A review of congenital tremor type A-II in piglets

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Abstract

Congenital tremor (CT) is a neurological disease that affects new-born piglets. It was described in 1922 and six different forms, designated type AI-V and type B, are described based on the causative agents, as well as specific histological findings in the central nervous system (CNS). The various forms present with identical clinical signs consisting of mild to severe tremor of the head and body, sometimes complicated with ataxia. By definition, all A-forms have hypomyelination of the CNS, whereas there are no histopathological lesions with the B-form. The cause of the A-II form was long unknown, however, at present several different viruses have been proposed as the causative agent: porcine circovirus-II (PCV-II), astrovirus, PCV-like virus P1, and atypical porcine pestivirus (APPV). Currently, APPV is the only virus that has been proven to fulfill Mokili's Metagenomic Koch's Postulates. Following infection of the pregnant sow, the virus passes the placental barrier and infects the fetus. Interestingly, no clinical signs of disease have been associated with APPV in adult pigs. Furthermore, other viruses cannot be ruled out as additional potential causes of CT. Given the increased interest and research in CT type A-II, the aim of this review is to summarize current knowledge.

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Introduction

Congenital tremor (CT) is a neurological disorder among newborn piglets that is clinically visible as a tremor of the head and body due to hypomyelination of the central nervous system (CNS). In severe cases the tremor may be complicated by ataxia, thus exacerbating the piglets' ability to suckle (Kinsley, 1922; Larsson, 1955; Done, 1968). The first description of the syndrome was made in the US in 1922 by Kinsley. An outbreak of unknown origin was noticed in a swine herd causing newborn piglets to shake and jump. The piglets looked like they were dancing, hence Kinsley designated the syndrome 'dancing pig disease' (Kinsley, 1922). The disease that is nowadays known as CT has since then been given a number of other names such as: 'trembles', 'trembling pigs', and 'myoclonia congenita' (Done, 1968, 1976).

The hypotheses regarding the causes of CT have changed over time, from the suggestion that it may be of dietary origin, to being an inherited disorder. In 1955, CT was described in Sweden and was suggested to be caused by a virus (Larsson, 1955) due to the epidemiological pattern of the disease. Later, several different agents and causes were related to CT, all inducing identical clinical signs in newborn piglets. Today, CT has been divided into six different subtypes – types AI-V and type B, based on the causal factor (Done, 1968, 1976).

The classification into A- and B-forms is based on the histopathological lesions in the CNS. Types AI-V are characterized by hypomyelination and vacuolization in the CNS, probably due to retarded myelination during fetal development, whereas type B has no evident lesions in the brain or in the spinal cord (Done, 1968, 1976; Done et al., 1986).

CT type A-I is associated with classical swine fever virus infection in the pregnant sow, leading to a transplacental infection of the fetus. In some of the literature, CT type A-I is described to sometimes be complicated by severe ataxia, compared to the other types of CT (Bradley et al., 1983). Type A-II is presumed to be caused by a newly discovered virus, atypical porcine pestivirus (APPV), that has been demonstrated in the CNS of CT-affected piglets. Furthermore, CT was induced by inoculation of pregnant sows with serum proven to be positive for APPV by the polymerase chain reaction (PCR) (Postel et al., 2016; Schwarz et al., 2017). The A-III and A-IV forms are hereditary within the British Landrace and Saddleback pig breeds, respectively (Done, 1976). The A-V-type is associated with metrifonate treatment of the pregnant sow, a drug that was previously used to treat ectoparasites in pigs (Larsson, 1955). The causative agent of the B-form is unknown; it has been suggested that the condition is idiopathic or at least not of infectious origin (Done, 1968, 1976). The nervous signs and histopathological lesions are, however, similar regardless of the cause; hence

laboratory diagnostics, e.g. molecular or toxicological methods, are needed to identify the causative agent.

In 1967, the first successful experimental infection inducing CT type A-II was performed. Sows at day 30 of gestation were injected intramuscularly with tissue from the CNS and spleen from piglets affected with a CT of unknown aetiology (Patterson et al., 1976; Done et al., 1986).

From the mid-1980s until the beginning of the 21st century, little research on CT was published until porcine circovirus-II (PCV-II), was isolated in piglets suffering from CT type A-II (Stevenson et al., 2001). Nervous signs such as tremor may occasionally be seen in piglets suffering from PCV-2 systemic disease (previously post-weaning multisystemic syndrome) caused by PCV-II. However, the role of PCV-II is now questioned as a presumptive cause of CT (Stevenson et al., 2001; Choi et al., 2002; Ha et al., 2005; Wen et al., 2018).

In recent years, a number of viruses have been detected in the CNS of pigs suffering from CT, by high-throughput sequencing. Of these, only APPV has been demonstrated worldwide (Hause et al., 2015; de Groof et al., 2016), whereas other viruses have been found in the CNS of piglets originating from specific geographical areas. For example, astrovirus was found in the brain of Swedish piglets suffering from CT (Blomstrom et al., 2014), LINDA virus (lateral-shaking inducing neurodegenerative agent-virus) was demonstrated in pigs from a farm in Austria (Schwarz et al., 2017) and a porcine circovirus-like virus P1 has been described in piglets from China (Wen et al., 2018).

Clinical signs

CT is present at birth and presents itself with tremor and, occasionally, ataxia. The tremor ranges from a slight shiver of the head to severe tremble of the entire piglet and may be complicated by ataxia or sometimes splay leg. In severe cases, the twitches can make the piglets jump off the floor (Kinsley, 1922; Done et al., 1986). There are no reports of fever or impaired demeanour associated with CT type A-II (Done, 1968; de Groof et al., 2016; Arruda et al., 2017; Schwarz et al., 2017).

CT is an action tremor characterized by an increased tremor when the muscles are activated, e.g. the piglet is moving or stressed, and an absence of neurological signs when the muscles are relaxed, e.g. when the piglet is asleep or at rest (Done et al., 1986). At present, there is no known medical treatment of CT, and whether a piglet will survive or not is dependent on its ability to grip the teat and suck as well as on its ability to avoid being crushed by the sow (Done, 1968, 1976). Most pigs recover spontaneously within 3 months of birth; however, some pigs might become chronic shakers (Done, 1976; Postel et al., 2016). Interestingly, in pigs where the shaking is no longer evident in the unstressed animal, external stimuli such as a sudden noise or handling might trigger the tremor; thus, recovered pigs can sometimes start shaking when exposed to stress, e.g. at the abattoir (Done, 1976; Schwarz et al., 2017).

A variety of infectious agents, intoxications, and conditions may induce neurological signs similar to those of CT. However, CT may be differentiated from these conditions, as it is an action-tremor present at birth and is absent or reduced at sleep or rest. Hypoglycemia and hypothermia of neonatal pigs are the most likely differential diagnosis as they will induce shivering within a short time after birth, although in these cases the tremor will commonly be present at sleep or at rest as well (Curtis, 1974). Other conditions such as PCV-2 systemic disease, Aujeszky's disease, porcine reproductive and respiratory syndrome, otitis media, aflatoxicosis, classical and African swine fever, and salt poisoning, may present with a large number of neurological signs, including tremor and ataxia, although the neurologic signs are not congenital and are usually combined with severe multisystemic disease (Baskerville, 1972; Liess, 1987; Done and Paton, 1995; Segales and Domingo, 2002; Schulz et al., 2017).

Epidemiology

The prevalence of CT type A-II is not well studied, but an estimate from China indicates that about 1–2% of all piglets are affected (Yuan et al., 2017). Typically, CT occurs on farms as outbreaks without any prior indication or clinical signs of infection among the sows (Done, 1976; Bolske et al., 1978; de Groof et al., 2016; Postel et al., 2016). The outbreak usually lasts for 2–3 months with a morbidity of 50–100%, being highest in litters born to first-parity sows. The mortality rates are generally low, but may occasionally approach 15–20% (Bolske et al., 1978; Postel et al., 2016; Schwarz et al., 2017). In some farms, however, losses have been reported to be as high as 50% during outbreaks (Bolske et al., 1978; Done et al., 1986; Lamp et al., 2017).

There are a few within-farm epidemiological studies of outbreaks of CT associated with APPV.

When CT outbreaks caused by APPV occur on a farm, piglets born to primiparous sows are more commonly affected, whereas piglets born to multiparous sows usually remain unaffected (de Groof et al., 2016; Postel et al., 2016). The within-litter prevalence of APPV PCR-positive piglets, during an outbreak of CT, varies from <10 to 100%, which is evenly distributed between sexes and does not affect the average number of live-born piglets (de Groof et al., 2016).

Gross and histological lesions

Gross examinations of the CNS, the peripheral nervous system or the skeletal muscles of affected piglets are, in general, without any significant findings (Done et al., 1986; Blomstrom et al., 2014; Postel et al., 2016). However, varying degrees of hypomyelination and mild vacuolization of the CNS are characteristic histologic findings, both in CT cases where APPV is detected and in CT cases where astrovirus is detected (Done et al., 1986; Blomstrom et al., 2014; Postel et al., 2016; Lamp et al., 2017).

The hypomyelination of the CNS presumably causes impaired impulse transmission and saltatory conduction, resulting in neurologic signs such as tremor or ataxia (Patterson et al., 1976; Done et al., 1986). The hypomyelination is commonly characterized by thinly myelinated axons and occasionally nonmyelinated axons; any myelin that is present, however, has a normal histological appearance (Duncan, 1987). Interestingly, the extent of the hypomyelination of the CNS varies between individuals as well as between studies and outbreaks.

Done et al. (1986) used a light microscope to measure the diameter of the spinal cord from piglets born with CT, and reported that the spinal cord from a CT-affected piglet had, on average, a 12% decrease of myelin compared to a healthy control piglet. On the other hand, others report only a mild, unquantifiable hypomyelination, visualized as a mildly reduced staining intensity by Luxol fast blue (commonly used to stain myelin for light microscopy) in the spinal cord from severely affected CT-piglets (Postel et al., 2016). The hypomyelination could also

be noted in the brain as numerous small vacuoles of the white matter (Blomstrom *et al.*, 2014).

More prominent and extensive vacuolization due to hypomyelination of the white matter in the brain was documented in piglets during a CT type A-II outbreak in Austria, where the LINDA virus was discovered. The remaining myelin in the CNS, however, had a normal constitution and structure (Lamp *et al.*, 2017). This extensive vacuolization corresponded to the LINDA virus-affected piglets being more atactic and presenting with tremor of higher intensity than commonly described in other outbreaks of CT type A-II (Lamp *et al.*, 2017).

A recent study from China reported not only the discovery of a new virus, a porcine circovirus-like virus P1, in the brain of CT-piglets, but also previously unreported histological lesions in the brain. In this particular outbreak, the predominant lesion was the dissolution of the nucleus of Purkinje cells affecting coordination and balance (Wen *et al.*, 2018). If these cells are damaged, the piglet will present with neurologic signs such as tremor and ataxia (Wen *et al.*, 2018).

Causative agents

Soon after CT type A-II was first recognized, it was suggested to be caused by a virus (Larsson, 1955). Hence, when Done *et al.* (1986) were able to induce CT in piglets by injecting pregnant sows with tissue from CT-affected piglets, it was taken as evidence to support this hypothesis. Since then, several viruses have been associated with CT type A-II. At present, a virus first described in 2015, APPV, is proposed as a cause of CT type A-II (Hause *et al.*, 2015; Arruda *et al.*, 2016; de Groof *et al.*, 2016; Postel *et al.*, 2017a). Interestingly, APPV has been detected in the brain of piglets with CT in Brazil as a co-infection together with porcine teschovirus (Possatti *et al.*, 2018b).

In 1994, PCV-II was suggested as the causative agent of CT type A-II (Hines, 1994), but since 2005 it is no longer generally regarded as a plausible causative agent. Because PCV-II is regularly detected in neurologically normal piglets, and rarely in piglets suffering from CT, it is usually considered as an incidental finding in piglets (Chae, 2005; Ha *et al.*, 2005).

In 2014, astrovirus was demonstrated in the brain of piglets suffering from CT type A-II. However, astrovirus was also detected in the brain from healthy piglets. Hence, the hypothesis of astrovirus as a plausible cause of CT type A-II could be neither confirmed nor rejected (Blomstrom *et al.*, 2014). However, 2 years later, astrovirus was described as an incidental finding together with APPV in brain-homogenate from piglets with CT (de Groof *et al.*, 2016).

A pestivirus provisionally named 'LINDA virus' was discovered in the brain of piglets from an Austrian farm, in connection with an outbreak of CT (Lamp *et al.*, 2017). By sequencing, the LINDA virus showed 60% identity to APPV and 68% identity to Bungowannh virus (Lamp *et al.*, 2017). The mortality rate during the LINDA virus outbreak was higher than in the average CT outbreak, due to the intense lateral shaking, impairing the piglets' capability to suckle. Only 22.4 piglets per sow were weaned during the year of the outbreak, compared to on average 25.8 piglets per sow per year (Lamp *et al.*, 2017). The outbreak stopped abruptly when all sows had each given birth to one CT-affected litter, which may indicate that they developed immunity to the virus (Lamp *et al.*, 2017). No new reports of the LINDA virus have been published since this outbreak.

The latest report of a virus that might be a causative agent of CT type A-II is the recent finding from China of PCV-like virus P1, which has been found in the brains of piglets with CT type A-II (Wen *et al.*, 2018). No follow-up studies on this virus have yet been published.

Atypical porcine pestivirus (APPV)

APPV was first detected in the US in 2015, by high-throughput sequencing of porcine sera. In a few publications, it has also been referred to as 'porcine pestivirus' (Blomstrom *et al.*, 2016). This previously unknown virus was found to be widespread in the US pig population (Hause *et al.*, 2015). Soon after the detection of the APPV, it was associated with CT type A-II. The virus was present in the CNS of newborn piglets with CT, whereas it was absent from the CNS in the vast majority of their healthy littermates and in the healthy control piglets (Arruda *et al.*, 2016; de Groof *et al.*, 2016; Postel *et al.*, 2016; Schwarz *et al.*, 2017; Gatto *et al.*, 2018a, 2018b).

Researchers were also able to induce CT by injecting pregnant sows with sera containing APPV (Arruda *et al.*, 2016; de Groof *et al.*, 2016). Hence, Mokili's Metagenomic Koch's Postulates were fulfilled (Mokili *et al.*, 2012) although Koch's postulates *per se* are yet to be fulfilled.

APPV is assigned to the family Flaviviridae, genus Pestivirus. It is an enveloped RNA virus with a positive sense, single-stranded genome of about 11–12 kb, and it has four structural proteins (C, Erns, E1, and E2) and eight non-structural proteins (Npro, P7, NS2, NS3, NS4B, NS5A, and NS5B) (Hause *et al.*, 2015). The genome is highly variable, but, so far, genomic clustering of the virus related to geographic areas has not been possible, because almost every farm with clinical disease has a distinct strain of APPV (de Groof *et al.*, 2016; Beer *et al.*, 2017; Munoz-Gonzalez *et al.*, 2017; Smith *et al.*, 2017). Changes in pestivirus nomenclature have been suggested, with APPV being designated as pestivirus K (Smith *et al.*, 2017).

Since the first finding of APPV (Hause *et al.*, 2015), the virus has been detected globally, e.g. in China, Sweden, Germany, Great Britain, Italy, Serbia, Switzerland, Taiwan, Brazil, Austria, Hungary, and Spain (Hause *et al.*, 2015; Blomstrom *et al.*, 2016; Postel *et al.*, 2016; Munoz-Gonzalez *et al.*, 2017; Denes *et al.*, 2018; Gatto *et al.*, 2018b). Furthermore, the oldest detection of APPV is currently from samples stored in 1997, originating from Spanish piglets with CT (Munoz-Gonzalez *et al.*, 2017).

Because APPV is a recent finding, the global prevalence has not been well defined, but both enzyme linked immunosorbent assay (ELISA) and PCR have been developed for diagnostic purposes (Hause *et al.*, 2015; Schwarz *et al.*, 2017; Yuan *et al.*, 2017; Postel *et al.*, 2017a, 2017b). No cross-reactivity with APPV-specific antibodies in the diagnosis of classical swine fever has been reported (Postel *et al.*, 2017b).

In 2017, serum samples from 1460 healthy pigs from Europe (Germany, Great Britain, Italy, Serbia, and Switzerland) and Asia (mainland China and Taiwan) were tested using an indirect APPV-ELISA and an APPV-specific PCR. The result indicated that 8.9% of the 1460 pigs were PCR-positive for APPV and 60% of the pigs were APPV-positive by serology (Postel *et al.*, 2017a). This is in line with other studies, e.g. from China, where 5.2% of 135 sampled pigs were PCR-positive for APPV (Yuan *et al.*, 2017), and in a screening of semen where 15% of 597 breeding boars in the United States were PCR-positive for the virus (Gatto *et al.*, 2018a).

The highest viral load of APPV has been detected in lymphoid organs such as tonsils, lymph nodes, spleen, and thymus, which are also the tissues where APPV is most commonly detected. The virus is also frequently found in the brainstem, cerebrum, cerebellum, sera and in nasal and rectal swabs (Arruda *et al.*, 2016; Postel *et al.*, 2016; Munoz-Gonzalez *et al.*, 2017; Yuan *et al.*, 2017; Gatto *et al.*, 2018*a*).

The main histopathological findings associated with APPV-infection are the demyelination and/or hypomyelination of the CNS. Additional lesions commonly observed in the CNS consist of neuronal necrosis of the brain, gliosis, and neuronophagia with satellitosis, lesions that are more suggestive of necrosis than of inflammation (Possatti *et al.*, 2018*b*).

The transmission route of APPV is yet to be determined. However, frequent detection of APPV in salivary glands, mandibular lymph nodes, duodenum, pancreas, and colon, together with prolonged shedding of virus in feces, might be suggestive of a fecal–oral route of transmission (de Groof *et al.*, 2016; Postel *et al.*, 2016).

Since APPV is difficult to propagate in cell culture, serum from piglets with clinical CT that were PCR-positive for APPV have been used to inject sows in two independent successful experiments (Arruda *et al.*, 2016; de Groof *et al.*, 2016). However, in 2017, APPV was successfully grown on Porcine Kidney cells-15 (Schwarz *et al.*, 2017). This was done using an APPV-positive serum sample originating from a piglet with signs of CT, where colostral antibodies were avoided since the serum was obtained before suckling.

Arruda *et al.* (2016) infected five sows at day 45 and 62 of pregnancy by simultaneous intravenous injection, intranasal inoculation, and injection of APPV directly into the amniotic vesicle, whereas de Groof *et al.* (2016) infected three sows at day 32 of gestation via intramuscular injection. In both experiments, none of the sows that were inoculated with APPV developed any clinical signs of disease, nor did they develop a PCR-detectable viremia. However, these sows gave birth to piglets with typical signs of CT, and the piglets were also PCR-positive for APPV in multiple organs (e.g., CNS, spleen, tonsils, and serum) (Arruda *et al.*, 2016; de Groof *et al.*, 2016). In these studies, the within-litter prevalence of piglets born with signs consistent with CT was 57–100% (Arruda *et al.*, 2016) and 0–100%, respectively (de Groof *et al.*, 2016). Interestingly, a proportion of the piglets in both studies also showed signs of splay-leg (0–40% of the pigs within the litter) in addition to CT (Arruda *et al.*, 2016; de Groof *et al.*, 2016). Splay leg is a condition characterized by impairment of the adducting muscles of the hindlimbs due to hypomyelination of the spinal cord and the nerves innervating the affected muscles (Thurley *et al.*, 1967; Szalay *et al.*, 2001). In both studies, all piglets born with CT were positive for APPV; however, the majority of healthy piglets born to APPV-inoculated sows were also PCR-positive for APPV (Arruda *et al.*, 2016; de Groof *et al.*, 2016). Arruda *et al.* (2016) reported that 10 out of 11 healthy piglets were APPV-positive and de Groof *et al.* (2016) reported that 26 out of 28 healthy piglets were APPV-positive. Why APPV may be present in the CNS of healthy piglets is yet unknown.

Since it was hypothesized that APPV infection can result in persistently infected animals, two studies were performed to elucidate this proposal. A longitudinal follow-up study was done in piglets with CT, originating from the experimental infection (de Groof *et al.*, 2016). Most of these piglets were free from signs of CT, and 20 out of 27 were PCR-negative for APPV in serum at 4.5 months of age (de Groof *et al.*, 2016). Interestingly, five piglets were found to be asymptomatic carriers of APPV at 8.5 months of age. These pigs were PCR-negative for APPV in serum but were shedding high amounts of the virus in feces, saliva, and in preputial fluid (de Groof *et al.*, 2016). In the second study, two pigs, one female and one male, were born with CT and sampled over a 6-month period. They had APPV NS3H-specific antibodies present until 12 weeks of age and signs of CT until 14 weeks of age (Schwarz *et al.*, 2017). At 6 months of age, the boar reached sexual maturity without any detectable levels of APPV NS3H-specific antibodies, but with high levels of APPV in both saliva and semen detected by the PCR. The viral concentration in serum, however, was low (Schwarz *et al.*, 2017). At 6 months of age, the sow also had high APPV loads in the saliva; however, the serum level of APPV was below the PCR detection level (Schwarz *et al.*, 2017).

Conclusion

APPV has been proven to cause both neurologic signs and histologic lesions in the CNS consistent with CT type A-II, as well as previously undescribed inflammatory lesions (de Groof *et al.*, 2016; Schwarz *et al.*, 2017; Gatto *et al.*, 2018*a*; Possatti *et al.*, 2018*a*). Interestingly, APPV is not the only viral finding in the brain of piglets suffering from CT; astrovirus, a pestivirus named 'LINDA virus', a porcine teschovirus, and porcine circovirus-like virus P1 have been detected, opening up the possibility of a number of potential virus and/or co-infections causing CT (Blomstrom *et al.*, 2014; de Groof *et al.*, 2016; Lamp *et al.*, 2017; Wen *et al.*, 2018; Possatti *et al.*, 2018*a*, 2018*b*). Because CT is classified into sub-groups based on the causative agent, it is possible that, in the years to come, more than six types will be identified. Further research is needed to elucidate if several viruses are capable of inducing CT and to elucidate their route of transmission. In our view, the most interesting tasks at present are to identify the transmission route of APPV and to determine if any additional viruses may induce CT.

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