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Caroline van Droogenbroeck, Delphine S.A. Beeckman, Taher Harkinezhad, Eric Cox, Daisy Vanrompay. Evaluation of the prophylactic use of ovotransferrin against chlamydiosis in SPF turkeys. *Veterinary Microbiology*, 2008, 132 (3-4), pp.372. 10.1016/j.vetmic.2008.05.028 . hal-00532438

HAL Id: hal-00532438

<https://hal.science/hal-00532438>

Submitted on 4 Nov 2010

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Accepted Manuscript

Title: Evaluation of the prophylactic use of ovotransferrin against chlamydiosis in SPF turkeys

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PII: S0378-1135(08)00213-7
DOI: doi:10.1016/j.vetmic.2008.05.028
Reference: VETMIC 4061

To appear in: *VETMIC*

Received date: 12-12-2007
Revised date: 21-5-2008
Accepted date: 26-5-2008

Please cite this article as: Van Droogenbroeck, C., Beeckman, D.S.A., Harkinezhad, T., Cox, E., Vanrompay, D., Evaluation of the prophylactic use of ovotransferrin against chlamydiosis in SPF turkeys, *Veterinary Microbiology* (2007), doi:10.1016/j.vetmic.2008.05.028

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1 **Evaluation of the prophylactic use of ovotransferrin against**
2 **chlamydiosis in SPF turkeys**

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24 **Abstract**

25 *Chlamydophila (C.) psittaci* infections are highly prevalent in turkeys and the
26 economical and public health importance of these infections has been recognized since
27 1950. As there are no vaccines, antibiotic treatment (tetracyclines, enrofloxacin) is often
28 needed to allow marketing of poultry. In this study, we explored the use of ovotransferrin
29 (ovoTF), a natural anti-microbial protein, in preventing an experimental *C. psittaci*
30 infection in specific pathogen free (SPF) turkeys. Turkeys were treated with aerosolized
31 ovoTF prior to the infection. Groups 1 and 2 received a single dose of 10 and 5 mg
32 ovoTF per turkey, respectively. Group 3 received a daily dose of 5 mg ovoTF per turkey
33 during twelve days. Group 4 served as untreated, infected control group. Turkeys were
34 aerosol infected using 10^6 TCID₅₀ of the virulent *C. psittaci* serovar/genotype D strain
35 92/1293. Birds were monitored (clinical signs, bacterial excretion) during twelve
36 subsequent days before being necropsied. At necropsy, pathology and *C. psittaci*
37 replication in various tissues was examined. A single dose of 10 mg ovoTF and a
38 repeated daily dose of 5 mg ovoTF could not prevent the birds from becoming infected
39 with *C. psittaci*, but they significantly reduced the outcome of the infection. A single dose
40 of 5 mg ovoTF had no influence on the outcome of the infection as compared to the non-
41 treated infected controls. Our results demonstrate the anti-chlamydial effect of ovoTF *in*
42 *vivo* and present a base for further research on practical applications of ovoTF on turkey
43 farms.

44

45

46 **Keywords:** Ovotransferrin; *Chlamydophila psittaci*; Chlamydiosis; Turkeys

47 **1. Introduction**

48 *Chlamydophila (C.) psittaci* is an obligate intracellular Gram-negative bacterium
49 causing respiratory disease in poultry, pet birds and humans (Andersen and Vanrompay,
50 2000). Recent *in vitro* results suggest a possible role for ovotransferrin (ovoTF) in
51 preventing chlamydiosis in birds. OvoTF inactivates extracellular *C. psittaci* and
52 significantly inhibits chlamydial entry in HD11 chicken macrophages by blocking actin
53 polymerization (Beeckman et al., 2007), which is crucial for chlamydial cell entry
54 (Dautry-Varsat et al., 2005). Thus, ovoTF exerts a bactericidal and bacteriostatic effect
55 on *C. psittaci*, that may be applied to prevent *C. psittaci* infections in poultry.

56 OvoTF is an avian extracellular iron-binding glycoprotein that belongs to the family of
57 transferrins. Egg-white OvoTF is synthesized by oviduct cells and is present in egg white
58 and in eggshell. Serum ovoTF is synthesized by the liver and released to the blood
59 (Stevens, 1991; Giansanti et al., 2002). Egg-white and serum OvoTF differ only in their
60 glycosylation patterns (Jacquinot et al., 1994). OvoTF shows marked sequence and
61 structural homologies with both mammalian serum transferrin (TF) and mammalian
62 lactoferrin (LF) (Williams et al., 1982; Metz-Boutigue et al., 1984; Jeltsch et al., 1987;
63 Kurokawa et al., 1999). Like mammalian serum TF, ovoTF is involved in iron transport
64 and delivery to cells and like mammalian LF, ovoTF is a component of innate immunity
65 (Giansanti et al., 2002; Giansanti et al., 2007). Egg-white ovoTF displays the same
66 antibacterial functions as LF in milk. The antibacterial activity can partially involve iron
67 sequestration (Valenti et al., 1982; Valenti et al., 1983) and can be related to the action of
68 OTAP-92, a short sequence located in the N-terminal domain of ovoTF (Ibrahim et al.,

69 1998; Ibrahim et al., 2000). OvoTF also displays anti-viral activity (Giansanti et al.,
70 2002; Giansanti et al., 2007).

71 In the present study, we explored the use of aerosolized ovoTF in preventing an
72 experimental *C. psittaci* genotype D infection in specific pathogen free (SPF) turkeys. In
73 fact, aerosolization of transferrins is not new as it was used in a sheep model of asthma,
74 where an abolishment of late-phase airway responses in allergic sheep could be observed
75 after administration of aerosolized lactoferrin (Elrod et al., 1997).

76

77 **2. Materials and methods**

78 *2.1. Chlamydophila and host cell line*

79 *C. psittaci* strain 92/1293, isolated from a severe outbreak of respiratory disease in a
80 commercial broiler turkey farm in the Netherlands, was used (Vanrompay et al., 1993).
81 The strain was isolated from a pooled homogenate of the lungs, the cloacae and the
82 spleens of diseased turkeys and was characterized as serovar D and genotype D (Geens et
83 al., 2005).

84 Bacteria were grown in Buffalo Green Monkey (BGM) cells as previously described
85 (Vanrompay et al., 1992) and the titration was performed by the method of Spearman and
86 Kaerber (Mayr et al., 1974). The bacterial stock showed a titer of 10^{11} 50 % tissue culture
87 infective dose (TCID₅₀) per ml.

88

89 *2.2. Ovotransferrin*

90 Ovotransferrin (ovoTF), purified from chicken egg white, was purchased from Sigma
91 (Bornem, Belgium). Non iron-saturated ovoTF was used.

92

93 *2.3. Experimental design*

94 Experiments were performed in negative pressure isolators (IM 1500, Montair
95 Sevenum, the Netherlands). The experimental design was evaluated and approved by the
96 Ethical Commission for Animal Experiments of Ghent University. At three weeks of age,
97 four groups of eight specific pathogen free (SPF) turkeys (CNEVA, Ploufragan, France),
98 each group in a separate isolator, were exposed for one hour to an ovoTF aerosol (5 µm
99 droplets; CirrusTM nebulizer; Laméris, Aartselaar, Belgium) prior to *C. psittaci* infection.
100 OvoTF was administered by aerosol as chlamydial replication starts in mucosal epithelial
101 cells and macrophages of the upper respiratory tract. The ventilation was switched off
102 during ovoTF treatment. Group 1 (High Dose Group) and 2 (Single Low Dose Group)
103 received a single dose of 10 and 5 mg ovoTF per turkey, respectively. Group 3 (Multiple
104 Low Dose Group) received a daily dose of 5 mg ovoTF per turkey during twelve
105 consecutive days. Group 4 served as untreated, infected, control group (No OvoTF
106 Group). At the end of the exposure time, ovoTF aerosols were drained from the isolator
107 by ventilation. After a ventilation period of two hours, turkeys were aerosol infected
108 during one hour using 10^6 TCID₅₀ of the *C. psittaci* genotype D strain 92/1293. Based on
109 the pathogenesis of *C. psittaci* in SPF turkeys (Vanrompay et al., 1995), birds were
110 monitored during 12 days post infection (p.i.).

111 Clinical signs were daily scored until necropsy at 12 days p.i. Clinical score 0 indicated
112 no clinical signs; score 1: conjunctivitis; score 2: rhinitis; score 3: dyspnoea; score 4:
113 conjunctivitis and rhinitis; score 5: conjunctivitis and dyspnoea; score 6: rhinitis and
114 dyspnoea; score 7: conjunctivitis, rhinitis and dyspnoea. Pharyngeal and cloacal excretion

115 of *C. psittaci* was monitored at 3, 6, 9 and 12 days p.i., using rayon-tipped, aluminium
116 shafted swabs (Colpan; Fiers, Kurne, Belgium) provided with *C. psittaci* transport
117 medium (sucrose 74,6 g/l (Acros Organics, Geel, Belgium); KH_2PO_4 5,1 g/l and K_2HPO_4
118 1,2 g/l (Sigma); L-glutamic acid mono potassium salt 0,9 g/l and gentamycin 50 $\mu\text{g}/\text{ml}$
119 (Invitrogen, Merelbeke, Belgium); vancomycin 100 $\mu\text{g}/\text{ml}$ and streptomycin 100 $\mu\text{g}/\text{ml}$
120 (Soenen, Merelbeke, Belgium); nystatin 25000 U/ml (Sigma) pH 7). Swabs were stored
121 at -80°C until processed. At 3, 6, 9 and 12 days p.i., two birds of each group were
122 necropsied and macroscopic lesions were scored as described in Table 1. Tissue samples
123 of the conchae, the trachea, the lungs, the abdominal and thoracic airsacs and the spleen
124 were immersed in methocel (Methocel MC, Sigma), snap frozen in liquid nitrogen and
125 stored at -80°C until processed.

126

127 2.4. *Chlamydophila* replication and excretion

128 Cryostat tissue sections (5 μm) of the conchae, the trachea, the lungs, thoracic and
129 abdominal airsacs and spleen were examined for *C. psittaci* replication by the
130 IMAGENTM immunofluorescence staining (IMAGENTM CHLAMYDIA, Oxoid,
131 Drongen, Belgium), as previously described (Vanrompay et al., 1994). *C. psittaci* positive
132 cells were enumerated in five randomly selected microscopic fields (400x, Nikon Eclipse
133 TE2000-E, Japan) and scored between 0 and 4: score 0 indicated no *C. psittaci* present;
134 score 1 was given when a mean of 1-5 elementary bodies (infectious, non-metabolic
135 morphological form) was present; score 2, 3 and 4 were given when a mean of 1-5, 6-10
136 and >10 inclusion (actively replicating organisms) positive cells was present.

137 All swabs were examined for *C. psittaci* excretion by bacterial culture in Buffalo Green
138 Monkey (BGM) cells (Vanrompay et al., 1992) and subsequent chlamydial identification
139 using the IMAGENTM direct immunofluorescence assay (Vanrompay et al., 1994). The
140 presence of *C. psittaci* was scored as for bacterial replication in different tissues.

141

142 2.5. Statistical analysis

143 The Mann-Whitney test was used for statistical analysis. Results were considered
144 significantly different at the level of $P < 0,05$.

145

146 3. Results

147 3.1. Clinical signs

148 Although the animals were daily monitored for clinical signs, Table 2 shows the
149 maximum clinical scores for SPF turkeys on p.i. days 3, 6, 9 and 12. Throughout the
150 experiment, severe respiratory signs were observed in the No OvoTF Group. The highest
151 maximal clinical scores (score 5 to 6) in this group were observed at p.i. days 6, 9 and 12,
152 when all birds showed dyspnoea, conjunctivitis (blepharospasm, scratching their eyes)
153 and/or rhinitis (head shaking, sneezing, scratching their nares). Sometimes they were
154 gasping. As for the No OvoTF Group, the Single Low Dose Group showed the highest
155 maximal clinical scores (5 to 6) at 6, 9 and 12 days p.i. On day 12 p.i., all birds in this
156 group showed conjunctivitis and dyspnoea. Turkeys of the Multiple Low Dose Group
157 showed no clinical signs throughout the experiment except for 11 days p.i., when mild
158 dyspnoea was observed in the remaining two turkeys. The following day however, at
159 necropsy, both turkeys appeared clinically healthy. The High Dose Group showed the

160 highest score (score 3) at 12 days p.i., with mild dyspnoea being observed for the first
161 time.

162

163 3.2. Macroscopic findings

164 The macroscopic findings, presented as the sum of the mean gross lesion scores for the
165 different tissues of two SPF turkeys necropsied at days 3, 6, 9 and 12 p.i., are shown in
166 Table 2. Macroscopic lesions were noticed in the No OvoTF Group from 3 days p.i. until
167 the end of the experiment at 12 days p.i. The highest lesion score (score of 10,50) was
168 observed at 12 days p.i. when severe congestion of the conjunctivae, the conchae and the
169 trachea was observed together with pneumonia, fibrous adhesive airsacculitis, serous
170 pericarditis and hepatosplenomegaly. At the same time, also at 12 days p.i., turkeys of the
171 Single Low Dose Group showed the highest score (score of 7) characterized by severe
172 congestion of the conjunctivae and the conchae, pneumonia, focal fibrin deposits in the
173 airsacs and moderate enlargement of the spleen. Interestingly, turkeys of the Multiple
174 Low Dose Group showed the highest lesion score (score of 1) at 9 days p.i. but only
175 slight congestion of the conjunctivae and the lungs were present. For the High Dose
176 Group, the highest lesion score (score of 1,50) was also observed at 12 days p.i.
177 However, only slight congestion of the conjunctivae and moderate congestion of the
178 lungs could be observed. In conclusion, there was no significant difference in
179 macroscopic findings between the High Dose Group and the Multiple Low Dose Group,
180 but the lesions were overall less severe compared to the Single Low Dose Group and the
181 No OvoTF Group.

182

183 3.3. *Chlamydophila* replication and excretion

184 Table 2 shows the sum of the mean scores of the direct immunofluorescence staining on
185 frozen tissue sections for *C. psittaci* replication of two turkeys of all groups, necropsied
186 on p.i. days 3, 6, 9 and 12. The No ovoTF group and the Single Low Dose Group showed
187 identical highest scores at 9 (score of 11) and 12 (score of 12) days p.i., although scores
188 for the Single Low Dose Group were lower at p.i. days 3 and 6. Repeating the ovoTF
189 administration daily for the Multiple Low Dose Group, resulted in the absence of *C.*
190 *psittaci* replication on day 6 p.i.. However, the infection could not be cleared as the upper
191 and lower respiratory tract became positive again at days 9 and 12 p.i. At day 3 p.i., no
192 chlamydial replication could be detected in the High Dose Group, while the other groups
193 were already positive in the tissue immunofluorescence staining. In conclusion, tissue
194 sections of the High Dose Group and the Multiple Low Dose Group showed significant
195 lower scores as compared to the Single Low Dose Groups and the No OvoTF Group,
196 with a highest score of 3 (9 days p.i.) and 3,50 (12 days p.i.), respectively.

197 Table 2 shows that pharyngeal swabs of the No OvoTF Group, the Single Low Dose
198 Group and the High Dose Group were positive on p.i. days 3, 6, 9 and 12, indicating
199 pharyngeal *C. psittaci* excretion. Cloacal shedding in these three groups still occurred at
200 12 days p.i (Table 2). However, at the given time points, culture scores of the No OvoTF
201 Group and the Single Low Dose Group were consistently higher than for the High Dose
202 Group. The Multiple Low Dose Group, receiving ovoTF daily, apparently shed the lowest
203 amount of bacteria as pharyngeal and cloacal swabs became negative on 9 and 12 days
204 p.i., respectively.

205

206 4. Discussion

207 *Chlamydophila (C.) psittaci* infections are highly prevalent in turkeys and the
208 economical and public health importance of these infections has been recognized since
209 1950. There are no vaccines. Thus, antibiotic treatment (tetracyclines, enrofloxacin) is
210 often needed to allow marketing of poultry. At present, we explored the use of
211 ovotransferrin (ovoTF), a natural anti-microbial protein, in preventing an experimental *C.*
212 *psittaci* infection in specific pathogen free (SPF) turkeys. The aerosol experimental
213 infection model has been established years ago and has proven its reproducibility several
214 times (Vanrompay et al., 1993; Vanrompay et al., 1995). The *C. psittaci* delivery method
215 reflects the natural way of infection and as in nature bacteria can get on the feathers and
216 into the eyes. Thus, opportunity for further infection by inhalation of aerosolized feather
217 dust after delivery is existent. Infection after delivery by this natural infection route is
218 always possible, as the infected birds will also excrete the bacterium in the surroundings
219 and on their feathers. Prognosis was greatly improved by a single prophylactic dose of 10
220 mg aerosolized ovoTF or by 5 mg ovoTF aerosol once a day for each of twelve days. Our
221 results demonstrate the anti-chlamydial effect of ovoTF *in vivo* and present a base for
222 further research on practical applications of ovoTF on turkey farms. One might consider
223 the use of ovoTF aerosols on turkeys at ages three to four weeks when residual *C. psittaci*
224 maternal antibody titres are extremely low and infection outbreaks occur (Van Loock et
225 al., 2005).

226 We used iron-unsaturated ovoTF, as iron-saturated transferrins normally transport iron
227 into the cells by receptor-mediated endocytosis (Dunn et al., 2007) and although
228 *Chlamydiales* live as intracellular 'parasites', they require iron to develop a productive

229 infection. Indeed, recently has been demonstrated that the intracellular growth of
230 *Chlamydia trachomatis* and *C. psittaci* in HEK293 T cells is regulated by the levels of
231 intracellular iron (Paradkar et al., 2008). In addition, they showed that iron-depletion
232 limited intracellular bacterial growth in mouse macrophages. The mechanism of iron
233 acquisition by chlamydial organisms is undefined, but is reasonable to assume that the
234 cytosolic iron pool is the source.

235 At present, several biological and immunomodulatory activities of ovoTF were
236 probably involved in influencing the outcome of the *C. psittaci* infection. It is well known
237 that Gram-negative bacteria can be killed through a membrane damage mechanism by the
238 defensin-like structural 92-amino acid N-terminal ovotransferrin peptide (OTAP-92)
239 (Ibrahim et al., 1998; Ibrahim et al., 2000). Thus, extracellular *C. psittaci* might have
240 been killed by this mechanism and possibly by other antimicrobial functions ascribed to
241 transferrins such as destabilization of the outer membrane of Gram-negative bacteria
242 through binding of bacterial LPS (Appelmelk et al., 1994; Brandenburg et al., 2001) and
243 selective permeation of ions (Aguilera et al., 2003). Additionally, transferrins can disrupt
244 the bacterial type III secretion system (TTSS), which promotes bacterial attachment and
245 internalization by inducing actin polymerization at the bacterial entry site (Gomez et al.,
246 2003; Ochoa et al., 2003). *In vitro*, OvoTF clearly inhibits actin polymerization at the *C.*
247 *psittaci* entry site (Beeckman et al., 2007). Thus, there is a reason to believe that it might
248 also occur *in vivo*.

249 Importantly, besides direct effects of ovoTF causing bacterial membrane damage and
250 disruption of the type III secretion machinery, ovoTF also acts as the major avian acute
251 phase protein (APP), which is normally elevated during inflammation and infection

252 (Hallquist and Klasing, 1994). OvoTF promotes IL-6 release by macrophages and
253 heterophils (Xie et al., 2002).

254 Recently, a key immunomodulatory function was assigned to lactoferrin, namely
255 linking the innate immunity with adaptive immunity through maturation of dendritic cells
256 (DCs) (Spadaro et al., 2008). Maybe, although not yet examined, ovoTF could have a
257 similar function.

258 Regarding the direct anti-chlamydial effects of ovoTF and its immunomodulatory
259 functions, one can understand that the group receiving a single high dose of 10 mg ovoTF
260 per turkey (High Dose Group), performed especially better in the beginning of the
261 experiment, at day 3 p.i., as compared to the group receiving a daily dose of only 5 mg
262 ovoTF per turkey during twelve days (Multiple Low Dose Group). However, the latter
263 group generally performed better by the end of the experiment as compared to the High
264 Dose Group. Probably the repeated dosing boosted the immunity, was able to kill bacteria
265 on the feathers and could inhibit the infection caused by contamination with group mates
266 and aerosolized feather dust. One single dose of 5 or 10 mg ovoTF could not prevent the
267 infection.

268 In conclusion, we provided evidence for an anti-chlamydial effect of ovoTF *in vivo* and
269 for the possibility to administer this natural anti-microbial protein aerogenically to
270 poultry. OvoTF aerosols could provide an additional strategy to prevent *C. psittaci*
271 infections in turkeys. However, further research on practical applications is needed.

272

273 **Conflict of interest statement**

274 None of the authors (Caroline Van Droogenbroeck, Delphine Beeckman, Taher
275 Harkinezhad, Eric Cox and Daisy Vanrompay) has a financial or personal relationship
276 with other people or organizations that could inappropriately influence or bias the paper
277 entitled “Evaluation of the prophylactic use of ovotransferrin against chlamydiosis in SPF
278 turkeys”.

279

280 **Acknowledgements**

281 The Belgian Federal Public Service for Health, Food Chain Safety and Environment
282 (RT-6177) granted present research. The Research Foundation – Flanders (FWO –
283 Vlaanderen) is acknowledged for providing a Ph.D. grant to Delphine S.A. Beeckman.
284 We gratefully thank R. Cooman for animal care taking.

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References

- 288 Aguilera, O., Quiros, L.M., Fierro, J.F., 2003. Transferrins selectively cause ion efflux
289 through bacterial and artificial membranes. FEBS Lett. 548, 5-10.
- 290 Andersen, A.A., Vanrompay, D., 2000. Avian chlamydiosis. Rev. Sci. Techn. Off. Int.
291 Epizoot. 19, 396-404.
- 292 Appelmelk, B.J., An, Y.Q., Geerts, M., Thijs, B.G., de Boer, H.A., MacLaren, D.M., de
293 Graaff, J., Nuijens, J.H., 1994. Lactoferrin is a lipid A-binding protein. Infect. Immun.
294 62, 2628-2632.
- 295 Beeckman, D.S.A., Van Droogenbroeck, C.M.A.D., De Cock, B.J.A., Van Oostveldt, P.,
296 Vanrompay, D.C.G., 2007. Effect of ovotransferrin and lactoferrins on *Chlamydomphila*
297 *psittaci* adhesion and invasion in HD11 chicken macrophages. Vet. Res. 38, 729-739.
- 298 Brandenburg, K., Jurgens, G., Muller, M., Fukuoka, S., Koch, M.H., 2001. Biophysical
299 characterization of lipopolysaccharide and lipid A inactivation by lactoferrin. Biol. Chem.
300 382, 1215-1225.

- 301 Dautry-Varsat, A., Subtil, A., Hackstadt, T., 2005. Recent insights into the mechanisms
302 of Chlamydia entry. *Cell Microbiol.* 7, 1714-1722.
- 303 Dunn, L.L., Rahmanto, Y.S., Richardson, D.R., 2007. Iron uptake and metabolism in the
304 new millennium. *Trends Cell Biol.* 17, 93-100.
- 305 Elrod, K.C., Moore, W.R., Abraham, W.M., Tanaka, R.D., 1997. Lactoferrin, a potent
306 tryptase inhibitor, abolishes late-phase airway responses in allergic sheep. *Am. J. Respir.*
307 *Crit. Care Med.* 156, 375-381.
- 308 Geens, T., Desplanques, A., Van Loock, M., Bonner, B.M., Kaleta, E.F., Magnino, S.,
309 Andersen, A.A., Everett, K.D., Vanrompay, D., 2005. Sequencing of the *Chlamydomphila*
310 *psittaci* *ompA* gene reveals a new genotype, E/B, and the need for a rapid discriminatory
311 genotyping method. *J. Clin. Microbiol.* 43, 2456-2461.
- 312 Giansanti, F., Giardi, M.F., Massucci, M.T., Botti, D., Antonini, G., 2007. Ovotransferrin
313 expression and release by chicken cell lines infected with Marek's disease virus.
314 *Biochem. Cell Biol.* 85, 150-155.
- 315 Giansanti, F., Rossi, P., Massucci, M.T., Botti, D., Antonini, G., Valenti, P., Seganti, L.,
316 2002. Antiviral activity of ovotransferrin discloses an evolutionary strategy for the
317 defensive activities of lactoferrin. *Biochem. Cell Biol.* 80, 125-130.
- 318 Gomez, H.F., Ochoa, T.J., Carlin, L.G., Cleary, T.G., 2003. Human lactoferrin impairs
319 virulence of *Shigella flexneri*. *J. Infect. Dis.* 187, 87-95.
- 320 Hallquist, N.A., Klasing, K.C., 1994. Serotransferrin, ovotransferrin and metallothionein
321 levels during an immune response in chickens. *Comp. Biochem. Physiol. Biochem. Mol.*
322 *Biol.* 108, 375-384.
- 323 Ibrahim, H.R., Iwamori, E., Sugimoto, Y., Aoki, T., 1998. Identification of a distinct
324 antibacterial domain within the N-lobe of ovotransferrin. *Biochim. Biophys. Acta* 1401,
325 289-303.
- 326 Ibrahim, H.R., Sugimoto, Y., Aoki, T., 2000. Ovotransferrin antimicrobial peptide
327 (OTAP-92) kills bacteria through a membrane damage mechanism. *Biochim. Biophys.*
328 *Acta* 1523, 196-205.
- 329 Jacquinet, P.M., Leger, D., Wieruszkeski, J.M., Coddeville, B., Montreuil, J., Spik, G.,
330 1994. Change in glycosylation of chicken transferrin glycans biosynthesized during
331 embryogenesis and primary culture of embryo hepatocytes. *Glycobiology* 4, 617-624.
- 332 Jeltsch, J.M., Hen, R., Maroteaux, L., Garnier, J.M., Chambon, P., 1987. Sequence of the
333 chicken ovotransferrin gene. *Nucleic Acids Res.* 15, 7643-7645.
- 334 Kurokawa, H., Dewan, J.C., Mikami, B., Sacchettini, J.C., Hirose, M., 1999. Crystal
335 structure of hen apo-ovotransferrin. Both lobes adopt an open conformation upon loss of
336 iron. *J. Biol. Chem.* 274, 28445-28452.

- 337 Mayr, A., Bachmann, P.A., Bibrack, B., Wittmann, G., 1974. Quantitative bestimmung
338 der virusinfektiosität. In: Mayr, A., Bachmann, P.A., Bibrack, B., Wittmann, G. (Eds.),
339 Virologische arbeitsmethoden Bd.I., Gustav Fisher Verlag, Jena, pp. 35-39.
- 340 Metz-Boutigue, M.H., Jolles, J., Mazurier, J., Schoentgen, F., Legrand, D., Spik, G.,
341 Montreuil, J., Jolles, P., 1984. Human lactotransferrin: amino acid sequence and
342 structural comparisons with other transferrins. Eur. J. Biochem. 145, 659-676.
- 343 Ochoa, T.J., Noguera-Obenza, M., Ebel, F., Guzman, C.A., Gomez, H.F., Cleary, T.G.,
344 2003. Lactoferrin impairs type III secretory system function in enteropathogenic
345 *Escherichia coli*. Infect. Immun. 71, 5149-5155.
- 346 Paradkar, P., De, D., I, Durchfort, N., Zohn, I., Kaplan, J., Ward, D.M., 2008. Iron-
347 depletion limits intracellular bacterial growth in macrophages. Blood, in press.
- 348 Spadaro, M., Caorsi, C., Ceruti, P., Varadhachary, A., Forni, G., Pericle, F., Giovarelli,
349 M., 2008. Lactoferrin, a major defense protein of innate immunity, is a novel maturation
350 factor for human dendritic cells. FASEB J., in press.
- 351 Stevens, L., 1991. Egg white proteins. Comp. Biochem. Physiol. B 100, 1-9.
- 352 Valenti, P., Antonini, G., Fanelli, M.R., Orsi, N., Antonini, E., 1982. Antibacterial
353 activity of matrix-bound ovotransferrin. Antimicrob. Agents Chemother. 21, 840-841.
- 354 Valenti, P., Antonini, G., Von Hunolstein, C., Visca, P., Orsi, N., Antonini, E., 1983.
355 Studies of the antimicrobial activity of ovotransferrin. Int. J. Tissue React. 5, 97-105.
- 356 Van Loock, M., Geens, T., De Smit, L., Nauwynck, H., Van Empel, P., Naylor, C.,
357 Hafez, H.M., Goddeeris, B.M., Vanrompay, D., 2005. Key role of *Chlamydophila psittaci*
358 on Belgian turkey farms in association with other respiratory pathogens. Vet. Microbiol.
359 107, 91-101.
- 360 Vanrompay, D., Ducatelle, R., Haesebrouck, F., 1992. Diagnosis of avian chlamydiosis:
361 specificity of the modified Giménez staining on smears and comparison of the sensitivity
362 of isolation in eggs and three different cell cultures. Zentralbl. Veterinarmed. B 39, 105-
363 112.
- 364 Vanrompay, D., Ducatelle, R., Haesebrouck, F., Hendrickx, W., 1993. Primary
365 pathogenicity of an European isolate of *Chlamydia psittaci* from turkey poults. Vet.
366 Microbiol. 38, 103-113.
- 367 Vanrompay, D., Mast, J., Ducatelle, R., Haesebrouck, F., Goddeeris, B., 1995. *Chlamydia*
368 *psittaci* in turkeys: pathogenesis of infections in avian serovars A, B and D. Vet.
369 Microbiol. 47, 245-256.
- 370 Vanrompay, D., Van Nerom, A., Ducatelle, R., Haesebrouck, F., 1994. Evaluation of five
371 immunoassays for detection of *Chlamydia psittaci* in cloacal and conjunctival specimens
372 from turkeys. J. Clin. Microbiol. 32, 1470-1474.

- 373 Williams, J., Elleman, T.C., Kingston, I.B., Wilkins, A.G., Kuhn, K.A., 1982. The
374 primary structure of hen ovomucoprotein. *Eur. J. Biochem.* 122, 297-303.
- 375 Xie, H., Huff, G.R., Huff, W.E., Balog, J.M., Rath, N.C., 2002. Effects of ovomucoprotein
376 on chicken macrophages and heterophil-granulocytes. *Dev. Comp. Immunol.* 26, 805-
377 815.
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382 **Tables**

383 Table 1

384 Scores for *C. psittaci* macroscopic lesions in turkeys

Tissue	Score 1	Score 2	Score 3
Conjunctivae	Slightly congested	Severely congested	Petechiae
Conchae	Slightly congested	Severely congested	Necrosis
Trachea	Slightly congested	Moderately congested	Severely congested
Lungs	Slightly congested	Severely congested	Grey foci
Thoracic airsacs	Diffuse opacity	Focal fibrin deposits	Severe fibrinous airsacculitis
Abdominal airsacs	Diffuse opacity	Few fibrin deposits	Severe fibrinous airsacculitis
Pericardium	Serous pericarditis	Sero-fibrinous pericarditis	Fibrinous adhesive pericarditis
Spleen	Slightly enlarged	Moderately enlarged	Severely enlarged
Liver	Slightly enlarged	Moderately enlarged	Severely enlarged

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390 Table 2

391 Clinical signs, macroscopic lesions and chlamydial excretion and replication in different turkey groups.

Group	Parameter	Day 3 p.i. ^e	Day 6 p.i.	Day 9 p.i.	Day 12 p.i.	Observed range
No OvoTF	Clinical signs ^a	3	6	5	6	3 - 6
	Macroscopic lesions ^b	2,50	6	10	10,50	2,50 - 10,50
	Pharyngeal excretion ^c	0,50	1,33	1,50	1	0,50 - 1,50
	Cloacal excretion ^c	0	0,67	1,25	1,50	0 - 1,50
	Replication ^d	5	12,50	11	12	5 - 12,50
Single Low Dose	Clinical signs	0	6	5	5	0 - 6
	Macroscopic lesions	0	4	7	7	0 - 7
	Pharyngeal excretion	0,88	1	1,25	1,50	0,88 - 1,50
	Cloacal excretion	0	1	0,75	1,50	0 - 1,50
	Replication	2,50	7	11	12	2,50 - 12
Multiple Low Dose	Clinical signs	0	0	0	0	0 - 0
	Macroscopic lesions	0,50	0,50	1	0	0 - 1
	Pharyngeal excretion	0,50	0,67	0	0	0 - 0,67
	Cloacal excretion	0	0	0,25	0	0 - 0,25
	Replication	1,50	0	2,50	3,50	0 - 3,50
High Dose	Clinical signs	0	0	1	3	0 - 3
	Macroscopic lesions	0	1	0,50	1,50	0 - 1,50
	Pharyngeal excretion	0,38	0,33	0,75	0,50	0,33 - 0,75
	Cloacal excretion	0	0	0,50	1	0 - 1
	Replication	0	2,50	3	1,50	0 - 3

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393 ^a Sum of all individual (n = 8) clinical scores per group on p.i. days 3, 6, 9 and 12.

394 ^b Macroscopic lesion scores (based on Table 1), presented as the sum of the mean lesion scores for all tissues examined for two turkeys per group, necropsied at
395 p.i. days 3, 6, 9 and 12.

396 ^c Swab culture scores for pharyngeal or cloacal excretion, presented as mean culture scores per group.

397 ^d Scores for chlamydial replication observed by DIF staining on tissue cryosections. Scores are presented as the sum of the mean scores for two turkeys per group
398 necropsied at p.i. days 3, 6, 9 and 12.

399 ^e p.i.: post infection.

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