$ TCID₅₀) or with diluent via the amniotic route. The
170 poults were allowed to hatch in separate hatchers and live poults counted at 28.5 days
171 of incubation. Clinical signs of aMPV were monitored post hatch. At 8, 12, and 43
172 days post hatch groups of 10 vaccinated or control poults were challenged with
173 virulent aMPV via the oculonasal route. Clinical signs of aMPV were monitored for
174 10 days post challenge.

175

176 **Statistical analyses**

177

178 A two-tailed T test was used to compare serological responses. P-values of less than
179 0.05 were considered to be significant.

180

181 **Results.**

182

183 SPF embryos were inoculated with graded doses of 11/94 vaccine *in ovo* or with a
184 high vaccine dose at day old to compare the aMPV-specific serological immune
185 response induced by these vaccination routes. At 22 days of age all of the *in ovo*
186 vaccinations, including 1000 fold less virus than given at day old, gave significantly
187 better responses than day old vaccination with $10^{4.5}$ TCID₅₀ ($10^{1.5}$ TCID₅₀ *in ovo* versus
188 $10^{4.5}$ TCID₅₀ at day old, $p=0.00006$) (figure 1) confirming previous results (Hess *et al.*,
189 2004). Furthermore, 100% of the birds vaccinated *in ovo* with $10^{4.5}$ TCID₅₀ had
190 seroconverted after 22 days, whereas only 60% of the birds vaccinated at day old had
191 seroconverted. It should be noted that the serological immune response to the subtype
192 B based vaccine was tested using a subtype A ELISA and higher responses may have
193 been detected had a homologous system been used.

194

195 To study the effect of MDA on the aMPV-specific serological response induced by
196 the different routes of vaccination, graded doses of vaccine were inoculated *in ovo* in
197 comparison to a low dose given at day old. At 43 days post hatch 25% of the placebo
198 vaccinated birds remained seropositive (titre $\geq 4.64 \log_2$) making it difficult to
199 establish the extent of seroconversion in the vaccinated groups (table 1A). However
200 62.5% of the chicks vaccinated *in ovo* with $10^{1.5}$ TCID₅₀ had titres greater than 4.64
201 \log_2 in comparison to 37.5% of the day old vaccinates suggesting a positive effect of
202 *in ovo* vaccination. Seroconversion increased in line with the vaccine dose with *in*

203 *ovo* vaccination doses of $10^{2.5}$ TCID₅₀ and $10^{3.5}$ TCID₅₀ giving 87.5% and 100%
204 seroconversion respectively.

205

206 To study the OOI in MDA positive chickens following *in ovo* vaccination, birds were
207 challenged at various time points post hatch. Hatched birds showed no clinical signs
208 of aMPV infection. At 5 days post vaccination all birds vaccinated *in ovo* were
209 protected regardless of dose (table 1B). The clinical signs in the group vaccinated
210 with $10^{1.5}$ TCID₅₀ *in ovo*, consisted of slight nasal discharge in three of the ten birds
211 for several days. In contrast the birds vaccinated at day old with $10^{1.5}$ TCID₅₀ showed
212 mild clinical signs in seven of the ten birds over a longer period (individual data not
213 shown) though these were significantly lower ($p=0.0000005$) than the clinical signs
214 observed in the placebo-vaccinated birds. From day 8 onwards all vaccinated groups
215 were considered protected against clinical signs up until the end of the experiment
216 though very minor nasal discharge was occasionally detected (table 1B). The clinical
217 signs in the placebo vaccinates reduced during the experiment confirming an age-
218 resistance (Williams *et al.*, 1991).

219

220 The serological immune response and onset of immunity following *in ovo* vaccination
221 was also studied for turkeys. At 43 days post hatch 10% of the placebo vaccinated
222 poults remained seropositive (titre ≥ 6.64 log₂) making it difficult to establish the
223 extent of seroconversion in the vaccinated groups (table 2A). However 50% of the
224 chicks vaccinated *in ovo* with $10^{1.5}$ TCID₅₀ had titres greater than 6.64 log₂.

225 Seroconversion increased in line with the vaccine dose with *in ovo* vaccination doses
226 of $10^{2.5}$ TCID₅₀ and $10^{3.5}$ TCID₅₀ both giving 100% seroconversion. Following
227 vaccination with the highest dose of 8544 vaccine ($10^{3.5}$ TCID₅₀) very mild clinical

228 signs consisting of slight nasal discharge were observed post hatch for 4 days (results
229 not shown). For this reason the first challenge was undertaken at 8 days post hatch.
230 All poult s were protected from challenge from 8 days until the end of the experiment
231 at 43 days post hatch irrespective of the dose confirming previous results
232 (Worthington *et al.*, 2003) (table 2B).

233

234 **Discussion.**

235

236 With the rapid spread of disease through an aMPV infected flock an early onset of
237 immunity could bring significant advantages to the poultry industry. We found that *in*
238 *ovo* vaccination of SPF chicken embryos induced a significantly higher antibody
239 response to aMPV than day old vaccination of chicks confirming previous results
240 (Hess *et al.*, 2004). However, a similar difference in the serological response was not
241 observed when vaccinating MDA positive chick embryos as has been demonstrated
242 for turkeys (Worthington *et al.*, 2003). Although MDA affects the serological
243 antibody response to aMPV it does not prevent successful vaccination (Cook *et al.*,
244 1989 and Naylor *et al.*, 1997) though it remains unknown whether MDA affects the
245 replication of aMPV in the respiratory tract. Regardless it is well established that the
246 serological response does not correlate with protection against virulent challenge and
247 that a local serological immune response (Worthington *et al.*, 2003) or cell mediated
248 immunity may be important in protection.

249

250 We studied the onset of immunity of the aMPV vaccines via virulent challenge in
251 time course experiments in MDA positive chicks. The reason for the earlier onset of
252 protection in MDA positive chicks, and the increased antibody response in SPF

253 chicks, observed following *in ovo* vaccination with the 11/94 vaccine has yet to be
254 established. However, improved antibody responses (Worthington *et al.*, 2003 and
255 Hess *et al.*, 2004) and earlier protection (Sharma and Burmester, 1982) have been
256 demonstrated after *in ovo* vaccination for various vaccines. The advantages achieved
257 by *in ovo* vaccination may be associated with replication of the viruses in the embryo
258 (Rautenschlein and Haase, 2005, Sharma, 1986, Sharma, 1987 and Sharma *et al.*,
259 1984) so it is feasible that replication of the 11/94 aMPV vaccine in the embryo could
260 have provided an earlier and/or higher antigenic load and therefore the induction of
261 better responses than day old delivery. Alternatively vaccination *in ovo* into the
262 amniotic fluid, which the embryo ingests, may have allowed the vaccine virus to
263 replicate in embryonic tissues that systemic delivery cannot provide access to.
264

265 Seroconversion of turkey poult vaccinated *in ovo* was dose dependent as the two
266 highest doses led to 100% seroconversion whereas the lowest dose led to 50%
267 seroconversion of poults. However, there was no significant difference between the
268 titres in any of the vaccinated groups. Unfortunately the very mild clinical signs
269 found following *in ovo* delivery of the turkey vaccine prevented a challenge at 5 days
270 of age. It should be noted that the onset of immunity of the turkey vaccine could be
271 earlier than 8 days though a comparison with day old vaccination was not included to
272 determine whether *in ovo* vaccination could induce a more rapid onset of immunity
273 than day old vaccination for turkeys. Regardless this data demonstrates the early
274 onset of immunity following *in ovo* vaccination, supplementing the previously
275 published data on this route of vaccination for turkeys (Worthington *et al.*, 2003).

276

277 Overall these results establish that although MDA can affect the serological immune
278 response following *in ovo* vaccination, the onset of immunity is still very early after
279 hatch, and in chickens vaccinated with 11/94 *in ovo* the onset of immunity is earlier
280 than can be achieved with day old vaccination with a comparative dose.

281

282 **References.**

283

284 Catelli, E., Cecchinato, M., Savage, C.E., Jones, R.C. Naylor, C.J. 2006.

285 Demonstration of loss of attenuation and extended field persistence of a live avian
286 metapneumovirus vaccine. *Vaccine*. 24, 6476-6482.

287

288 Cook, J. K. A., Darbyshire, J.H. & Peters, R.W. 1976. The use of tracheal organ
289 cultures for the isolation and assay of avian infectious bronchitis virus. *Archives of*
290 *Virology*. 50, 109-118.

291

292 Cook, J. K. A., Holmes, H. C., Finney, P. M., Dolby, C. A., Ellis, M. M. & Huggins,
293 M. B. 1989. A live attenuated turkey rhinotracheitis virus vaccine. 2. The use of the
294 attenuated strain as an experimental vaccine. *Avian Pathology*. 18, 523-34.

295

296 Cook, J. K. A., Huggins, M. B., Woods, M. A., Orbell, S. J. & Mockett, A. P. A.
297 1995. Protection provided by a commercially available vaccine against different
298 strains of turkey rhinotracheitis virus. *Vet. Record*. 136, 392-3.

299

300 Cook, J.K.A., Orthel, F., Orbell, S., Woods, M.A. & Huggins, M.B.

301 1996. An experimental turkey rhinotracheitis (TRT) infection in breeding turkeys and
302 the prevention of its clinical effects using live attenuated and inactivated TRT
303 vaccines. *Avian Pathology*. 25, 231– 243.

304

305 Cook, J. K. A., Huggins, M. B., Orbell, S. J. & Senne, D. A. 1999. Preliminary
306 antigenic characterization of an avian pneumovirus isolated from commercial
307 turkeys in Colorado, USA. *Avian Pathology*. 28, 607–17.

308

309 Hess, M., Huggins, M.B. and Heincz, U. 2004. Hatchability, serology and virus
310 excretion following *in ovo* vaccination of chickens with an avian metapneumovirus
311 vaccine. *Avian Pathology*. 33, 576-80.

312

313 Naylor, C. J. and Jones, R.C. 1994. Demonstration of a virulent subpopulation in a
314 prototype live attenuated turkey rhinotracheitis vaccine. *Vaccine*. 12, 1225-1230.

315

316 Naylor, C. J., Worthington, K. J. & Jones, R. C. 1997. Failure of maternal antibodies
317 to protect young turkey poults against challenge with turkey rhinotracheitis
318 virus. *Avian Dis*. 41, 968–71.

319

320 Rautenschlein, S., and Haase, C. 2005. Differences in the immunopathogenesis of
321 infectious bursal disease virus (IBDV) following *in ovo* and post-hatch vaccination of
322 chickens. *Vet. Immunol. Immunopathol*. 106, 139-150.

323

324 Reed, L.J., & Muench, H. 1938. A simple method of estimating fifty per cent
325 endpoint. *Am. J. Hyg*. 27, 493-497

326

327 Sharma, J.M., and Burmester, B.R. 1982. Resistance to Marek's disease at hatching in
328 chickens vaccinated as embryos with the turkey herpesvirus. *Avian Dis.* 26, 134-139.

329

330 Sharma, J.M. 1986. Embryo vaccination of specific-pathogen-free chickens with
331 infectious bursal disease virus: tissue distribution of the vaccine virus and protection
332 of hatched chickens against disease. *Avian Dis.* 30, 776-780.

333

334 Sharma, J.M. 1987. Delayed replication of Marek's disease virus following *in ovo*
335 inoculation during late stages of embryonal development. *Avian Dis.* 31, 570-576.

336

337 Sharma, J.M., Lee, L.F., and Wakenell P.S. 1984. Comparative viral, immunologic,
338 and pathologic responses of chickens inoculated with herpesvirus of turkeys as
339 embryos or at hatch. *Am. J. Vet. Res.* 45, 1619-23.

340

341 Toquin, D., Bayon-Auboyer, M. H., Jestin, V. & Eterradosi, N. 1999. Réponse
342 sérologique et protection croisée vis à vis de l'infection par une souche non-A non-B
343 du virus de la rhinotrachéite infectieuse de la dinde. *Comptes-rendus des 3èmes*
344 *Journées de la Recherche Avicole*, St Malo, France, 23-5 Mars, pp 223-4.

345

346 Williams, R.A., Savage, C.E., Worthington, K.J., Jones, R.C. 1991. Further studies
347 on the development of a live attenuated vaccine against turkey rhinotracheitis. *Avian*
348 *Pathology.* 20, 589-96.

349

350 Worthington, K.J., Sargent, B.A., Davelaar, F.G. and Jones, R.C. 2003. Immunity to
351 avian pneumovirus infection in turkeys following *in ovo* vaccination with an
352 attenuated vaccine. *Vaccine*. 21,1355-1362.
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Table 1: The serological immune response of MDA positive chicks following *in ovo* or day old vaccination (A) The geometric mean titre and Standard deviation are shown. Percentage seropositive is shown in parentheses. Protection of chicks against clinical signs following virulent aMPV challenge (B) The percentage of birds with clinical signs is shown in parentheses. Total cumulative scores per group are shown.

A

| Age | $10^{3.5}$ TCID ₅₀ <i>in ovo</i> | $10^{2.5}$ TCID ₅₀ <i>in ovo</i> | $10^{1.5}$ TCID ₅₀ <i>in ovo</i> | $10^{1.5}$ TCID ₅₀ at day old | Placebo in ovo |
|-------------|--|--|--|---|--------------------|
| 5 | 8.96±3.82 (100) | 8.75±2.97 (100) | 9.54 ±2.79 (100) | 8.39±2.36 (100) | 8.91±2.53 (100) |
| 8 | 8.96±1.38 (100) | 9.10±2.32 (100) | 8.51±2.41 (100) | 7.16±2.45 (100) | 7.76±3.23 (100) |
| 12 | 8.10±1.34 (100) | 8.05±1.37 (100) | 7.35±1.89 (90) | 6.01±1.46 (100) | 6.42±1.74 (90) |
| 15 | 8.47±1.23 (100) | 8.07±0.7 (100) | 7.85±1.00 (90) | 6.64±2.35 (80) | 5.92±1.89 (80) |
| 22 | 6.81±0.91 (100) | 7.53±2.61 (90) | 6.17±2.03 (70) | 4.76±0.88 (30) | 4.29±0.70 (30) |
| 43 (n=8) | 7.61±2.42 (100) | 8.03±3.97 (87.5) | 6.34±2.05 (62.5) | 5.41 ±1.56 (37.5) | 4.27±1.61 (20) |

B

| Age | $10^{3.5}$ TCID ₅₀ <i>in ovo</i> | $10^{2.5}$ TCID ₅₀ <i>in ovo</i> | $10^{1.5}$ TCID ₅₀ <i>in ovo</i> | $10^{1.5}$ TCID ₅₀ at day old | Placebo in ovo |
|-------------|--|--|--|---|------------------------|
| 5 | 2 (10) ^a | 5 (20) ^a | 10 (30) ^a | 40 (70) ^a | 131 (100) ^b |
| 8 | 4 (20) ^a | 0 (0) ^a | 0 (0) ^a | 2 (20) ^a | 95 (100) ^b |
| 12 | 1 (10) ^a | 4 (20) ^a | 3 (20) ^a | 4 (30) ^a | 91 (100) ^b |
| 15 | 1(10) ^a | 1 (10) ^a | 1 (10) ^a | 6 (20) ^a | 79 (100) ^b |
| 43 (n=8) | 0 (0) ^a | 4 (25) ^a | 3 (12.5) ^a | 3 (12.5) ^a | 52 (100) ^b |

Different superscripts represent a significant difference $p \leq 0.05$.

Table 2: The serological immune response of MDA positive poult following *in ovo* vaccination (A). The geometric mean titre and standard deviations are shown. Percentage seropositive is shown in parentheses. Protection of poult against clinical signs following virulent aMPV challenge (B). Total cumulative scores per group are shown.

A

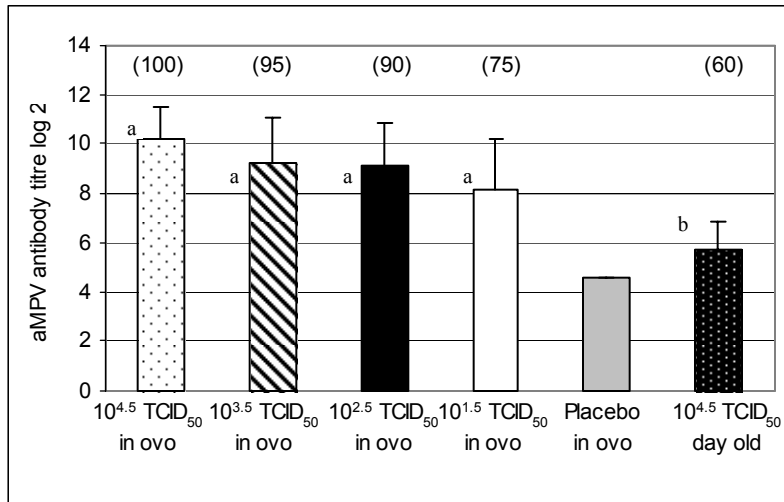
| Age | $10^{3.5}$ TCID ₅₀ <i>in ovo</i> | $10^{2.5}$ TCID ₅₀ <i>in ovo</i> | $10^{1.5}$ TCID ₅₀ <i>in ovo</i> | Placebo <i>in ovo</i> |
|-----|---|---|---|-----------------------|
| 8 | 9.35±3.42 (100) | 9.25±2.87 (100) | 9.6±2.36 (100) | 9.4±2.07 (100) |
| 12 | 10.15±3.01 (100) | 8.25±1.36 (100) | 9.2±2.01 (100) | 8.6±1.31 (90) |
| 43 | 9.28±2.22 (100) | 8.48±2.34 (100) | 6.99±3.1 (70) | 4.93±1.43 (30) |

B

| Age | $10^{3.5}$ TCID ₅₀ <i>in ovo</i> | $10^{2.5}$ TCID ₅₀ <i>in ovo</i> | $10^{1.5}$ TCID ₅₀ <i>in ovo</i> | Placebo <i>in ovo</i> |
|-----|---|---|---|-----------------------|
| 8 | 0 ^a | 0 ^a | 0 ^a | 177 ^b |
| 12 | 0 ^a | 0 ^a | 0 ^a | 284 ^b |
| 43 | 0 ^a | 1 ^a | 1 ^a | 86 ^b |

Different superscripts represent a significant difference $p \leq 0.05$.

Figure 1.



The serological immune response following *in ovo* vaccination of SPF embryos. Groups of twenty SPF chicken eggs or day old chicks were inoculated as shown. Twenty two days post hatch the sera were tested for aMPV responses by ELISA. Figures in parenthesis indicate the percentage seroconversion within each group. Different superscripts demonstrate significant differences in response ($p \leq 0.05$).