



HAL
open science

Torque Teno virus (TTV) infection in sows and suckling piglets

M. Sibila, L. Martínez-Guinó, E. Huerta, M. Mora, L. Grau-Roma, T. Kekarainen, J. Segalés

► To cite this version:

M. Sibila, L. Martínez-Guinó, E. Huerta, M. Mora, L. Grau-Roma, et al.. Torque Teno virus (TTV) infection in sows and suckling piglets. *Veterinary Microbiology*, 2009, 137 (3-4), pp.354. 10.1016/j.vetmic.2009.01.008 . hal-00485530

HAL Id: hal-00485530

<https://hal.science/hal-00485530>

Submitted on 21 May 2010

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Accepted Manuscript

Title: Torque Teno virus (TTV) infection in sows and suckling piglets

Authors: M. Sibila, L. Martínez-Guinó, E. Huerta, M. Mora, L. Grau-Roma, T. Kekarainen, J. Segalés



PII: S0378-1135(09)00009-1
DOI: doi:10.1016/j.vetmic.2009.01.008
Reference: VETMIC 4322

To appear in: *VETMIC*

Received date: 6-10-2008
Revised date: 12-12-2008
Accepted date: 2-1-2009

Please cite this article as: Sibila, M., Martínez-Guinó, L., Huerta, E., Mora, M., Grau-Roma, L., Kekarainen, T., Segalés, J., Torque Teno virus (TTV) infection in sows and suckling piglets, *Veterinary Microbiology* (2008), doi:10.1016/j.vetmic.2009.01.008

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15

Short communication

**TORQUE TENO VIRUS (TTV) INFECTION IN SOWS AND SUCKLING
PIGLETS**

M. Sibila ^{a*}, L. Martínez-Guinó ^a, E. Huerta ^a, M. Mora ^a, L. Grau-Roma ^{a,b}, T.
Kekarainen ^a, J. Segalés ^{a,b}

^a *Centre de Recerca en Sanitat Animal (CRESA), UAB-IRTA, Campus de la Universitat
Autònoma de Barcelona, 08193 Bellaterra, Barcelona, Spain.* ^b *Departament de Sanitat
i Anatomia Animals, Universitat Autònoma de Barcelona, 08193 Bellaterra, Barcelona,
Spain*

* Corresponding author at: Centre de Recerca en Sanitat Animal (CRESA), UAB-IRTA,
Campus de la Universitat Autònoma de Barcelona, 08193 Bellaterra, Barcelona, Spain.
E-mail address: marina.sibila@cresa.uab.cat (M. Sibila)

16 **Abstract**

17 Torque Teno virus (TTV) is a single stranded DNA virus that has been detected in
18 serum of primate and non-primate species including swine. Little information on swine
19 TTV infection and transmission dynamics is nowadays available. The goal of this study
20 was to gain insight into the potential role of the sow in transmitting TTV to piglets and
21 the infection dynamics of both swine TTV genogroups (TTV1 and TTV2) during the
22 lactation period. Serum samples from 44 sows at 1 week post-farrowing and 215 piglets
23 at 1 and 3 weeks of age were tested using TTV1 and TTV2 PCR methods. Sow parity
24 distribution and the number of delivered piglets (liveborn, stillborn and mummified) per
25 each studied sow were recorded. TTV1 was detected in higher percentages than TTV2
26 in both sows (75% vs 43%, respectively) and piglets at 1 (17% vs 7%, respectively) and
27 3 (32% vs 12%, respectively) weeks of age. TTV1 and TTV2 co-infections were
28 observed in higher percentages in sows (34%) than in piglets (2% and 4% at 1 and 3
29 weeks of age, respectively). Detection of swine TTV genogroups in sows was not
30 associated with their detection in piglets. Moreover, there were piglets infected at 1
31 week of age with a swine TTV genogroup different from the one detected in their dam.
32 The number of sows delivering stillborns and the mean number of stillborns per sow
33 tended to be higher in the TTV2 infected sows; this value was significantly higher when
34 co-infected sows (TTV1 and TTV2) were compared with non-co-infected ones. Old
35 parity sows had a higher percentage of TTV1 infected 1 week-old piglets. Results of the
36 present study showed that the TTV infection occurs early in the production system and
37 that these viruses may be transmitted from sow-to-piglet but also from piglet-to-piglet
38 in farrowing facilities.

39

40 *Keywords: Torque Teno virus (TTV), piglet, sow, PCR, serum, lactation period*

41

42

43 **1. Introduction**

44 Torque Teno virus (TTV) is a non-enveloped, circular, single-stranded DNA virus
45 able to infect several vertebrate species, including human, swine, poultry, sheep, cattle,
46 dogs and cats (Kekarainen and Segalés, 2008).

47 In humans, several TTV genogroups have been described (Peng et al., 2002). The
48 frequency of their detection in serum is very variable between countries and increases
49 with age (Saback et al., 1999). Apart from serum, human TTV has been detected in
50 saliva, nasal secretions and faeces, suggesting the faecal-oral as the main transmission
51 route (Maggi et al., 2003; Okamoto et al., 1998; Xuewen Deng, 2000). Nevertheless,
52 detection of this virus in serum of mother-to-child pairs (Kazi et al., 2000), cord blood
53 (Matsubara et al., 2001) and in breast milk (Schröter et al., 2000) indicates that TTV
54 transmission may also occur vertically. To date, no definitive association between
55 human TTV infection and a specific disease or pathology has been reported.

56 In swine, two TTV genogroups (TTV1 and TTV2) have been described so far (Niel
57 et al., 2005; Okamoto et al., 2002). Up to now, swine TTV has been detected in pigs
58 from different countries (McKeown et al., 2004; Bigarré et al., 2005), ages (Kekarainen
59 et al., 2007; Kekarainen et al., 2006; Martelli et al., 2006), sex (Kekarainen et al., 2006;
60 Segalés et al., 2008) and production systems (Martelli et al., 2006). Like its human
61 counterpart, swine TTV has not been clearly linked to any specific pathology. However,
62 TTV2 has been more frequently found in animals suffering from postweaning
63 multisystemic wasting syndrome (PMWS), a disease caused by PCV2 (Segalés et al.,
64 2005), than in healthy pigs (Kekarainen et al., 2006).

65 At present, transmission routes of swine TTVs are still unknown. However, evidence
66 of potential vertical transmission exists since both swine TTVs have been recently

67 detected in colostrum samples and in sera of sows and their stillborns (Martínez-Guinó
68 et al., 2008). Moreover, swine TTVs have been detected in semen of boars suggesting
69 that the sexual route may contribute to the viral dissemination (Kekarainen et al., 2007).

70 The main objective of this work was to investigate the potential role of the sow in
71 transmitting swine TTVs to piglets and the infection dynamics of TTV genogroups
72 during the lactation period.

73

74 **2. Materials and Methods**

75 ***2.1. Animals and farm***

76 Eleven batches from a total of 7 Spanish multi-site herds were investigated
77 (Table 1). Four sows of different parity numbers (parities 1 to 10) per batch and 5
78 healthy piglets per sow were randomly selected and included in this study. A total of 44
79 sows and 215 piglets (from five of the sows, 4 piglet sera were available) were finally
80 included in the study. Blood samples from cava vein (5 ml Venoject, Terumo Europe,
81 Madrid, Spain) were taken from sows at 1 week post-farrowing and from piglets at 1
82 and 3 weeks of age. Data of delivered pigs (liveborn, stillborn and mummified) of the
83 sows included in the study were recorded. Treatments, housing, and husbandry
84 conditions conformed to the European Union Guidelines and Good Clinical Practices.

85

86 ***2.2. Swine TTV1 and TTV2 PCR methods***

87 DNA was extracted from 200 µl of serum using a commercial kit (Nucleospin®
88 Blood, Macherey-Nagel GmbH & Co KG Düren, Germany), according to
89 manufacturer's instructions. Presence of TTV1 and TTV2 DNA was assessed with two
90 previously described specific polymerase chain reaction (PCR) methods (Segalés et al.,
91 2008). To minimize the risk of contaminations, each stage of the PCR process (DNA

92 extraction, DNA amplification and electrophoresis) was carried out in separated rooms.
93 The amplified products (305 bp for TTV1 and 250 bp for TTV2) were run in a 2%
94 agarose gel with 0.05 mg/ml of ethidium bromide.

95

96 **2.3. Statistical analyses**

97 Sows were grouped by parity numbers into two different groups: young (from 1st
98 to 3rd parity, n=21) and old (from 4th to 10th, n=23) sows. Bivariate analyses using
99 contingency tables (Chi-square statistics or Fisher's exact test for 2x2 tables) were used
100 to compare: 1) prevalence of TTV1 and/or TTV2 in serum of sows according to their
101 parity group and the presence or absence of stillborns and 2) prevalence of both TTV
102 genogroups in piglets at different ages and between piglets and sows. Moreover, a non-
103 parametric statistical analyses (Kruskal-Wallis test) was used to test differences in the
104 mean number of liveborn, stillborn and mummified piglets according to the TTV
105 infection status of the sow. Statistical analyses were performed with the SAS system for
106 Windows version 9.0 (SAS Institute Inc, Cary, North Carolina, USA). Statistical
107 significance level was set at $\alpha=0.05$.

108

109 **3. Results**

110 From the 44 sows analyzed, 33 (75%) and 19 (43%) were PCR positive to TTV1
111 and TTV2, respectively (Fig. 1). However, this difference was not statistically
112 significant ($p>0.05$). While 15 (34%) out of the 44 sows were co-infected with both
113 TTV genogroups, 7 (16%) sows were negative to both PCR methods. The number of
114 sows that delivered stillborn piglets tended to be higher ($p=0.051$) among those TTV2
115 PCR positive sows than negative ones (Table 2). Moreover, this parameter was
116 significantly higher ($p=0.0479$) among sows co-infected with both TTV genogroups

117 compared to non-co-infected sows. On the other hand, TTV2 PCR positive sows tended
118 to have higher ($p=0.0502$) mean number of stillborns than the negative ones. For this
119 parameter, similar results ($p=0.0582$) were obtained when comparing co-infected sows
120 versus non-co-infected ones. In regards TTV1 PCR positive and negative sows, no
121 significant differences were observed in both parameters (number of sows delivering
122 stillborns and mean number of stillborns) ($p>0.2$). On the other hand, the mean number
123 of liveborn and mummified piglets was independent of the TTV infection status of the
124 sow ($p>0.05$).

125 In piglets, TTV1 was globally (at 1 and/or 3 weeks of age) detected in higher
126 percentages (92/215, 43%) than TTV2 (41/215, 19%), although this difference was not
127 significant ($p>0.05$) (Table 3). From the 215 piglets studied, 36 (17%) and 69 (32%)
128 were positive to TTV1 at 1 and 3 weeks of age, respectively. From these 36 TTV1 PCR
129 positive piglets at 1 week of age, only 13 were also positive at 3 weeks of age. On the
130 contrary, from the 179 piglets TTV1 PCR negative at 1 week of age, 56 (31%) were
131 positive at 3 weeks of age. Regarding TTV2, 15 (7%) and 26 (12%) out of the 215
132 piglets were positive at 1 and 3 weeks of age, respectively. None of the 15 piglets TTV2
133 PCR positive at 1 week of age was positive at 3 weeks of age. From the 200 piglets
134 PCR negative to TTV2 at 1 week of age, 26 (13%) became positive two weeks later.
135 The rate of new infections at 3 weeks of age was significantly higher for TTV1 than for
136 TTV2 ($p<0.05$). Co-infections with both genogroups were sporadic since only 3 (2%)
137 and 8 (4%) animals were positive to both TTV PCRs at 1 and 3 weeks of age,
138 respectively. On the contrary, 96 (45%) out of 215 piglets were negative to both TTV
139 genogroups at both sampling times.

140 Detection of TTV1 and/or TTV2 in sows at 1 week post-farrowing was not
141 statistically associated with virus detection in their piglets at 1 and/or 3 weeks of age

142 ($p>0.05$) (Table 3). The percentage of infected pigs at 1 week of age coming from
143 TTV1 PCR positive sows (26/163, 16%) was not different from the one coming from
144 negative sows (10/52, 19%) ($p>0.05$). On the other hand, no significant differences
145 ($p>0.05$) were observed between the percentage of 1 week-old TTV2 PCR positive
146 piglets coming from TTV2 PCR positive (8/94, 8.5%) and negative (7/121, 6%) sows.
147 Moreover, there were 5 out of the 36 (14%) TTV1 PCR positive piglets at 1 week of
148 age that came from sows PCR negative to TTV1 but PCR positive to TTV2 (data not
149 shown). Similarly, 4 out of the 15 (27%) TTV2 PCR positive piglets at 1 week of age
150 came from sows PCR negative to this TTV genogroup but PCR positive to TTV1.

151 Swine TTV genogroups were detected in higher percentages in young parity sows
152 (18/21 [86%] and 10/21 [47%] for TTV1 and TTV2, respectively) than in old parity
153 sows (15/23 [65%] and 9/23 [39%] for TTV1 and TTV2, respectively). No association
154 between parity number and TTV1 and/or TTV2 infection in sows was found ($p>0.05$).
155 Old sows had higher number of 1 week-old TTV1 PCR positive piglets (25/111, 23%)
156 than young ones (11/104, 11%) ($p=0.01$). On the contrary, at 3 weeks of age, the
157 proportion of TTV1 PCR positive piglets from young sows (40/104, 39%) tend to be
158 higher ($p=0.052$) than old ones (29/111, 26%). TTV2 infection in piglets was
159 independent of the sow parity.

160

161 **4. Discussion**

162 Results of the present study confirm that TTV1 and TTV2 are able to infect sows and
163 their suckling piglets. Detection of swine TTVs in sows is in agreement with two
164 studies in which these viruses were retrospectively (Segalés et al., 2008) and
165 contemporaneously (Martínez-Guinó et al., 2008) found in sows coming from several
166 Spanish farms. Therefore, it seems that TTV1 and TTV2 infections are widespread in

167 the sow population, as it has been demonstrated in boars as well (Kekarainen et al.,
168 2007). This latter point reinforces the notion that pig could serve as an animal model for
169 human TTV infection as has been suggested (Kekarainen and Segalés, 2008), since the
170 prevalence of human TTV infection increases with age, being high in adults (Saback et
171 al., 1999).

172 An interesting outcome of this study is the fact that the number of sows delivering
173 stillborns and the mean number of stillborns per sow tended to be higher in the TTV2
174 infected sows; this value was significantly higher when co-infected sows (TTV1 and
175 TTV2) were compared with non-co-infected ones. This result might indicate a potential
176 role of these viruses in reproductive failure. It must be taken into account, however, that
177 only 44 sows were analyzed. Therefore, such potential relationship with increased
178 number of stillborns must be taken with caution and deserves further investigations,
179 especially in regards potential co-infection with other well-known pathogens linked to
180 reproductive failure. On the human side, although infection in pregnant women (ranging
181 from 28 to 83%) and young babies have been described (Kazi et al., 2000; Saback et al.,
182 1999; Schröter et al., 2000; Sugiyama et al., 2001), no association between TTV
183 infections and gestation disturbances has been reported so far.

184 Parity number of the sow was not associated with TTV1 or TTV2 infection in sows,
185 as has been previously suggested (Martínez-Guinó et al., 2008). Surprisingly, old sows
186 (parity 4-10) had a higher percentage of 1 week-old TTV1 infected piglets. There is no
187 clear explanation of this finding; it is assumed that the potential transmission from sow
188 to piglet should be similar in both young and old sows, since their rates of infection
189 were not significantly different. It can not be ruled out that such result might be a
190 spurious effect due to sample.

191 The higher prevalence of TTV1 than TTV2 in sows and piglets reported in this study
192 differs from results obtained in two previous studies in which TTV2 was more prevalent
193 than TTV1 (Kekarainen et al., 2006; Segalés et al., 2008). These discrepancies are
194 probably due to the variability of origin and animal ages of the samples analyzed.

195 Simultaneous detection of swine TTVs in sows and their newborn piglets (1 week of
196 age) is suggestive of vertical transmission. However, swine TTV infection in piglets
197 was independent of the TTV infection status of the sow. These apparently controversial
198 results have been also found in their human counterpart (Schröter et al., 2000; Kazi et
199 al., 2000; Lin et al., 2002). Nevertheless, sow-to-piglet transmission is probably the most
200 likely transmission route taking into account the existence of piglets already infected at
201 1 week of age. If the observed viral dissemination was related to transplacental,
202 intrauterine, colostrum-feeding or by daily contact transmission was not elucidated in
203 this study.

204 Apart from the potential sow-to-piglet transmission route, results of the present work
205 also support the existence of piglet-to-piglet viral dissemination. This latter route of
206 transmission could be supported by two indirect facts. Firstly, the existence of piglets
207 negative at 1 week of age that were infected two weeks later. And secondly, the
208 detection of piglets infected with a swine TTV genogroup different from the one
209 detected in their mother. This latter situation has also been described in humans (Lin et
210 al., 2002; Sugiyama et al., 2001) and also in other swine ssDNA viruses such as PCV2
211 (Grau-Roma et al., 2008). Both facts would explain the existence of *de novo* infections
212 and the apparently increasing frequency of swine TTV infection with age, as has been
213 shown in humans (Saback et al., 1999).

214 In conclusion, this study showed that swine TTV infection occurs at early stages of
215 the production system and suggests that these viruses are transmitted from sow-to-

216 piglet, although horizontal transmission piglet-to-piglet in the farrowing units is also of
 217 importance.

218

219 Acknowledgments

220 This work was funded by the grants AGL2006-02778/GAN, TRT2006-00018
 221 and CONSOLIDER-PORCIVIR CSD2006-00007 from Spanish government.

222

223 References

- 224 Bigarré, L., Beven, V., de Boisseson, C., Grasland, B., Rose, N., Biagini, P., Jestin, A., 2005. Pig anelloviruses are
 225 highly prevalent in swine herds in France. *J. Gen. Virol.* 86, 631-635.
- 226 Grau-Roma, L., Crisci, E., Sibila, M., Lopez-Soria, S., Nofrarias, M., Cortey, M., Fraile, L., Olvera, A., Segales, J., 2008.
 227 A proposal on porcine circovirus type 2 (PCV2) genotype definition and their relation with postweaning
 228 multisystemic wasting syndrome (PMWS) occurrence. *Vet. Microbiol.* 128, 23-35.
- 229 Kazi, A., Miyata, H., Kurokawa, K., Khan, M.A., Kamahora, T., Katamine, S., Hino, S., 2000. High frequency of postnatal
 230 transmission of TT virus in infancy. *Arch. Virol.* 145, 535-540.
- 231 Kekarainen, T., Lopez-Soria, S., Segales, J., 2007. Detection of swine Torque teno virus genogroups 1 and 2 in boar
 232 sera and semen. *Theriogenology* 68, 966-971.
- 233 Kekarainen, T., Segalés, J., 2008. Torque teno virus infection in the pig and its potential role as a model of human
 234 infection. *Vet. J.* doi:10.1016/j.tvjl.2007.12.005
- 235 Kekarainen, T., Sibila, M., Segales, J., 2006. Prevalence of swine Torque teno virus in post-weaning multisystemic
 236 wasting syndrome (PMWS)-affected and non-PMWS-affected pigs in Spain. *J. Gen. Virol.* 87, 833-837.
- 237 Lin, H.H., Kao, J.H., Lee, P.I., Chen, D.S., 2002. Early acquisition of TT virus in infants: possible minor role of maternal
 238 transmission. *J. Med. Virol.* 66, 285-290.
- 239 Maggi, F., Pifferi, M., Fornai, C., Andreoli, E., Tempestini, E., Vatteroni, M., Presciuttini, S., Marchi, S., Pietrobelli, A.,
 240 Boner, A., Pistello, M., Bendinelli, M., 2003. TT Virus in the Nasal Secretions of Children with Acute
 241 Respiratory Diseases: Relations to Viremia and Disease Severity. *J. Virol.* 77, 2418-2425.
- 242 Martelli, F., Caprioli, A., Di Bartolo, I., Cibir, V., Pezzotti, G., Ruggeri, F.M., Ostanello, F., 2006. Detection of Swine
 243 Torque Teno Virus in Italian Pig Herds. *J. Vet. Med. B. Infect. Dis. Vet. Public Health.* 53, 234-238.
- 244 Martínez-Guinó, L., Sibila, M., Kekarainen, T., Martín-Valls, G., Segalés, J., 2008. Evidence of Torque Teno virus (TTV)
 245 vertical transmission in swine. In: *International Pig Veterinary Society, Durban, South Africa*, p. 97.
- 246 Matsubara, H., Michitaka, K., Horiike, N., Kihana, T., Yano, M., Mori, T., Onji, M., 2001. Existence of TT virus DNA and
 247 TTV-like mini virus DNA in infant cord blood: mother-to-neonatal transmission. *Hepatology Research* 21, 280-
 248 287.
- 249 McKeown, N.E., Fenaux, M., Halbur, P.G., Meng, X.J., 2004. Molecular characterization of porcine TT virus, an orphan
 250 virus, in pigs from six different countries. *Vet. Microbiol.* 104, 113-117.
- 251 Niel, C., Diniz-Mendes, L., Devalle, S., 2005. Rolling-circle amplification of Torque teno virus (TTV) complete genomes
 252 from human and swine sera and identification of a novel swine TTV genogroup. *J. Gen. Virol.* 86, 1343-1347.
- 253 Okamoto, H., Akahane, Y., Ukita, M., Fukuda, M., Tsuda, Y., Miyakawa, Y., Mayumi, M., 1998. Fecal excretion of a
 254 nonenveloped DNA virus (TTV) associated with posttransfusion non-A-G hepatitis. *J. Med. Virol.* 56, 128-132.

- 255 Okamoto, H., Takahashi, M., Nishizawa, T., Tawara, A., Fukai, K., Muramatsu, U., Naito, Y., Yoshikawa, A., 2002,
256 Genomic characterization of TT viruses (TTVs) in pigs, cats and dogs and their relatedness with species-
257 specific TTVs in primates and tupaias. *J. Gen. Virol.* 83, 1291-1297.
- 258 Peng, Y.H., Nishizawa, T., Takahashi, M., Ishikawa, T., Yoshikawa, A., Okamoto, H., 2002. Analysis of the entire
259 genomes of thirteen TT virus variants classifiable into the fourth and fifth genetic groups, isolated from viremic
260 infants. *Arch. Virol.* 147, 21-41.
- 261 Saback, F., Gomes, S.A., de Paula, V., da Silva, R.R., Lewis-Ximenez, L.L., Niel, C., 1999. Age-specific prevalence and
262 transmission of TT virus. *J. Med. Virol.* 59, 318-322.
- 263 Schröter, M., Polywka, S., Zollner, B., Schafer, P., Laufs, R., Feucht, H.H., 2000. Detection of TT virus DNA and GB
264 virus type C/Hepatitis G virus RNA in serum and breast milk: determination of mother-to-child transmission. *J*
265 *Clin. Microbiol.* 38, 745-747.
- 266 Segalés, J., Allan, G.M., Domingo, M., 2005, Porcine circovirus diseases. *Animal Health Research Reviews* 6, 119-142
- 267 Segalés, J., Martínez, L., Cortey, M., Navarro, N., Huerta, E., Sibila, M., Pujols, J., Kekarainen, T., 2008. Retrospective
268 study on swine Torque teno virus genogroups 1 and 2 infection from 1985 to 2005 in Spain. *Vet. Microbiol.*
269 doi:10.1016/j.vetmic.2008.08.002
- 270 Sugiyama, K., Goto, K., Ando, T., Mizutani, F., Terabe, K., Yokoyama, T., 2001. Highly diverse TTV population in infants
271 and their mothers. *Virus Res.* 73, 183-188.
- 272 Xuewen Deng, H.T., Ray Handema, Minoru Sakamoto, Takatoshi Kitamura, Masahiko Ito, Yoshihiro Akahane., 2000.,
273 Higher prevalence and viral load of TT virus in saliva than in the corresponding serum: Another possible
274 transmission route and replication site of TT virus. *J. Med. Virol.* 62, 531-537.
- 275
- 276

Table 1

Summary of data on the seven Spanish multi-site farms included in the study and information of the 4 sows tested per batch such as sow parity distribution and mean of delivered piglets

Farms	Farm size (No. of sows)	Batch reference	Parity distribution of the 4 sows tested per batch		Mean of piglets delivered of the studied sows per batch \pm standard deviation		
			Parity 1 to 3	Parity 4 to 10	Liveborn	Stillborn	Mummified
			A	1000	1	2	2
B	850	2	3	1	12 \pm 1.41	0.5 \pm 1	0
C	2400	3	2	2	13.75 \pm 1.25	0.75 \pm 0.95	0
		4	2	2	12.5 \pm 1.91	0	0
D	2400	5	2	2	10.75 \pm 0.5	0.25 \pm 0.5	0
		6	4	0	11.25 \pm 0.95	0	0
E	600	7	2	2	12 \pm 1.15	0.5 \pm 0.577	0
		8	2	2	13.75 \pm 1.5	0.25 \pm 0.5	0.25 \pm 0.5
F	1500	9	1	3	12 \pm 1.41	0.5 \pm 1	0
G	950	10	1	3	10.5 \pm 1.29	0	0
		11	0	4	11 \pm 2.94	0	0

Table 2

Number of sows that delivered stillborns (percentages) and mean number of stillborns distributed according to the TTV infection status. P-values mean differences between PCR positive and negative sows for a given TTV genogroup. Letters in superscript means statistically significant differences ($p < 0.05$)

	TTV genogroup	PCR	Number of sows with stillborns/ Total number of sows	Mean number of stillborn* \pm standard deviation
Sows	TTV1	Positive	9/33 (27%)	0.42 \pm 0.15
		Negative	1/11 (9%)	0.18 \pm 0.18
	TTV2	Positive	7/19 (37%)	0.63 \pm 0.24
		Negative	3/25 (12%)	0.16 \pm 0.09
	Co-infection	Positive	6/15 (40%) ^a	0.66 \pm 1.11
		Negative	4/29 (14%) ^b	0.20 \pm 0.55

* The mean number of stillborns refers to the total number of sows according to their TTV infection status

Table 3

TTV1 and TTV2 PCR results in piglets at 1 and 3 weeks of age distributed according TTV detection in sows. *PCR status of the piglets at 1 and 3 weeks of age (i.e., 1+3- means positive PCR result at 1 week of age and negative at 3).

		Sows				Total	
		TTV1		TTV2			
		Positive (%)	Negative (%)	Positive (%)	Negative (%)		
		n=33	n=11	n=19	n=25		
Piglets*	TTV1	1+3+	9 (5.5)	4 (7.7)	3 (3.2)	10 (8.3)	13 (6.0)
		1+3-	17 (10.4)	6 (11.5)	11 (11.7)	12 (9.9)	23 (10.7)
		1-3+	48 (29.4)	8 (26.0)	25 (26.6)	31 (25.6)	56 (26.0)
		1-3-	89 (54.6)	34 (57.2)	55 (58.5)	68 (56.2)	123 (57.2)
		Total	163 (100)	52 (100)	94 (100)	121 (100)	215 (100)
	TTV2	1+3+	0 (0)	0 (0)	0 (7.0)	0 (0)	0 (0)
		1+3-	12 (7.4)	3 (5.8)	8 (12.1)	7 (5.8)	15 (7.0)
		1-3+	23 (14.1)	3 (5.8)	10 (80.9)	16 (13.2)	26 (12.1)
		1-3-	128 (78.5)	46 (88.5)	76 (100)	98 (81.0)	174 (80.9)
		Total	163 (100)	52 (100)	94 (100)	121 (100)	215 (100)

Figure 1

Percentage of TTV1 (in black) and TTV2 (in grey) PCR positive serum samples in sows at 1 week post-farrowing and in piglets at 1 and 3 weeks of age.

