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# SWINE TORQUE TENO VIRUS (TTV) INFECTION AND EXCRETION DYNAMICS IN CONVENTIONAL PIG FARMS

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1           **SWINE TORQUE TENO VIRUS (TTV) INFECTION AND EXCRETION**

2                           **DYNAMICS IN CONVENTIONAL PIG FARMS**

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13

14 **Abstract:**

15 *Torque teno virus* (TTV) is a non-enveloped, single stranded DNA (ssDNA)  
16 virus infecting human and non-primate species. Two genogroups of TTV (TTV1 and  
17 TTV2) have been described in swine so far. In the present study, TTV1 and TTV2  
18 prevalences in serum, and nasal as well as rectal swabs of 55 randomly selected piglets  
19 from seven Spanish multi-site farms, were monitored from 1 to 15 weeks of age. Also,  
20 blood from their dams (n=41) were taken at 1 week post-farrowing. Samples were  
21 tested by means of two TTV genogroup specific PCRs. Although prevalence of TTV1  
22 and TTV2 in sows was relatively high (54% and 32%, respectively), it was not directly  
23 associated to their prevalence in the offspring. Percentage of viremic pigs for both TTV  
24 genogroups followed similar dynamics, increasing progressively over time, with the  
25 highest rate of detection at 11 weeks of age for TTV1 and at 15 weeks for TTV2. Forty-  
26 two (76%) and 33 (60%) of the 55 studied pigs were TTV1 and TTV2 PCR positive in  
27 serum, respectively, in more than one sampling time. TTV1 and TTV2 viremia lasted in  
28 a number of animals up to 15 and 8 weeks, respectively. Co-infection with both TTV  
29 genogroups in serum was detected at all sampling points, but at 1 week of age. On the  
30 contrary, there were animals PCR negative to both genogroups in serum at all sampling  
31 times but at 15 weeks of age. During the study period, TTV1 and TTV2 nasal shedding  
32 increased also over time and faecal excretion was intermittent and of low percentage  
33 (<20%). In conclusion, the present study describes for the first time the infection  
34 dynamics of TTV1 and TTV2 as well as the nasal and faecal excretion throughout the  
35 life of in pigs from conventional, multi-site farms. Moreover, results indicate that both  
36 swine TTV genogroups are able to establish persistent infections in a number of pigs.

37 *Keywords: Torque teno virus (TTV), pig, infection dynamics, serum, nasal swab, rectal*  
38 *swab*

## 39 1. Introduction

40 *Torque teno virus* (TTV) is a non-enveloped, circular, single-stranded DNA  
41 (ssDNA) virus that infects human, non-primate and domestic species, including swine  
42 (Kekarainen and Segalés, 2009). In humans, TTV infection is ubiquitous and several  
43 genogroups have been identified (Bendinelli et al., 2001). However, up to now, a  
44 definitive proof linking infection in humans and a specific disease is missing (Jelicic et  
45 al., 2004; Peng et al., 2002; Maggi et al., 2003). Frequency of human TTV detection in  
46 serum is very variable between countries and increases with age (Komatsu et al., 2004;  
47 Saback et al., 1999). Detection of the virus in serum of mother-to-child pairs, cord  
48 blood and breast milk has led to think that it can be transmitted vertically (Komatsu et  
49 al., 2004). Moreover, its detection in saliva, nasal secretions and faeces has suggested  
50 that it also spreads horizontally (Bendinelli et al., 2001).

51 In swine, two species-specific TTV genogroups (TTV1 and TTV2) have been so far  
52 described (Niel et al., 2005). Swine TTVs are ubiquitous and considered non-  
53 pathogenic. Nevertheless, a relation between TTV infection and postweaning  
54 multisystemic wasting syndrome (PMWS) development has been suggested. On one  
55 hand, Kekarainen et al. (2006) found a higher TTV2 prevalence in PMWS-affected pigs  
56 than in healthy ones. On the other hand, Ellis et al. (2008) reported that a TTV1  
57 inoculation prior to porcine circovirus type 2 (PCV2) infection in gnotobiotic pigs  
58 facilitated PMWS development. Furthermore, co-infection of TTV1 with porcine  
59 reproductive and respiratory syndrome virus (PRRSV) has been linked with the  
60 development of a porcine dermatitis and nephropathy syndrome-like condition in  
61 gnotobiotic pigs (Krakowka et al., 2008).

62 To date, TTV1 has been detected by PCR in sera from pigs from different  
63 countries including Canada, China, France, Italy, Spain, Thailand and USA, with

64 prevalences ranging from 24% to 100% (Bigarré et al., 2005; Kekarainen et al., 2006;  
65 Martelli et al., 2006; McKeown et al., 2004). On the contrary, presence of TTV2 DNA  
66 in sera has only been described in two countries, Brazil (Niel et al., 2005) and Spain  
67 (Kekarainen et al., 2006). Apart from serum, swine TTVs have been also detected by  
68 PCR in plasma, semen, faeces and colostrum (Brassard et al., 2008; Kekarainen et al.,  
69 2007; Martínez-Guinó et al., 2009).

70 Little information on epidemiology of TTV infection in pig herds is nowadays  
71 available. In Italy, number of TTV1 infected pigs in farms was very variable, ranging  
72 from 0 to 53% but was not related to herd size, sanitary status or biosecurity procedures  
73 (Martelli et al., 2006). TTV infections occur early in the production system and may be  
74 transmitted from sow-to-piglet or from piglet-to-piglet in the farrowing crates (Sibila et  
75 al., 2009). Besides, both swine TTVs have been detected in colostrum samples and in  
76 stillborns indicating vertical route of transmission (Martinez-Guinó et al., 2009).  
77 However, dynamics of infection and excretion of swine TTVs throughout the  
78 productive life of pigs is still unknown. Therefore, the purpose of this work was to study  
79 the infection and excretion evolution of TTV1 and TTV2 in healthy, naturally infected  
80 animals under field conditions between 1 and 15 weeks of age.

81

## 82 2. Materials and methods

### 83 2.1. Samples

84 Eleven batches from seven Spanish multi-site farms were included in this study  
85 (Table 1). Five healthy piglets randomly selected per farm (n=55) were monitored from  
86 1 to 15 weeks of age. The piglets came from a total of 41 sows (3 to 5 sows/farm).  
87 Blood (Vacutainer, Becton-Dickinson, Meylan Cedex, France) samples as well as nasal  
88 and rectal swabs (Collection swab, Eurotubo, Rubí, Spain) from all piglets were taken at

89 1, 3, 7, 11 and 15 weeks of age (but from 2 pigs from farm 7 and one from farm 6 the  
90 sample at 15 weeks of age was missing). Blood from sows was collected at 1 week post-  
91 farrowing (same day as piglet sampling at 1 week of age). Samples were individually  
92 identified and transported in refrigeration to the laboratory. Pigs were weaned at 3  
93 weeks of age and moved to fattening units at 8-9 weeks of age. Treatments, housing and  
94 husbandry conditions conformed to the European Union Guidelines and Good Clinical  
95 Practices.

## 96 **2.2. Swine TTV1 and TTV2 PCR methods**

97 Blood samples were centrifuged at 1500 g for 10 minutes at 4°C, and obtained  
98 sera were stored at -80°C until processing. Nasal and rectal swabs were suspended in 1  
99 ml of sterile PBS and vigorously vortexed; swab suspensions were stored at -80°C until  
100 further processing. To minimize the risk of contaminations, each stage of the PCR  
101 process (DNA extraction, DNA amplification and electrophoresis) was carried out in  
102 separated rooms. DNA was extracted from 200 µl of serum samples and from 300 µl of  
103 nasal swab suspensions according to manufacturer's instructions (Nucleospin® Blood,  
104 Macherey-Nagel GmbH & Co KG Düren, Germany). DNA from rectal 300 µl of swabs  
105 was extracted according to manufacturer's instructions (QIAamp DNA Stool mini kit,  
106 QIAGEN GmbH, Germany). To assess for potential contaminations during the  
107 extraction procedure, a negative control was included using PBS as extraction substrate  
108 in each group of extracted samples. Presence of TTV1 and TTV2 DNA was assessed  
109 with two previously described specific polymerase chain reaction (PCR) methods  
110 (Segalés et al., 2009). A PCR positive serum to TTV1 but PCR negative to TTV2  
111 served as positive control for the TTV1 PCR and a PCR positive serum to TTV2 but  
112 PCR negative to TTV1 was used as positive control for the TTV2 PCR. The negative  
113 controls used in the PCR methods consisted of distilled water instead of extracted DNA

114 and they were included every fourth samples. The sensitivity of both tests is  $3.3 \times 10^3$   
115 molecules/ml equal to 6 molecules/reaction (data not shown).

116 The amplified products (305 bp for TTV1 and 250 bp for TTV2) were run in a  
117 2% agarose gel with 0.05 mg/ml of ethidium bromide.

### 118 **2.3. Statistical analysis**

119 Sows were grouped by parity into two different groups: young (from 1<sup>st</sup> to 4<sup>th</sup>  
120 parity) (n=34) and old (from 5<sup>th</sup> to 10<sup>th</sup>) (n=7) parity sows (Sibila et al., 2007). Bivariate  
121 analyses using contingency tables were used to compare 1) prevalence of both TTV  
122 genogroups in serum between sows and piglets at 1 week of age, 2) prevalence of both  
123 genogroups in sows between different parity groups and 3) prevalence of both  
124 genogroups in sows between different production systems. Mean prevalence of both  
125 TTVs in serum, nasal and rectal swabs at the different sampling was compared using a  
126 T-student test. Moreover, a GENMOD procedure was used to analyse the influence of  
127 the age on the prevalence of TTV1 and TTV2 in the different samples tested. Statistical  
128 analyses were performed with SAS system for Windows version 8.0 (SAS Institute Inc,  
129 Cary, North Carolina, USA). Statistical significance level was set at  $\alpha=0.05$ .

## 130 **3. Results**

### 131 **3.1. TTV1 and TTV2 PCR detection in serum of sows**

132 TTV1 and TTV2 were detected in sera of sows from all batches and farms  
133 analyzed. No significant differences between farms and batches regarding TTV1 and  
134 TTV2 prevalence in sows were found ( $p>0.05$ ). Overall, from the 41 tested sows, 22  
135 (54%) and 13 (32%) were TTV1 and TTV2 PCR positive, respectively ( $p>0.05$ ) (Table  
136 2). Eight (19.5%) sows were co-infected with both genogroups and 14 (34%) sows were  
137 negative to both TTVs. No association between TTV1 and TTV2 detection in sows was  
138 found ( $p>0.05$ ).



139           Regarding the production system, a higher number of TTV1 positive sows was  
140 found in 3-sites farms (16/23, 69.6%) compared to 2-site ones (6/18, 33.3%) ( $p<0.05$ ).  
141 On the contrary, no significant differences between TTV2 PCR positive sows between  
142 3- (5/23, 21.7%) and 2- (8/18, 44.4%) site farms were observed ( $p>0.05$ ).

143           From the 34 younger sows (parity 1<sup>st</sup> to 4<sup>th</sup>), 19 and 12 were TTV1 and TTV2  
144 PCR positive, respectively. From the 7 older sows (5<sup>th</sup> to 10<sup>th</sup> part), 3 were TTV1 PCR  
145 positive and only 1 was TTV2 PCR positive. No statistically significant differences  
146 between prevalence of TTV genogroups among different parity groups were found.

### 147           3.2. *TTV1 and TTV2 PCR detection in piglets*

148           TTV1 and TTV2 were detected in serum, nasal and/or rectal swabs of piglets  
149 from all studied batches and farms studied. No statistically significant differences on  
150 TTV1 and TTV2 prevalences in any of the sample type between piglets of different  
151 batches and farms were observed. Results of PCR detection in serum, nasal and rectal  
152 swabs are globally represented in figures 1, 2 and 3, respectively.

#### 153           3.2.1. *TTV1 and TTV2 PCR detection in serum of piglets*

154           Detection of TTV genogroups in serum increased with age (Figure 1). The  
155 lowest percentage of PCR detection was found in 1 week-old piglets (6/55, 11% for  
156 TTV1 and 5/55, 9% for TTV2) and the highest one at 11 weeks of age for TTV1 (42/55,  
157 76%) and at 15 weeks of age for TTV2 (34/52, 65%). TTV1 was detected to higher  
158 percentages than TTV2 at all sampling ages, being significantly different ( $p<0.05$ ) at 3,  
159 7 and 11 weeks of age (Figure 1).

160           From the 55 analyzed pigs, 3 animals (5.5%) were TTV1 PCR positive in serum  
161 at 5 different sampling times while only 2 pigs (3.6%) remained PCR negative in serum  
162 throughout the study. On the other hand, 42 out of the 55 animals (76%) were positive

163 to TTV1 in more than one sampling; 35 out of the 42 pigs (83%) had consecutive PCR  
164 positive results. The mean number of TTV1 PCR positive sera samples per animal was  
165 2.52 ( $\pm 1.21$ ).

166 Regarding TTV2, none of the tested pigs was TTV2 PCR positive at all ages,  
167 while 8 piglets remained PCR negative in serum throughout the study. On the contrary,  
168 33 pigs (60%) were TTV2 PCR positive in serum in more than one sampling; 27 out of  
169 the 33 pigs (82%) had consecutive PCR positive results. The mean number of TTV2  
170 PCR positive sera samples per animal was 1.72 ( $\pm 1.26$ ).

171 Co-infection with TTV genogroups in serum was detected at all sampling points  
172 except at 1 week of age. On the contrary, there were PCR negative pigs to both TTV  
173 genogroups in serum at all sampling times except at 15 weeks of age.

### 174 ***3.2.2. Relationship between TTV1 and TTV2 PCR detection in serum of sows*** 175 ***and 1 week-old piglets***

176 No relationship between swine TTV infection in sows and their piglets in serum  
177 at 1 week of age was found ( $p > 0.05$ ). In fact, from the 6 TTV1 PCR positive pigs at that  
178 age, only one came from a TTV1 PCR positive sow. Similar results were obtained for  
179 TTV2, since from the 5 TTV2 PCR positive 1 week-old piglets, only one came from a  
180 TTV2 PCR positive sow.

### 181 ***3.2.3. TTV1 and TTV2 PCR detection in nasal swabs***

182 Like in serum, percentage of TTV1 and/or TTV2 PCR positive pigs in nasal  
183 swabs increased significantly ( $p < 0.05$ ) with age (Figure 2). Percentage of PCR  
184 detection of TTV1 from 1 to 7 weeks of age was higher than TTV2. Afterwards, the  
185 proportion of infected pigs changed, and percentage of TTV2 PCR positive pigs was  
186 higher than TTV1. Differences in TTV prevalences in nasal cavity were significant only

187 at 11 and 15 weeks of age ( $p < 0.05$ ). Significant differences in percentage within TTV1  
188 and TTV2 PCR detection in nasal swabs throughout the study are represented in figure  
189 2.

190 Most of the animals studied were either TTV1 PCR negative (21/55, 38%) or  
191 positive at only one sampling time (18/55, 33%). The mean number of TTV1 PCR  
192 positive nasal swabs per animal was 1.1 ( $\pm 1.2$ ). Similar results were observed for TTV2,  
193 with 15 (27.2%) TTV2 PCR negative pigs and 17 (31%) TTV2 PCR positive pigs on a  
194 single sample. The mean number of TTV2 PCR positive nasal swabs per animal was  
195 1.34 ( $\pm 1.12$ ).

196 Co-infections with both TTV genogroups in nasal cavity were observed at all  
197 sampling times but at 1 and 3 weeks of age. On the contrary, there were PCR negative  
198 pigs to both genogroups in nasal swabs at all samplings.

#### 199 **3.2.4. TTV1 and TTV2 PCR detection in rectal swabs**

200 In rectal swabs, overall detection of TTV1 and TTV2 was intermittent and of  
201 low percentage ( $< 20\%$ ) (Figure 3). Percentage of TTV1 positive pigs was fairly  
202 constant throughout the study, while the percentage of TTV2 positive pigs increased  
203 progressively from 7 weeks of age onwards. Significant differences between TTV1 and  
204 TTV2 prevalence in rectal swabs were found at 3 and 7 weeks of age.

205 Most of the animals studied were TTV1 PCR negative (27/55, 41%) or TTV  
206 PCR positive at only one sampling time (22/55, 40%). The mean number of TTV1 PCR  
207 positive rectal swabs per animal was 0.69 ( $\pm 0.9$ ). Similar results were observed for  
208 TTV2, with 40 (73%) TTV2 PCR negative pigs and 14 (26%) TTV2 PCR positive pigs  
209 on a single sample. The mean number of positive TTV2 PCR positive rectal swabs per  
210 animal was 0.29 ( $\pm 0.49$ ).

211 Co-infections with both TTV genogroups in faeces were observed at all  
212 sampling times except at 1 and 3 weeks of age. On the contrary, there were PCR  
213 negative pigs to both genogroups in rectal swabs at all samplings

#### 214 **4. Discussion**

215 In the present study, swine TTV genogroups were detected in serum of sows and  
216 pigs as well as in nasal cavity and faeces of pigs from seven Spanish farms. As far as  
217 the authors know, this is the first longitudinal study in which TTV viremia and shedding  
218 throughout the life of pigs have been investigated. Both swine TTV genogroups were  
219 present in the eleven batches of the seven studied multi-site farms confirming that swine  
220 TTV infections are ubiquitous in the pig population (Kekarainen et al., 2006; Martelli et  
221 al., 2006; McKeown et al., 2004; Segalés et al., 2009).

222 Prevalence of swine TTV in sows was relatively high as indicated in other  
223 studies (Martínez-Guinó et al., 2009; Martínez et al., 2006; Segalés et al., 2009).  
224 Nevertheless, detection was not directly associated to the prevalence in the offspring, as  
225 recently described (Sibila et al., 2009). Although prevalence of TTV1 and TTV2  
226 infection was higher in young sows, a lack of correlation between TTVs infection and  
227 parity distribution has been described (Sibila et al., 2009). Such result was expectable  
228 also in the present study, since serum samples from a number of sows (n=25) and  
229 piglets (n=27) tested by Sibila et al., (2009) were also included in the present  
230 longitudinal work. As reported, transmission from sow-to-piglet but also from piglet-to-  
231 piglet could explain the existence of TTV viremic piglets in farrowing crates coming  
232 from TTV non-viremic sows (Sibila et al., 2009).

233 In the present study, prevalence of TTV1 infected sows was higher in 3-site than  
234 2-site production systems, while such effect was not observed for TTV2 infection. The  
235 higher prevalence of TTV1 in 3-sites production systems is in accordance with Martelli

236 et al., (2006), who described a higher TTV1 prevalence in finishing (40%) than in  
237 farrow-to-finish (11%) herds. However, this putative difference in TTV1 prevalence  
238 according to the production system should be taken with caution since it can be a  
239 spurious effect due to the low number of 2-sites (n=2) and 3-sites (n=5) production  
240 systems included in the current study.

241 Percentage of viremic pigs, for both TTV genogroups, increased progressively  
242 over time, with the highest detection rate at 11 weeks of age for TTV1 and at 15 weeks  
243 for TTV2. The high percentage of TTV infection in adults, both in sows (Segalés et al.,  
244 2009; Sibila et al., 2009) and boars (Kekarainen et al., 2007), would support this  
245 increasing rate of infection over time. However, earlier studies have reported higher  
246 prevalences in young than adult pigs and wild boars (Martelli et al., 2006; Martínez et  
247 al., 2006). These discrepancies may be due to different factors such as: 1) the type of  
248 study; the present work is of longitudinal nature, while Martelli et al., (2006) and  
249 Martínez et al., (2006) were cross-sectional ones and, therefore, animals were sampled  
250 once; 2) the PCR method applied and its sensitivity; 3) the age of the animals studied; in  
251 the present study, the last sampling week was at 15 weeks of age and TTV prevalence in  
252 finishing or slaughter age pigs is unknown; and 4) the potential prevalence variations in  
253 different geographic regions (McKeown et al., 2004). Nevertheless, it is worthy to  
254 remark that human TTV infection is very common and increases with age, with peaks in  
255 young adults or later in life (Bendinelli et al., 2001) .

256 The duration of the TTV viremia in pigs under field conditions is still an  
257 unknown issue. In the present study, TTV1 and TTV2 viremia lasted in a number of  
258 animals up to 15 and 8 weeks, respectively. Although the virus was consecutively  
259 detected in most of the pigs, it was not possible to assess if it was due to virus  
260 persistence or re-infection. Long-lasting viremia in apparently healthy pigs suggests that

261 the immune responses elicited by swine TTV infection might be ineffective in clearing  
262 the virus. Long lasting infections are very common in the TTV human counterpart, in  
263 which viremia may last several years, suggesting lifelong persistence (Bendinelli et al.,  
264 2001). Besides, PCV2, another circular, ssDNA virus of swine, is able to persist up to  
265 21 weeks in some animals (Rodríguez-Arriola et al., 2002). Further studies focused on  
266 infection and seroconversion will help to elucidate the importance of humoral responses  
267 in controlling the TTV infection.

268         Apart from sera, swine TTV has been so far detected in different samples such  
269 as plasma, semen, faeces and colostrum (Brassard et al., 2008; Kekarainen et al., 2007;  
270 Martínez-Guinó et al., 2009). The present study represents the first description of swine  
271 TTV detection in nasal cavity. Detection of both genogroups in nasal swabs increased  
272 with age. Whether detection of TTV virus in nasal cavity corresponds to replication site  
273 or an entry or excretion route is still unknown. In humans, the ability of TTV to infect  
274 respiratory cells indicates that the respiratory tract is the primary infection and  
275 replication site (Maggi et al., 2003). Moreover, it is known that TTV is able to disrupt  
276 the mucociliary escalator (Pifferi et al., 2008). In the case of PCV2, nasal shedding has  
277 also been described (Sibila et al., 2004) but there is no report suggesting a role or an  
278 effect on the mucociliary system of the respiratory tract.

279         TTV genogroups were also detected intermittently in stools throughout the  
280 observation period. Rates of TTV faecal excretion detected in the present study (<20%)  
281 were lower than the ones reported by Brassard et al. (2008), who found 60.3% of TTV1  
282 PCR positive faeces from pigs of unknown age. Nevertheless, TTV faecal shedding  
283 observed in the present study resembles that observed in humans, which is also  
284 considered intermittent and to a low percentage (Okamoto et al., 1998; Ross et al.,  
285 1999). Indeed, faeces from TTV viremic humans are infective in tissue cultures (Maggi

286 et al., 2001), suggesting that this virus can be transmitted non-parentally and that the  
287 oro-fecal route can be the most common mode of transmission (Bendinelli et al., 2001).  
288 In PCV2, the faecal shedding has been reported to occur to higher rates (Grau-Roma et  
289 al., 2009) than the ones presented for TTV here, but no information regarding the  
290 infectivity of this material does exist.

291 In conclusion, this study describes for the first time the infection and excretion  
292 dynamics of swine TTVs throughout life of pigs from conventional, multi-site farms.  
293 Moreover, obtained results confirm the ubiquitous nature of the virus in the pig  
294 population and point to the existence of long-lasting viremia.

295

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300

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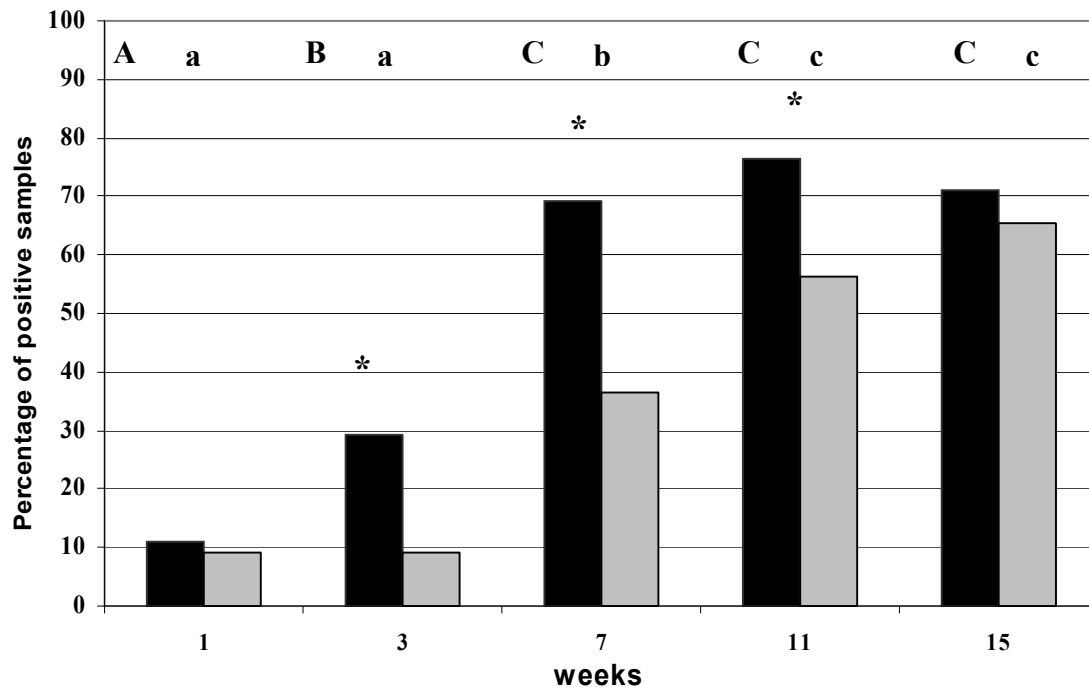


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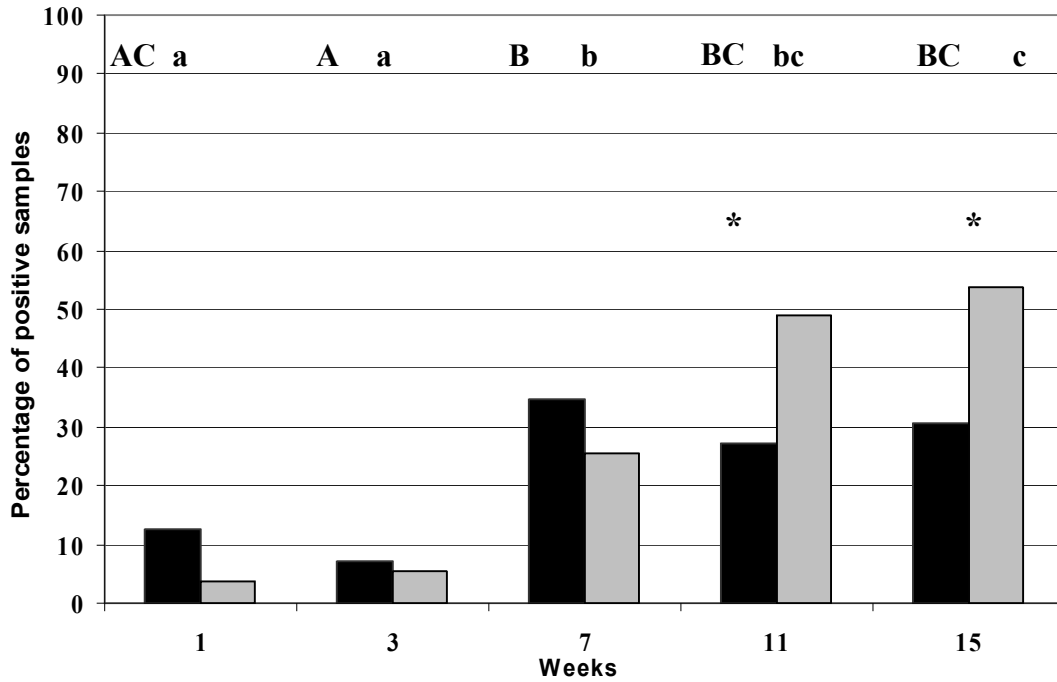
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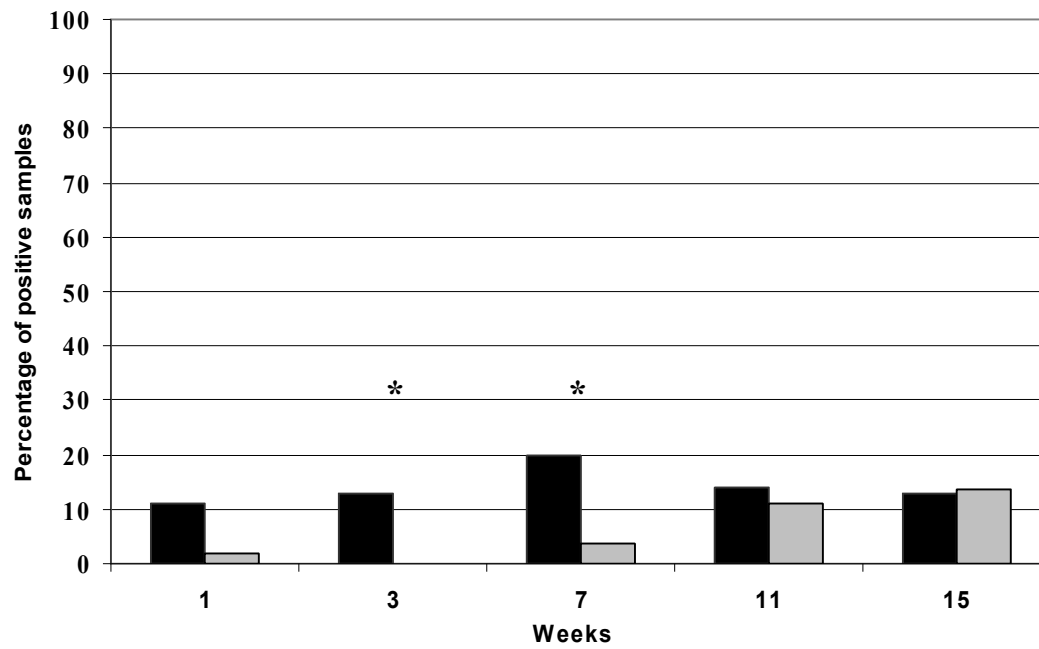
**Figure 1: Percentage of positive serum samples by PCR to TTV1 (black bars) and TTV2 (grey bars) at different sampling ages. Asterisks mean statistically significant differences between percentage of TTV1 and TTV2 positive pigs at a given age. Different letters mean significant differences within the percentage of TTV1 (capital letters) or TTV2 (case letters) PCR positive pigs throughout the different tested ages.**



**Figure 2: Percentage of positive nasal swabs by PCR to TTV1 (black bars) and TTV2 (grey bars) at different sampling ages. Asterisks mean statistically significant differences between percentage of TTV1 and TTV2 positive pigs at a given age. Different letters mean significant differences within the percentage of TTV1 (capital letters) or TTV2 (case letters) PCR positive pigs throughout the different tested ages**



**Figure 3: Percentage of positive rectal swabs by PCR to TTV1 (black bars) and TTV2 (grey bars) at different sampling ages. Asterisks mean a statistically significant difference between percentages of TTV1 and TTV2 positive pigs at a given age.**



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**Table 1: Data summary of farms, batches and parity distribution of sows included in the study. Health status describes other pathogens known to be present (+) or absent (-) in the sow-farm by serological determination at the time the study was performed. Porcine circovirus type 2 (PCV2), Porcine respiratory and reproductive syndrome virus (PRRSV), porcine parvovirus (PPV), *Mycoplasma hyopneumoniae* (Myc), swine influenza virus (SIV), *Salmonella* spp. (Salm), Aujeszky disease virus (ADV).**

Farm	Production system	Farm size (n° of sows)	Batch	Parity distribution of the tested sows per batch		Farm health status
				1 <sup>st</sup> to 4 <sup>th</sup>	5 <sup>th</sup> to 8 <sup>th</sup>	
A	3 sites	1000	1	2	2	PCV2+, PRRSV+, PPV+
B	3 sites	850	2	2	1	PCV2+, PRRSV+, PPV+, Myc+, SIV+, Salm+, ADV+
C	3 sites	2400	3	3	0	PCV2+, PRRSV+, PPV+, Myc+, SIV+, Salm+, ADV+
			4	5	0	
D	3 sites	2400	5	4	1	PCV2+, PRRSV+, PPV+, ADV-
E	2 sites	600	6	3	0	PCV2+, PRRSV+, PPV+, Myc+, SIV+, Salm+, ADV+
			7	3	0	
			8	4	0	
F	3 sites	1500	9	0	3	PRRSV+, PPV+, ADV-
G	2 sites	950	10	4	0	PRRSV-, ADV-, Myc-, PPV+, SIV+, Salm+
			11	4	0	

**Table 2: Number of PCR positive sows to TTV1 and TTV2, distributed according to the parity groups.**

PCR	Parity		Total
	1 <sup>st</sup> to 4th	5 <sup>th</sup> to 8 <sup>th</sup>	
TTV1 + / TTV2 +	8	0	8
TTV1 + / TTV2 -	11	3	14
TTV1 - / TTV2 +	4	1	5
TTV1 - / TTV2 -	11	3	14
Total	34	7	41

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