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1 Retrospective study on transmissible viral proventriculitis and Chicken proventricular necrosis virus

2 (CPNV) in the UK

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24 **Abstract**

25 Chicken proventricular necrosis virus (CPNV) is a recently described birnavirus, which has been
26 proposed to be the cause of transmissible viral proventriculitis (TVP). The understanding of the
27 epidemiology of both the virus and the disease is very limited. A retrospective investigation on TVP
28 and CPNV in broiler chicken submissions from the UK from between 1994 and 2015 was performed
29 with the aims of assessing the longitudinal temporal evolution of TVP and CPNV and to review the
30 histological proventricular lesions in the studied chickens. Ninety-nine of the 135 included submissions
31 (73.3%) fulfilled the TVP-diagnostic criteria, while the remaining 36 submissions (26.7%) displayed only
32 lymphocytic proventriculitis (LP). The first detection of CPNV by PCR dated from 2009. Results showed
33 a rise in the number of both TVP and positive CPNV RT-PCR submissions from 2009 with a peak in
34 2013, suggesting that they may be an emerging or re-emerging disease and pathogen, respectively.
35 Twenty-two out of the 99 submissions displaying TVP lesions (22%) and 4 out of the 36 (11%) ones
36 with LP gave positive CPNV RT-PCR results, further supporting the association between CPNV and TVP
37 and confirming that CPNV is present in a low proportion of proventriculi that do not fulfil the TVP
38 diagnostic criteria. In addition, intranuclear inclusion bodies were observed in 22 of the submissions
39 with TVP. The vast majority of these cases (21 of 22, 96%) gave negative CPNV RT-PCR results, raising
40 the question of whether another virus different from CPNV is responsible for some of these TVP-
41 affected cases.

42 **Research highlights**

43 • TVP and CPNV are present in the British broilers since at least 1994 and 2009, respectively
44 • TVP and CPNV seem to be an emerging and re-emerging disease and pathogen, respectively
45 • CPNV was detected in proventriculi with both TVP and LP-lesions
46 • Other viruses different from CPNV may be responsible for some TVP-affected cases

47 **Keywords**: Birnavirus; Chicken proventricular necrosis virus (CPNV); transmissible viral proventriculitis
48 (TVP); natural infection; poultry; Retrospective study.

49 **Introduction**

50 Transmissible viral proventriculitis (TVP) typically affects broiler chickens and is characterized by
51 specific histological lesions, which include oxynticopeptic cell necrosis, lymphocytic inflammation and
52 ductal epithelial cell hyperplasia of the submucosal glands (Hafner & Guy, 2013). Because of the
53 lesions in the glandular stomach, affected birds suffer from maldigestion, poor feed conversion and
54 stunted growth (Dormitorio *et al.*, 2007). The aetiology of the disease has been debated since its first
55 description in 1978 (Kouwenhoven *et al.*, 1978). In 2011, Guy et al. described the detection of a new
56 birnavirus in field and experimentally reproduced cases affected by the disease, which they tentatively
57 named *Chicken proventricular necrosis virus* (CPNV) (Guy *et al.*, 2007; J. S. Guy *et al.*, 2011b). Later on,
58 few other works supported the association between CPNV and TVP ( Marguerie *et al.*, 2011; Noiva *et*
59 *al.*, 2015; Grau-Roma *et al.*, 2017).

60 Although it has been suggested to have a significant economic impact, the available information about
61 both TVP and CPNV is very limited (Dormitorio, et al., 2007; Hafner & Guy, 2013), and most of the TVP
62 reports are based on low number of cases or on experimental infections (Guy *et al.*, 2011b; Kim *et al.*,
63 2015; Noiva, et al., 2015). In the past few years, TVP has been reported to occur in several countries
64 from North and South America, Europe and Asia (Grau-Roma *et al.*, 2010; Guy, et al., 2011b;
65 Marguerie, et al., 2011; Kim, et al., 2015), suggesting for it to be an emerging or re-emerging disease.
66 A recent non-peer reviewed retrospective study performed in California broiler flocks shows that TVP
67 is a frequent disease (Hauck et al., 2016). Even though lesions compatible to TVP had been seen in the
68 UK since at least the 90s (Randall & Reece, 1996), the first peer-reviewed report describing the
69 presence of the disease in the UK is very recent (Grau-Roma, et al., 2017). The latter work reported a
70 strong association between the CPNV and the TVP-affected chickens, and detected the presence of
71 CPNV in a low number of birds affected by lymphocytic proventriculitis (LP), which lacked the necrosis
72 of oxynticopeptic cells and therefore did not fulfil all the TVP diagnostic criteria.

73 The understanding of the epidemiology of both the virus and the disease is scarce and, as far as the
74 authors are aware, there is no retrospective study on TVP and CPNV. The present work is a
75 retrospective investigation in broiler chicken submissions received by the Animal and Plant Health
76 Agency (APHA) Lasswade with the following aims: (i) to assess the longitudinal temporal evolution of
77 TVP, LP and CPNV in the studied British broiler chickens; (ii) To retrospectively review the
78 proventricular histological lesions in a high number of TVP-affected proventricular sections; (iii) to
79 assess the presence of CPNV in chickens affected by TVP or LP.

80 **Material and Methods**

81 **Study design.** A retrospective study in the archive of submissions received by the Animal and Plant
82 Health Agency (APHA) Lasswade was performed between the years 2000 and 2015. The study was
83 based on formalin-fixed, paraffin-embedded (FFPE) proventricular tissues of broiler chickens. All
84 submissions containing the term ´proventriculitis´ on the microscopic description and/or on the
85 morphologic diagnosis were selected. All histology slides were then re-examined by 1 Pathologist (LG)
86 and these showing lesions compatible with TVP or LP were selected. Submissions compatible with TVP
87 were those showing lesions of lymphocytic and necrotizing proventriculitis affecting the
88 proventricular glands within the submucosa, while submissions of LP showed lesions of lymphocytic
89 proventriculitis without oxynticopeptic cell necrosis (Grau-Roma, et al., 2017). In addition, a single
90 case archived from 1994, which corresponded to the case illustrated by Randall end Reece (1996), was
91 also included in the study. A total of 135 cases were identified. Each submission contained between 1
92 and 6 FFPE tissue blocks, with between 1 and 5 proventricular sections per block. Typically, each
93 section corresponded to a different bird.

94 **Histopathology.** Proventricular sections were histologically re-examined by 2 European College of
95 Veterinary Pathologists’ board-certified veterinary pathologists (SdB, LG), and they were all assessed
96 following a previously reported system with minor modifications (Grau-Roma et al., 2017). Briefly,
97 proventriculi were assessed for the following 3 histopathological lesions: i) glandular lymphocytic

98 infiltration; ii) glandular hyperplasia and metaplasia; iii) necrosis of oxynticopeptic cells. These
99 parameters were semi-quantified as follows: 0 (absence), 1 (>0% to 10% of the glands affected), 2
100 (>10–50% of the glands affected), and 3 (>50% of the glands affected). If only few (<10) small
101 multifocal follicular aggregates of lymphocytes or few (<10) small multifocal clusters of necrotic cells
102 were scattered through the proventricular glands, these parameters were graded as '1' even if they
103 were present in more than 10% of glands. Based on the histopathological results, each section was
104 classified as: i) TVP-affected chicken: lymphocytic infiltration and necrosis present in the
105 proventriculus; ii) LP-affected chicken: lymphocytic infiltration without necrosis present in the
106 proventriculus; iii) 'Without proventriculitis' (WP): neither lymphocytic infiltration nor necrosis
107 present in the proventriculus. Subsequently, those submissions with at least one section fulfilling the
108 TVP criteria were classified as such, while the remaining ones were classified as LP. No submissions
109 contained only WP sections. All sections were histologically analysed blindly, without knowing the
110 CPNV RT-PCR results.
111 **RNA extraction, RT-PCR, sequencing and phylogenetic analysis.** RNA was extracted from all the FFPE
112 proventriculi and subsequently tested by reverse transcriptase polymerase chain reaction (RT-PCR)
113 for CPNV. RNA extraction was done using four 25 µm-sections of each FFPE tissue following a
114 previously described protocol (Grau-Roma et al., 2017). A RT-PCR procedure was performed to amplify
115 a 171 nucleotide (nt) sequence within the VP1 gene of CPNV using primers and protocols described
116 previously (Guy et al., 2011b). RNA extracted material from FFPE tissues known to be positive for CPNV
117 from a previous study (Grau-Roma, et al., 2017) were used as positive controls.
118 For sequencing, the amplified products from the positive CPNV RT-PCR cases were purified using Mini
119 Elute Gel Extraction Kit (Qiagen, Valencia, CA, USA). Sequencing reactions were performed with ABI
120 Prism BigDye Terminator Cycle Sequencing v.31 Ready Reaction (Applied Biosystems, Foster City, CA,
121 USA), and analysed using an ABI Prism model 3730 automated sequencer (Applied Biosystems).
122 Positive and negative controls of extraction and amplification were added to each batch of samples
123 tested. Partial VP1 CPNV obtained sequences were compared with the previously reported CPNV

124 sequences using MEGA X (Molecular Evolutionary Genetics Analysis version X Center for Evolutionary
125 Medicine and Informatics, Biodesign Institute, Arizona, USA) software (Tamura et al., 2013).
126 Sequences were compared with the partial VP1 sequence of the American CPNV isolate R11/3 (Guy et
127 al., 2011a) available at Genbank under accession number HM038436.1 and 9 sequences from the
128 United Kingdom (Grau-Roma et al., 2017) available under Genbank accession numbers KU933595 to
129 KU933603. Sequences were aligned using muscle method. A nucleotide distance matrix between
130 sequences was computed to infer phylogenies and a neighbour-joining phylogenetic tree was
131 generated. The partial VP1 CPNV sequences reported in this work have been deposited at GenBank
132 under accession numbers MK531122 to MK531131.
133
134 **Statistical analyses.** IBM SPSS Statistics software (version 24.0, Armonk, NY: IBM Corp.) was used for
135 statistical analyses. The distribution of the age variable was assessed by the Shapiro-Wilk test. Mann-
136 Whitney test was used to compare the age between the histopathological (TVP and LP) and CPNV RT-
137 PCR categories of the studied submissions. Chi-square was used to compare the proportions of
138 histopathological lesions and of CPNV RT-PCR results between groups.
139
140 **Results**
141 Table 1 summarizes the number of submissions within each histopathological category, its age and
142 the CPNV RT-PCR results. One hundred and thirty-five submissions were identified. Ninety-nine of
143 them (73.3%) had at least 1 chicken with lesions compatible with TVP. The remaining 36 submissions
144 (26.7%) contained no TVP-affected proventricular sections but included at least 1 proventriculus with
145 LP lesions. Thirty-one out of the 135 submissions (23%) contained a mixture of TVP as well as LP
146 proventricular sections, while the remaining ones contained only either TVP or LP-affected
147 proventriculi. The youngest submission was 13 days old, corresponding to a TVP-affected case, while
148 the oldest one was 59 days old, corresponding to a LP-affected case. The mean±SD and range of age

149 within the TVP and LP groups was 28.2±8.0 (13 to 49) and 30.0±7.7 (15 to 56) days, respectively. No
150 statistically significant differences were found between the mean of age in each group (p=0.705).
151 Figure 1 depicts the yearly distribution of the total number of submissions as well as of CPNV RT-PCR
152 positive cases. Data shows less than 5 submissions per year until 2009, when there was a rise in the
153 total number as well as of CPNV RT-PCR positive submissions, reaching a peak in 2013. The earliest
154 CPNV RT-PCR positive submission dates from 2009, and the most recent one from 2015.

155 A total of 452 proventricular sections were assessed histologically. Two hundred and forty-eight of
156 them (54.9%) fulfilled the TVP diagnostic criteria, 166 (36.7%) were classified as LP and the remaining
157 38 (8.4%) showed no inflammation or necrosis and were classified as 'without proventriculitis' (WP)
158 (Grau-Roma, et al., 2017). Each submission contained between 1 and 17 proventricular sections. The
159 submissions consisted of formalin-fixed tissues with very variable, often limited, information about
160 the macroscopical findings. Therefore, no data about the macroscopic lesions of the proventriculi was
161 included in the study.

162 Table 2 summarizes the histopathological results in the 3 studied categories. Per definition, necrosis
163 of oxynticopeptic cells was only observed in the group of TVP-affected animals, and lymphocytic
164 infiltration was not present in the proventricular sections classified as WP. The proportion of lesional
165 grades of lymphocytic infiltration and tubular metaplasia/hyperplasia was significantly different
166 between the TVP and the LP-affected samples, with the TVP-affected proventricular sections showing
167 a higher proportion of moderate (2) and severe (3) lesions than in LP-affected ones in both categories
168 ($p<0.001$). Tubular metaplasia and hyperplasia was only present in 18% of the WP sections, and it was
169 graded as mild (1) in most (5 out of the 7 cases) of them.

170 The characteristic histological lesions of the TVP-affected proventricular sections were either
171 multifocal or diffuse, the former typically affecting several submucosal glands and sparing other
172 intermingled glands in between. In addition to the characteristic TVP-lesions, other histological lesions
173 were observed within the interstitium of the submucosal glands in both TVP and LP-affected

174 proventricular sections. These included the presence of oedema (in 10 out of the 99 submissions with
175 TVP and in 3 out of the 36 submissions with LP), which in few cases was accompanied with fibrin
176 deposition (in 4 and 1 of the submissions with TVP and LP, respectively), an increased number of
177 spindle cells (likely fibroblasts) with or without fibrosis (in 1 and 2 of the TVP and LP-affected cases,
178 respectively), and the presence of myxomatous stroma (2 TVP- and 2 LP-affected). No haemorrhages
179 were observed. Moreover, in 25 out of the 99 submissions with TVP (22%), low to moderate numbers
180 of oxynticopeptic cells within or adjacent to the areas of necrosis showed intranuclear structures
181 compatible to inclusion bodies, which were characterized by a pale eosinophilic centre and peripheral
182 margination of the chromatin (Figure 2). The inclusions were only observed in proventricular sections
183 with TVP-compatible lesions. No inclusions were observed in the submissions containing only LP-
184 affected proventricular sections.

185 Twenty-two out of the 99 submissions classified as TVP (22%) and 4 out of the 36 (11%) classified as
186 LP gave positive CPNV RT-PCR results, showing no statistically significant differences between groups
187 (p=0.148). The youngest CPNV positive case was 21 days old (a TVP-affected case), while the oldest
188 one was 56 days old (a LP-affected case). The oldest CPNV-positive TVP-affected submission was 49
189 days old. The mean of age of the CPNV RT-PCR positive submissions (32.8±9.6) was statistically higher
190 compared to the CPNV RT-PCR negative ones (27.7±7.2) (p=0.032). Within the positive CPNV RT-PCR
191 submissions, the mean±SD and range of age in the TVP and LP groups was 32.1±8.7 (21 to 49) and
192 36.0±14.5 (27 to 56) days, respectively. Within the negative CPNV RT-PCR submissions, the mean±SD
193 and range of age in the TVP and LP groups was 27.1±7.5 (13 to 49) and 29.2±6.3 (19 to 42), respectively.
194 No statistically significant differences were found between the mean of age of these groups. Only 1
195 out of the 25 TVP-submissions with intranuclear inclusions (4%) showed positive CPNV RT-PCR results,
196 making the proportion of positive CPNV RT-PCR submissions within the 'TVP-submissions without
197 inclusions' significantly higher [(21 out of 74, (28%)] than the one in the group of 'TVP-submissions
198 with intranuclear inclusions' (p=0.01). All the submissions with the additional above-described lesions
199 (oedema, fibrin, fibrosis and myxoid stroma) gave negative CPNV RT-PCR results.

200 A total of 10 sequences were obtained from the positive CPNV RT-PCRs. Phylogenetical analysis is
201 depicted in Figure 3, where two clear clusters are observed. One of them is composed by the UK
202 sequences from samples obtained in an study conducted in 2014-15 (Grau-Roma et al., 2017), the
203 American reference sequence and the four more recent sequences of the present study, obtained
204 from samples taken from 2013-2015. The other cluster includes sequences obtained from the oldest
205 samples of the present study, collected from 2009-2011. The British sequences obtained in the present
206 study showed a 78-100% nt similarity among them, whereas the percentage of similarity compared
207 with the reference CPNV American sequence ranges from 75-92%.

208 **Discussion**

209 As far as the authors are aware, this is the first retrospective study on both TVP and CPNV. The study
210 includes a TVP-affected case from 1994 and dates the first detection of CPNV back to 2009. This is five
211 years before its first detection in the UK, where CPNV-positive TVP-cases were reported in a single
212 prospective study from 2014 (Grau-Roma, et al., 2017), and 2 years before its first detection outside
213 the USA (Marguerie, et al., 2011). The work performed by Marusak et al. (2012) in the USA included
214 cases from between 2005 and 2009, without specifying the year of collection of the CPNV-positive
215 TVP cases. The other few works reporting CPNV detection in TVP field cases did not provide the year
216 of collection of the studied animals (Guy, et al., 2011b; Noiva, et al., 2015).

217 Present results show a rise in the number of TVP and positive CPNV RT-PCR submissions from 2009,
218 with a peak in 2013, which suggests that they may be an emerging or re-emerging disease and
219 pathogen, respectively. However, data about the total number of submissions to the APHA Lasswade
220 laboratory during the period of the study was not available and therefore an eventual increase in the
221 number of submissions, for example related to an increased awareness of the disease, cannot totally
222 be ruled out.

223 This study confirms the recent detection of CPNV not only in TVP-affected cases but also in LP-affected
224 cases (Grau-Roma, et al., 2017). As reported in the previous work, it seems likely for the group of LP-

225 affected cases to correspond to a mixture of cases with different aetiologies. Amongst them, a number
226 of LP-affected cases with negative CPNV RT-PCR results may correspond to chronically affected TVP
227 cases, where the virus is not detectable (Guy, et al., 2011b). In addition, cases with only multifocal
228 lymphoid aggregates in the proventricular glands are considered to be 'normal findings' (Fletcher &
229 Riddell, 2008), which may be therefore the case of some LP-cases showing only mild follicular
230 aggregates. The latter may also explain the lower proportion of histopathological scores 2 and 3 in the
231 group of LP-affected cases compared to the TVP-affected ones.

232 VP1 Partial sequencing of the CPNV RT-PCR positive samples showed differences in terms of nt
233 percentage similarity and clustering. A temporal distribution was observed, so that "old" UK strains
234 (from 2009-2012) clustered together and "new" (from 2013 onwards) made another branch together
235 with the reference American strain. The previously suggested geographical different lineages between
236 the European CPNV and the American CPNV sequences was not observed in the present study (Grau-
237 Roma et al., 2017). A larger study including more and larger sequences would be needed in order to
238 make further conclusions about the spatiotemporal evolution of this virus.

239 The here presented 22% of CPNV RT-PCR detection within the submissions showing TVP is lower than
240 the 47% reported in the previous prospective study (Grau-Roma, et al., 2017). In both studies, the
241 CPNV detection was performed in FFPE blocks following similar protocols of RNA extraction and RT-
242 PCR. As the cases are derived from field material, the post-mortem examination procedure, tissue
243 handling, type of fixative used as well as the length of fixation and storage were not standardised and
244 are likely to have reduced the sensitivity of the RT-PCR (Lewis et al., 2001). This may account for some
245 of the differences in these results (Lewis et al., 2001). In any case, the number of TVP cases or
246 submissions with negative CPNV RT-PCR results is relevant in both studies (53% in Grau-Roma et al.,
247 (2017) and 78% in the present one). Certainly, TVP cases with negative CPNV RT-PCR results may be
248 due to chronic stages of the disease or to the reduced RT-PCR sensitivity on formalin-fixed paraffin-
249 embedded tissues. However, given the relatively high number of them, it seems reasonable to also

250 consider the possibility that other pathogens different from CPNV may cause similar proventricular
251 lesions to the ones attributed to CPNV. Indeed, the number of pathogen factors that have over the
252 years been suggested as cause of TVP is broad, and includes several viruses, bacteria, fungi, or
253 parasites (Dormitorio, et al., 2007). In this regards, in addition to the characteristic TVP histological
254 lesions, we observed the presence of other proventricular lesions. These included oedema, fibrin
255 deposition, spindle cell proliferation and a myxomatous stroma, all of which gave negative CPNV RT-
256 PCR results. No haemorrhages, which were previously described in some TVP-affected cases with
257 positive CPNV RT-PCR results (Grau-Roma, et al., 2017), were observed in this study. Interestingly,
258 intranuclear inclusion bodies were observed in 22% of the TVP cases. The inclusions were similar to
259 the viral inclusions occasionally described in other works (Goodwin *et al.*, 1996; Grau-Roma, et al.,
260 2010). Certainly, the number of structures within each case was often not abundant, and some may
261 be non-viral, alternatively corresponding to degenerative features associated to the areas of necrosis.
262 Nevertheless, the fact that the vast majority of submissions with intranuclear inclusions gave negative
263 CPNV RT-PCR results (all but 1) contrasted with a CPNV RT-PCR positivity of more than one fourth
264 (28%) within the group of submissions with TVP which did not show intranuclear inclusions. All
265 together raises the question of whether another virus, different from the CPNV, is related to some of
266 these TVP-affected cases. On this line, several other virus including adenovirus (Goodwin, et al., 1996),
267 Gyrovirus (Li *et al.*, 2018) and picornavirus (Kim, et al., 2015), have been reported to be associated
268 with TVP, although none of them could be definitively proved as cause of TVP. The investigation of
269 such potential different aetiologies was however beyond the aims of the current work, and further
270 studies are needed to get further insights in this area.

271 In summary, the present retrospective study indicates that TVP and CPNV are present in the British
272 broilers since at least 1994 and 2009, respectively. Both the virus and the disease seem to have
273 emerged or re-emerged in the recent years. CPNV was detected in a significant number of submissons
274 with TVP, providing further evidence for this virus to be cause of TVP.

275 **Disclosure statement**

276 No potential conflict of interest was reported by the authors.

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295 **Table 1**. Number of submissions classified as transmissible viral proventriculitis (TVP) and lymphocytic

296 proventriculitis (LP) as well as showing positive *Chicken proventricular necrosis virus* (CPNV) RT-PCR

297 results.

| | Number and percentage of submissions | Age (days, mean±SD) | Positive CPNV RT-PCR (%) |
|---|---|---|---|
| TVP | 99 (73.3%) | 28.2±8.0 | 22 (22%) |
| LP | 36 (26.7%) | 30.0±7.7 | 4 (11%) |
| Total | 135 | 28.7±7.9 | 26 (20%) |

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**Table 2**. Histopathological scoring results in the 3 histologically established categories: transmissible viral proventriculitis (TVP), lymphocytic proventriculitis (LP) and without proventriculitis (WP). The values correspond to the studied proventricular sections and the percentage is given within each category.

| | Score | TVP | LP | WP |
|---|---|---|---|---|
| Oxynticopeptic cell necrosis | 0 | 0 | 166 (100%) | 38 (100%) |
| | 1 | 132 (53%) | 0 | 0 |
| | 2 | 77 (31%) | 0 | 0 |
| | 3 | 39 (16%) | 0 | 0 |
| Lymphocytic infiltration | 0 | 0 | 0 | 38 (100%) |
| | 1 | 15 (6%) | 63 (38%) | 0 |
| | 2 | 73 (29%) | 67 (40%) | 0 |
| | 3 | 160 (65%) | 36 (22%) | 0 |
| Tubular metaplasia and hyperplasia | 0 | 4 (2%) | 22 (13%) | 31 (82%) |
| | 1 | 26 (10%) | 26 (16%) | 5 (13%) |
| | 2 | 62 (25%) | 46 (28%) | 0 (0%) |
| | 3 | 156 (63%) | 72 (43%) | 2 (5%) |

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360 **References**


361 Dormitorio, T.V., Giambrone, J.J. & Hoerr, F.J. (2007). Transmissible proventriculitis in broilers. *Avian*
362 *Pathology,* 36, 87-91.
363 Fletcher, O.J. & Riddell, C. (2008). Avian histopathology. *American Association of Avian Pathologists.*
364 *3rd edn (pp.* 25-36)*.* Omni Press, Madison, WI 53704.
365 Goodwin, M.A., Hafner, S., Bounous, D.I., Latimer, K.S., Player, E.C., Niagro, F.D., et al. (1996). Viral
366 proventriculitis in chickens. *Avian Pathology,* 25, 369-379.
367 Grau-Roma, L., Marco, A., Martinez, J., Chaves, A., Dolz, R. & Majo, N. (2010). Infectious bursal disease-
368 like virus in cases of transmissible viral proventriculitis. *The Veterinary Record,* 167, 836.
369 Grau-Roma, L., Reid, K., de Brot, S., Jennison, R., Barrow, P., Sanchez, R., et al. (2017). Detection of
370 transmissible viral proventriculitis and chicken proventricular necrosis virus in the UK. *Avian*
371 *Pathology,* 46, 68-75.
372 Guy, J.S., Smith, L.G., Evans, M.E. & Barnes, H.J. (2007). Experimental reproduction of transmissible
373 viral proventriculitis by infection of chickens with a novel adenovirus-like virus (isolate R11/3).
374 *Avian Diseases,* 51, 58-65.
375 Guy, J.S., West, A.M. & Fuller, F.J. (2011a). Physical and genomic characteristics identify chicken
376 proventricular necrosis virus (R11/3 virus) as a novel birnavirus. *Avian Diseases,* 55, 2-7.
377 Guy, J.S., West, M.A., Fuller, F.J., Marusak, R.A., Shivaprasad, H.L., Davis, J.L., et al. (2011b). Detection
378 of chicken proventricular necrosis virus (R11/3 virus) in experimental and naturally occurring
379 cases of transmissible viral proventriculitis with the use of a reverse transcriptase-PCR
380 procedure. *Avian Diseases,* 55, 70-75.
381 Hafner, S. & Guy, J.S. (2013). Proventriculitis and proventricular dilatation of broiler chickens. *In:*
382 *Diseases of Poultry, 13th Edition, Swayne, D. E., Glisson, J. R., McDouglald, L. R., Nolan, L. K.,*
383 *Suarez, D. L. and Nair, V. L. ,* Eds. Ames, USA: Wiley-Blackwell Publishing, 1328-1332.
384 Hauck R, Senties-Cue G, Stoute S, Carnaccini S, Chin R P, Noiva R, Gallardo R A, Shivaprasad H L (2016).
385 Retrospective study of transmissible viral proventriculitis in broiler chickens in central
386 California: 2000-2014. *In*: *Proceedings of the 65th Western Poultry Disease Conference,*
387 *Vancouver, Canada,* 99-100.
388 Kim, H.-R., Yoon, S.-J., Lee, H.-S. & Kwon, Y.-K. (2015). Identification of a picornavirus from chickens
389 with transmissible viral proventriculitis using metagenomic analysis. *Archives of Virology,* 160,
390 701-709.
391 Kouwenhoven, B., Davelaar, F.G. & Van Walsum, J. (1978). Infectious proventriculitis causing runting
392 in broilers. *Avian Pathology*, 183-187.
393 Lewis, F., Maughan, N.J., Smith, V., Hillan, K., Quirke, P. (2001). Unlocking the archive--gene expression
394 in paraffin-embedded tissue. *The Journal of Pathology*, 195, 66-71.
395 Li, G., Yuan, S., He, M., Zhao, M., Hao, X., Song, M., et al. (2018). Emergence of gyrovirus 3 in
396 commercial broiler chickens with transmissible viral proventriculitis. *Transboundary Emerging*
397 *Diseases,* 65, 1170-1174.
398 Marguerie, J., Leon, O., Albaric, O., Guy, J.S. & Guerin, J.-L. (2011). Birnavirus-associated
399 proventriculitis in French broiler chickens. *The Veterinary Record,* 169, 394-396.
400 Marusak, R.A., West, M.A., Davis, J.F., Fletcher, O.J. & Guy, J.S. (2012). Transmissible viral
401 proventriculitis identified in broiler breeder and layer hens. *Avian Diseases,* 56, 757-759.
402 Noiva, R., Guy, J.S., Hauck, R. & Shivaprasad, H.L. (2015). Runting Stunting Syndrome Associated with
403 Transmissible Viral Proventriculitis in Broiler Chickens. *Avian Diseases,* 59, 384-387.
404 Randall, C.R. & Reece, R.L. (1996). Color atlas of avian histopathology. 1$^{st}$ edn (p. 57). *Mosby-Wolfe,*
405 *imprint Times Mirror International Publishers Limited, London, UK*.
406 Tamura, K., Stecher, G., Peterson, D., Filipski, A., Kumar, S. (2013). MEGA6: Molecular Evolutionary
407 Genetics Analysis version 6.0. Molecular Biology and Evolution, 30, 2725-2729.

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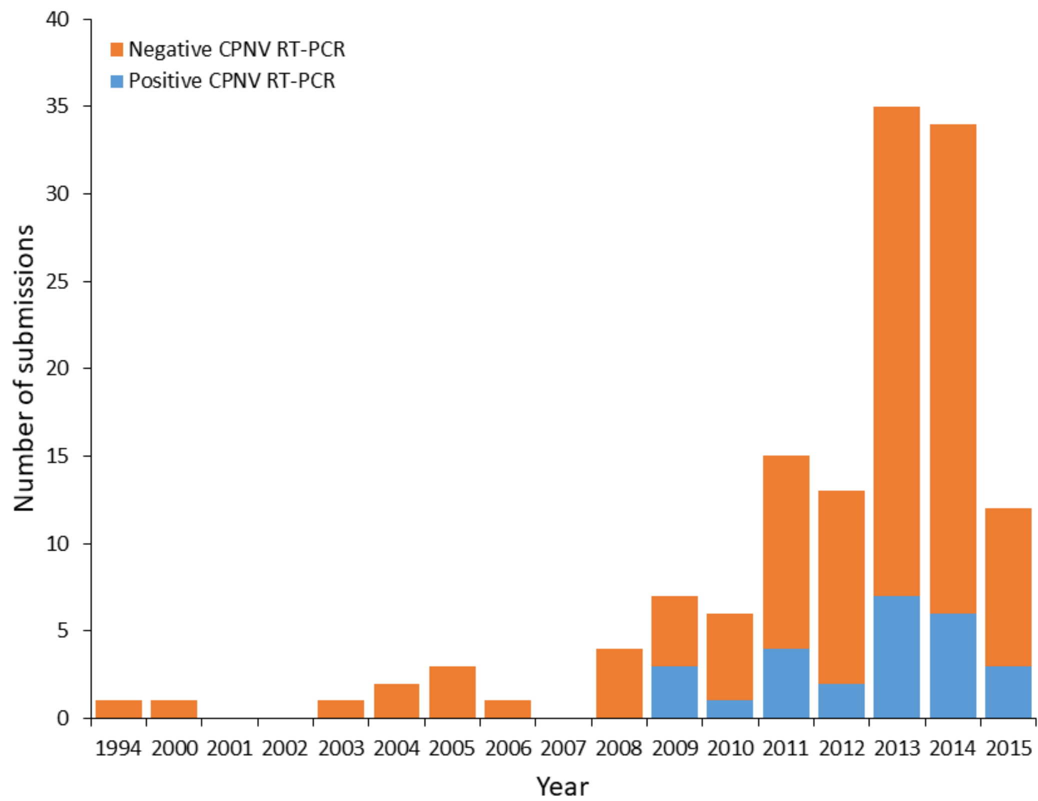


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413 Figure 1. Number of submissions included in the study as well as number of positive Chicken
414 proventricular necrosis virus (CPNV) RT-PCR per year.
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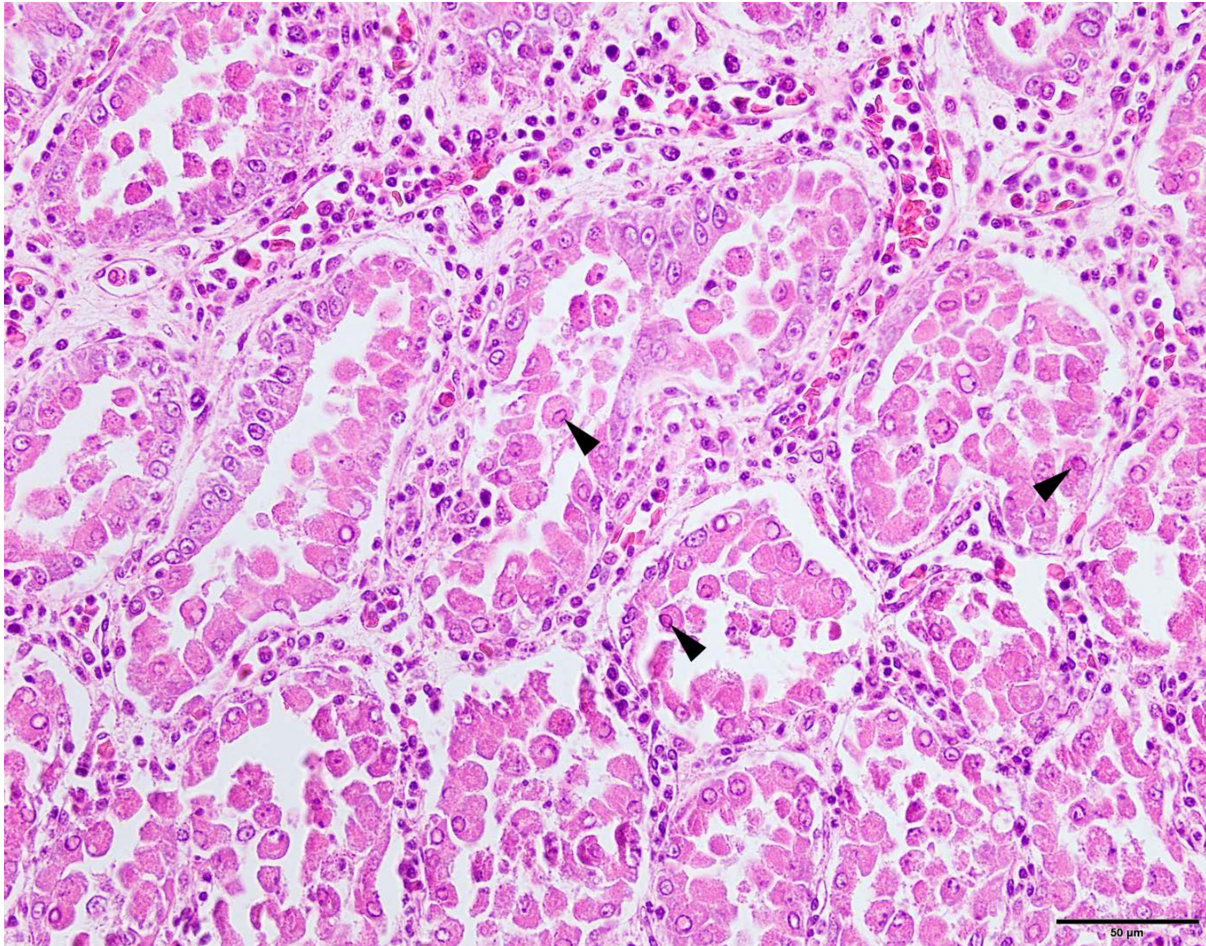

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420 Figure 2. Proventriculus, transmissible viral proventriculitis-affected broiler chicken.
421 Photomicrograph showing the presence of moderate numbers of pale eosinophilic intranuclear
422 inclusion bodies with marginated chromatin in oxynticopeptic cells within areas of glandular necrosis
423 (arrowheads). Haematoxylin and eosin.

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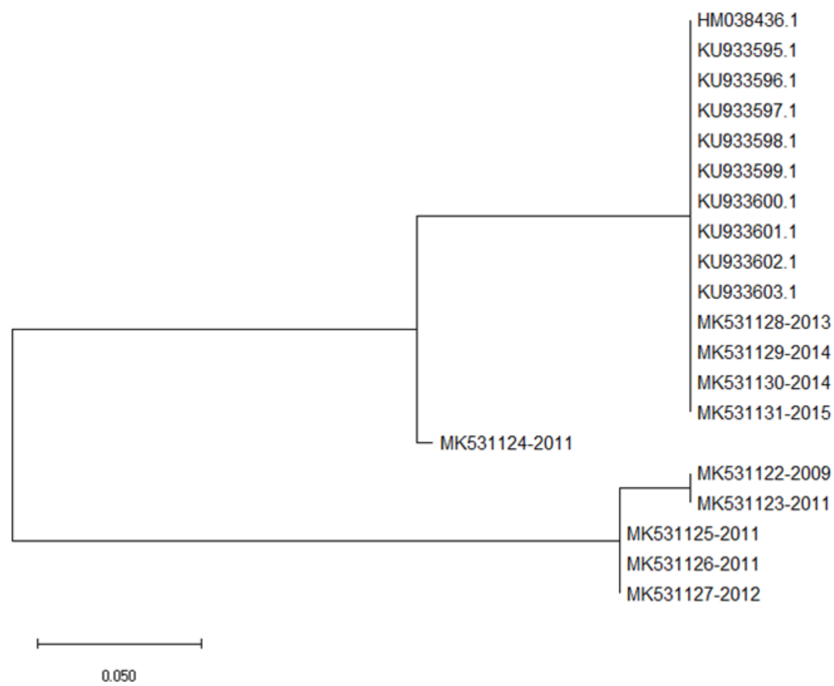


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431 Figure 3. Phylogenetic tree-based on the neighbour-joining method for 20 partial (171 nt) VP1 CPNV

432 sequences. Sequences originate from: USA (HM038436.1), a previous study on UK samples (Grau-
433 Roma et al., 2017) (KU933595 to KU933603) and samples from the present study (MK531122 to
434 MK531131).

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