Detection of Swine Torque Teno Virus in Italian Pig Herds

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Summary

Anellovirus is a recently created, floating genus of viruses. Torque teno virus (TTV), the type species in the genus, was first discovered in a human patient with a post-transfusion hepatitis of unknown aetiology. Recently, TTV genetically related to but distinct from those discovered in humans have also been found in animals, including pigs. The aims of this study were to estimate the prevalence of swine TTV in Italian pig herds and some risk factors possibly associated with this infection. Serum samples from 179 healthy pigs from 10 farms located in north-central Italy were tested by polymerase chain reaction for the presence of swine TTV DNA. Viral DNA was found in the sera of 43 pigs (24.0%), coming from eight of the 10 farms examined. Prevalence was significantly higher in finishing herds (40.1%) than in farrow-to-finish herds (11.0%) and did not depend on the size of the herd. Within the finishing herds the prevalence was significantly higher in weaners (57.4%) than in fatteners (22.9%), but this difference was not observed in farrow-to-finish herds. No relationship was observed between the prevalence of swine TTV and the implementation of some general hygiene practices and biosecurity procedures within the herds.

Introduction

Torque teno virus (TTV) was first discovered in 1997 in a human patient with a post-transfusion hepatitis of unknown aetiology and was named after the initials of the index patient (Nishizawa et al., 1997). TTV is a small (30–32 nm) non-enveloped virus with a single-stranded circular DNA genome of negative polarity, 3.4–3.9 kb in length (Okamoto et al., 2002). The virus carries an untranslated region and at least three major overlapping open reading frames (Biagini, 2005). Initially, because of the circular genome, TTV was associated with the Circoviridae family, along with porcine circoviruses 1 and 2, psittacine beak and feather disease virus, chicken anaemia virus and TTV-like minivirus, but it has been recently reclassified into a novel floating genus called Anellovirus (Biagini et al., 2005).

Torque teno virus has been frequently detected in human plasma worldwide (Niel et al., 1999) showing a high genomic diversity (Mushahwar et al., 1999). At the time of writing, virus variants have been officially classified into five main phylogenetic groups (Peng et al., 2002). Co-infection with different viral strains, even highly divergent, has been frequently described (Biagini, 2005). Clinical significance of TTV is uncertain: TTV viraemia is highly prevalent in patients with cryptogenic hepatitis, but it can be frequently observed also in healthy subjects (Bendinelli et al., 2001). At present time there is no clear relationship with any given pathology, despite numerous hypotheses: rhinitis, asthma, liver diseases, pancreatic cancer, diabetes mellitus, lupus erythematosus, idiopathic inflammatory myopathies and chromosomal translocation (Biagini, 2005). For these reasons, TTV can be considered an ‘orphan’ virus, a virus still waiting to be clearly linked to a given disease (Bendinelli et al., 2001).

Torque teno virus genetically related but distinct from those discovered in humans have been recently described in farm animals (pigs, chickens, cows and sheep) (Leary et al., 1999; Okamoto et al., 2002; McKeown et al., 2004; Bigarré et al., 2005; Niel et al., 2005; Kekarainen et al., 2006) and in dogs and cats (Okamoto et al., 2002). TTV has also been detected in non-human primates (Leary et al., 1999; Abe et al., 2000; Xiao et al., 2002) and in Tupaia belangeri chinensis, which shares characteristics with both primates and insectivores (Okamoto et al., 2001). Genomic length of TTV isolates appears to be smaller as the order of the animals decreases. In particular, swine strains showed a genome of 2.9 kb in length, instead of 3.4–3.9 kb of the human isolates (Okamoto et al., 2002).

Porcine TTV have been described in different countries (Leary et al., 1999; Okamoto et al., 2002; McKeown et al., 2004; Bigarré et al., 2005; Niel et al., 2005; Kekarainen et al., 2006), and sequence analyses have shown that the strains share between 71% and 100% nucleotide sequence identity (McKeown et al., 2004; Bigarré et al., 2005). As shown for human strains, intra-organ sequence heterogeneity has also been reported in swine (Bigarré et al., 2005). Phylogenetic analysis has also shown that apparently, clustering of porcine strains is not associated with their geographical origin (McKeown et al., 2004).

So far, the presence of swine TTV in European pig herds has been poorly investigated (McKeown et al., 2004; Bigarré et al., 2005; Kekarainen et al., 2006). Moreover, the epidemiology of the infection within and between swine herds and the associated risk factors are still unknown.
The aims of this study were a preliminary estimation of the prevalence of swine TTV in Italian pig herds by testing serum samples for the presence of viral DNA and the evaluation of some possible risk factors associated with this infection.

Materials and Methods

Sample collection
One hundred and seventy-nine serum samples were collected from pigs of 3–4 months of age (weaners) and 8–9 months of age (fatteners) from 10 different farms located in north-central Italy. To an external examination all the pigs sampled appeared clinically healthy. Blood was taken aseptically from the vena cava and the serum obtained by centrifugation was stored at −20°C until tested. For each farm, at least seven weaners and seven fatteners were sampled. This scheme provides a systematic sampling of different pig classes present within the herds and can estimate, with a 95% probability, the prevalence of swine TTV-positive animals with an expected prevalence of 30% and an accepted error of 25%.

A filled-in questionnaire was obtained from each farm tested. The information collected included herd typology (farrow-to-finish or finishing), herd size (number of fattening pigs), and issues regarding general hygiene practices and biosecurity procedures such as: rats extermination procedures, presence of perimetrical fences, control of the access, all-in/all-out, periodic rearing suspensions, and cleaning and disinfection between cycles.

Polymerase chain reaction detection of swine TTV DNA
Total DNA was extracted from 200 μl of each serum using a QIAamp DNA minikit (Qiagen, Hilden, Germany), according to manufacturer’s instructions. TTV DNA was amplified using a polymerase chain reaction (PCR) technique with primers targeting two conserved domains within an untranslated region of the viral genome (Bigarre et al., 2005). Amplified products were visualized on a 2% agarose gel; to confirm their identity, nucleotide sequence analysis was performed on four of the samples yielding a band of the correct size (115 bp in length), randomly chosen from different farms. Amplicons were gel purified using the High pure PCR product purification Kit (Roche Diagnostics, Mannheim, Germany) and sequenced using the ABI PRISM BigDye Terminator kit version 2.0 (Applied Biosystems, Foster City, CA, USA). The DNASIS Max 2.0 software (Hitachi Software Engineering Company, Alameda, CA, USA) was used to compare the nucleotide sequences obtained, 60 bp in length, with sequences from swine and human TTV isolates available in GenBank.

Statistical analysis
Data were analysed with the spss software for Windows 12.0 (SPSS Inc., Chicago, IL, USA). Kolmogorov–Smirnov test for goodness of adaptation was used to verify distribution normality. On the basis of the results of Kolmogorov–Smirnov test, Student’s t-test was used to compare quantitative data.

To evaluate factors related to the herd size, farms were divided into two categories on the basis of the average number of fattening pigs: large farms, with a number of fattening pigs ≥1700 and small farms, with a number of fattening pigs <1700.

To determine a possible correlation between swine TTV prevalence and general hygiene practices and biosecurity procedures implemented within the herds, the 10 farms were arbitrarily subdivided into two categories: farms implementing less than four of the above-mentioned hygiene and biosecurity practices (farms with a low sanitary standard) and farms implementing at least four of them (farms with a good sanitary standard).

Results
Positive PCR reactions were observed for 43 of 179 serum samples tested (24.0%, 95% CI: 18.1–31.1%). Nucleotide sequence analysis was performed on four PCR products, which were all confirmed to be swine TTV DNA. The relative sequences were deposited in the GenBank database with accession numbers DQ342283–DQ342286. In particular, sequence comparison showed that the four Italian strains shared to each other a nucleotide identity between 90.3% and 97% and a best fit (between 88.5% and 93%) with a Japanese TTV swine strain (GenBank accession number: AB076004). Positive animals were found in eight of the 10 farms tested, with prevalence values varying from 7.1% to 53.3% (Table 1). When the mean of the prevalence was calculated, the proportion of positive pigs was significantly higher in finishing herds (40.1%) than in farrow-to-finish herds (11.0%) (t: −3.398; P = 0.009) (Fig. 1).

Table 1. Prevalence of swine Torque teno virus infection in the farms examined

<table>
<thead>
<tr>
<th>Farm no.</th>
<th>Typology</th>
<th>No. fattens in the herd</th>
<th>No. pigs examined</th>
<th>No. positive pigs (%)</th>
<th>No. pigs examined</th>
<th>No. positive pigs (%)</th>
<th>No. pigs examined</th>
<th>No. positive pigs (%)</th>
<th>No. pigs examined</th>
<th>No. positive pigs (%)</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Farrow-to-finish</td>
<td>2000</td>
<td>15</td>
<td>2 (13.3)</td>
<td>15</td>
<td>2 (13.3)</td>
<td>30</td>
<td>4 (13.3)</td>
<td>5.1–26.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Farrow-to-finish</td>
<td>3500</td>
<td>7</td>
<td>0 (0.0)</td>
<td>7</td>
<td>0 (0.0)</td>
<td>14</td>
<td>0 (0.0)</td>
<td>0.1–0.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Farrow-to-finish</td>
<td>2000</td>
<td>7</td>
<td>1 (14.3)</td>
<td>14</td>
<td>1 (7.1)</td>
<td>21</td>
<td>2 (9.5)</td>
<td>3.1–23.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Farrow-to-finish</td>
<td>850</td>
<td>7</td>
<td>0 (0.0)</td>
<td>7</td>
<td>1 (14.3)</td>
<td>14</td>
<td>1 (7.1)</td>
<td>1.1–23.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Farrow-to-finish</td>
<td>250</td>
<td>7</td>
<td>1 (14.3)</td>
<td>7</td>
<td>4 (57.1)</td>
<td>14</td>
<td>5 (35.7)</td>
<td>18.1–57.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Farrow-to-finish</td>
<td>1100</td>
<td>7</td>
<td>0 (0.0)</td>
<td>7</td>
<td>0 (0.0)</td>
<td>14</td>
<td>0 (0.0)</td>
<td>0.1–0.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Finishing</td>
<td>3200</td>
<td>15</td>
<td>13 (66.7)</td>
<td>15</td>
<td>3 (20.0)</td>
<td>30</td>
<td>16 (53.3)</td>
<td>37.1–68.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Finishing</td>
<td>3500</td>
<td>7</td>
<td>4 (57.1)</td>
<td>7</td>
<td>2 (28.6)</td>
<td>14</td>
<td>6 (42.9)</td>
<td>23.1–64.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Finishing</td>
<td>9000</td>
<td>7</td>
<td>3 (42.9)</td>
<td>7</td>
<td>0 (0.0)</td>
<td>14</td>
<td>3 (21.4)</td>
<td>5.1–51.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Finishing</td>
<td>3000</td>
<td>7</td>
<td>3 (42.9)</td>
<td>7</td>
<td>3 (42.9)</td>
<td>14</td>
<td>6 (42.9)</td>
<td>23.1–64.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>86</td>
<td>27 (31.4)</td>
<td>93</td>
<td>16 (17.2)</td>
<td>179</td>
<td>43</td>
<td>24 (0.0)</td>
<td>18.1–31.1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Within finishing herds, the prevalence was significantly higher in weaners (57.4%) than in fatteners (22.9%) ($t$: 2.525; $P = 0.045$), although this difference was mainly due to the high prevalence (86.7%) detected in farm no. 7.

No statistically significant difference between weaners (7.0%) and fatteners (15.3%) was observed in farrow-to-finish herds ($t$: 0.898; $P = 0.390$) (Fig. 1).

No statistically significant difference in the swine TTV prevalence was observed between large and small herds ($t$: 1.676; $P = 0.132$).

The possible relationship between the implementation of general hygiene practices and biosecurity procedures and the prevalence of swine TTV infection was evaluated (Table 2). The prevalence of swine TTV infection was not significantly different between farms with a low sanitary standard and those with a good sanitary standard ($t$: 0.883; $P = 0.403$).

**Table 2.** Relationships between swine Torque teno virus (TTV) prevalence and the implementation of general hygiene practices and biosecurity procedures in the farms

<table>
<thead>
<tr>
<th>Farm no.</th>
<th>Rats extermination procedures</th>
<th>Presence of perimetrical fences</th>
<th>Control of the access</th>
<th>All-in/all-out</th>
<th>Periodic rearing suspension</th>
<th>Cleaning and disinfections between cycles</th>
<th>TTV prevalence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>G</td>
<td>F</td>
<td>G</td>
<td>G</td>
<td>F</td>
<td>F</td>
<td>13.3</td>
</tr>
<tr>
<td>2</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>0.0</td>
</tr>
<tr>
<td>3</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>9.5</td>
</tr>
<tr>
<td>4</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>7.1</td>
</tr>
<tr>
<td>5</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>35.7</td>
</tr>
<tr>
<td>6</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>0.0</td>
</tr>
<tr>
<td>7</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>53.3</td>
</tr>
<tr>
<td>8</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>42.9</td>
</tr>
<tr>
<td>9</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>21.4</td>
</tr>
<tr>
<td>10</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>42.9</td>
</tr>
</tbody>
</table>

G, growing section; F, finishing section; -, procedure not implemented; +, procedure implemented.

**Discussion**

This study represents the first survey on animal TTV conducted in Italy and confirms that these infections are common in intensively reared swine herds (Leary et al., 1999; Okamoto et al., 2002; McKeown et al., 2004; Bigarre et al., 2005; Niel et al., 2005; Kekarainen et al., 2006). Overall, the swine TTV individual prevalence observed in our study (24%) was lower than those reported in surveys conducted in other European countries. In Spanish herds, swine TTV DNA was found in 18 of 20 serum samples by McKeown et al. (2004), while a survey, recently conducted on serum samples from postweaning multisystemic wasting syndrome (PMWS) and non-PMWS affected pigs, revealed a 83.0% overall prevalence of infection (Kekarainen et al., 2006). In France, a study conducted examining different organs showed the presence of swine TTV DNA in 93.0% and 73.0% of the herds and the pigs tested respectively (Bigarre et al., 2005). The different prevalence values reported in these studies could be due to several factors, like the age of the animals and the type of specimen tested, the typology and size of the farm or the sensitivity of the PCR method used. On the other hand, the surveys conducted so far involved a limited number of animals and provided no information on either the characteristics of the farms investigated or the age of the animals tested.

At present, very little is known about the natural history of swine TTV infection in pigs, possible risk factors and mechanisms of transmission of the infection within and between herds. In this study, we tried to evaluate some of the factors that might influence the presence of swine TTV infection in swine herds.

With respect to the type of the herds considered, we found that swine TTV infection was significantly more frequent in finishing herds (40.1%) than in farrow-to-finish herds (11.%). This difference was mainly due to the higher prevalence observed among weaners in finishing herds, and could be due to the increased risk for naïve susceptible pigs to become infected upon contact with newly introduced animals harbouring the virus.

In this regard, it is of interest to notice that two of the 10 farms examined in this study appeared to be swine TTV negative and that both of them were farrow-to-finish farms. This suggests that swine TTV infection is not totally widespread in Italian herds and that negative herds could be maintained TTV free by controlling the introduction of new animals.

The observation of a significantly higher prevalence in weaners (57.4%) than in fatteners (22.9%) in finishing herds...
might also suggest that younger pigs could be more susceptible to swine TTV infection than older animals. However, the difference was mainly due to the high prevalence detected (86.7%) in weaners in a single farm and was not observed in farrow-to-finish herds. These conflicting data do not clarify the dynamic of swine TTV infection during the growing cycle of pigs. On the other hand, the dynamic of swine TTV infection with respect to the age is still unclear also in humans, despite the numerous studies conducted (Bendinelli et al., 2001).

Other factors considered in this study, like the herd size and the implementation of general hygiene practices and biosecurity procedures, did not seem to influence the prevalence of swine TTV, although they probably still need to be further evaluated.

The apparent ubiquitous nature of TTV in swine and the lack of geographical clustering of the strains have led to hypothesize a worldwide dissemination of the virus through contaminated swine vaccines (McKeown et al., 2004). However, this hypothesis appears to be in contrast with the heterogeneity of the viral genome reported in several papers (Bigarré et al., 2005; Niel et al., 2005; Kekarainen et al., 2006) and confirmed in this study, where nucleotide sequence analysis of four of the positive samples suggested that different porcine TTV strains circulate in Italian herds. The degree of genetic identity observed between our strains ranged in fact from 90.3% to 97% and was similar to that reported in other studies (McKeown et al., 2004; Bigarré et al., 2005). However, the limited size of the genomic fragment analysed, the possibility of PCR artefacts and the low number of strains sequenced does not allow further conclusions about the extent of diversity of swine TTV strains in Italy and the relationships with other animal and human strains.

Another issue that still remains unclear is whether swine TTV represents a sanitary risk to its natural host or other hosts. To date, there is no evidence that TTV is pathogenic for the swine. In our survey, the animals tested appeared to be clinically healthy, even in the farms with the highest prevalence of infection, thus supporting the hypothesis that swine TTV infection does not cause overt disease in pigs. On the other hand, the situation of TTV in swine may be reminiscent of the high worldwide prevalence of porcine circovirus type 2. This virus, which has coexisted with pigs for a long time, has been now associated with several swine diseases (Allan and Ellis, 2000). Moreover, the possibility that swine TTV may enhance coinfectant infections with other swine pathogens cannot be excluded. In this regard, a recent study (Kekarainen et al., 2006) has reported that PMWS affected pigs are more likely to be infected with swine TTV than non-PMWS affected animals.

Porcine TTV have been demonstrated to be genetically related to human TTV and to share a similar genomic organization. Although few studies conducted to date seem to indicate that TTV has host specificity and shows only poor viability in heterologous species (Mushahwar et al., 1999; Okamoto et al., 2000), the possible sanitary risk associated with frequent professional exposure of human beings to herds with a high prevalence of virus shedding should not be totally neglected (Bigarré et al., 2005).

In conclusion, our results confirm that swine TTV infections are common in intensively reared pigs, particularly in finishing herds. Further studies are needed to understand the epidemiology of porcine TTV infection, their pathogenic potential, and the possible interactions with other infectious agents. Experimental infections and longitudinal studies in infected herds could contribute to better understand these aspects. The genetic characterization of different porcine isolates will help in understanding if zoonotic transmission of swine TTV to human beings may occur.

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References


