UGONNA HENRY UZOKA

PESTIVIRUS IN PIGS: A SYSTEMATIC REVIEW AND OCCURRENCE IN THE MICRO-REGION OF PONTE NOVA, BRAZIL

Dissertation submitted to Veterinary Medicine Graduate program of the Universidade Federal de Viçosa, in partial fulfillment of the requirements for the degree of *Magister Scientiae.*

Advisor: Abelardo Silva Junior

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I give thanks to God my creator for the success of this work

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ABSTRACT

UZOKA, Ugonna Henry, M.Sc., Universidade Federal de Viçosa, February, 2023. **Pestivirusin pigs: a systematic review and occurrence in the Micro-region of Ponte Nova, Brazil**. Adviser: Abelardo Silva Júnior.

Pestiviruses are worldwide in distribution, causing a significant economic loss in the livestock industry. Atypical porcine pestivirus (APPV) affects piglets and causes high mortality as a result of the neurological signs that make them unable to suckle. Bovine Viral diarrhea virus (BVDV) is another pestivirus that affects pigs and has cattle as the major host and mixed farming of both species can lead to transmission of the disease leading to severe economic loss and difficulty in diagnosis of classical swine fever. Thus, the first study comprises a systematic review of the prevalence of APPV in pigs in the world. In the systematic review, we collectedas many relevant citations as possible regarding different countries that have reported the prevalence of APPV from 2015 to 2022. From the selected articles, data from the year of sample collection, the diagnostic technique, the samples used, the animal stage sampled, and the clinical signs were extracted. This systematic review found that real-time RT-PCR was used most often to detect the APPV genome in serum and/or tissue samples, also APPV is linked to CT in piglets. We also found that APPV infection can occur in herds with no clinicaldisease and that boars may play a critical role in APPV epidemiology. The review suggests that researchers in different countries study healthy pigs, CT piglets, and boars to find out the prevalence of APPV which will enhance the epidemiology survey. The second study on the occurrence and distribution of antibodies against BVDV-1 and BVDV-2 using a viral neutralization test in nine swine farms in the micro-region of Ponte Nova, Minas Gerais State,Brazil to be informed on its prevalence in sows and finished pigs. Four hundred serum samples from sows and finished pigs (200 each) were collected from nine farms in six municipalities; Jequeri, Rio Casca, Urucania, Ponte Nova, Teixeras, and Coimbra all in the microregion of Ponte Nova revealed low antibody titers (between 2 - 3.32 in log2). A BVDV prevalence of 4.75% was recorded and a herd prevalence of 44.4%. BVDV-1 had a prevalence of 0.25% and BVDV-2 4.5%. No prevalence was recorded in the farms from Rio Casca and Ponte Nova. Finished pigs recording more prevalence. These positive regions should be under close observation by the veterinary inspection services department as the presence of BVDVantibodies in swine serum can lead to false positive results of classical swine fever because of serological cross-reaction which can hamper CSF eradication programs.

Keywords: Bovine viral diarrhea virus. Revalence. Atypical porcine pestivirus. Virus. Congenital tremor. Swine.

RESUMO

UZOKA, Ugonna Henry, M.Sc., Universidade Federal de Viçosa, February, 2023**. Pestivírus em suínos: uma revisão sistemática e ocorrência na micro-região de Ponte Nova, Brasil.** Orientador: Abelardo Silva Júnior.

Os pestivírus estão em distribuição mundial, causando uma perda econômica significativa na indústria pecuária. O pestivírus suíno atípico (APPV) afeta leitões e causa alta mortalidade como resultado dos sinais neurológicos. O vírus da diarreia viral bovina (BVDV) é outro pestivírus que acomete suínos e tem os bovinos, o principal hospedeiro e a criação mista de ambas as espécies pode levar à transmissão da doença, levando a graves perdas econômicas e dificuldade no diagnóstico da peste suína clássica. Assim, o primeiro estudo compreende umarevisão sistemática da prevalência de APPV em suínos no mundo. Na revisão sistemática, coletamos o maior número possível de citações relevantes sobre diferentes países que relataram a prevalência de APPV de 2015 a 2022. Dos artigos selecionados, foram extraídos dados do ano de coleta da amostra, da técnica diagnóstica, amostras utilizadas, país e dos sinais clínicos. Esta revisão sistemática descobriu que a RT-PCR em tempo real foi usada com mais frequênciapara detectar o genoma da APPV em amostras de soro e / ou tecido, também a infecção por APPV está ligado ao tremo congênito em leitões. Também descobrimos que a infecção por APPV pode ocorrer em rebanhos sem doença clínica e que os javalis podem desempenhar um papel crítico na epidemiologia. A revisão sugere que pesquisadores em diferentes países estudem porcos saudáveis, leitões clinicamente afetados e javalis para descobrir a prevalênciade APPV, o que aumentará a pesquisa epidemiológica. O segundo estudo investigou a ocorrência e distribuição de anticorpos contra BVDV-1 e BVDV-2 utilizando um teste de neutralização viral em nove granjas da microrregião de Ponte Nova, Minas Gerais, Brasil. Quatrocentas amostras de soro de porcas e suínos acabados foram coletadas em seis municípios; Jequeri, Rio Casca, Urucania, Ponte Nova, Teixeras e Coimbra. Os resultados demonstraram que os animais positivos apresentavam baixos títulos de anticorpos (entre 2 - 3,32 em log2). Registrou-se prevalência de BVDV de 4,75%. Sendo que a prevalência de BVDV-1 foi de 0,25% e para o BVDV-2 de 4,5%. Nenhuma prevalência foi registrada nas fazendas de Rio Casca e Ponte Nova. Suínos de terminação apresentaram maior prevalência. Estas regiões positivas devem ser observadas de perto pelo serviço de inspecção veterinária, uma vez que a presença de anticorpos BVDV no soro suíno pode levar a resultados falso- positivos da peste suína clássica.

Palavras-chave: Vírus da diarreia viral bovina. Prevalência. Pestivírus atípico suíno. Vírus. Tremor congênito. Suíno.

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LIST OF ABBREVIATIONS

CSFV	Classical Swine Disease Virus
BVDV	Bovine Viral Disease Virus
BDV	Border Disease Virus
OIE	International Epizootic Office
ELISA	Enzyme-linked immunosorbent assay
SNT	Serum neutralization test
RT-PCR	Reverse transcription-polymerase chain reaction
GMT	Geometric Mean Titer
NCR	Non-coding region
UTR	Untranslated region
N-pro	N-terminal auto protease
CP	Cytopathogenic
NCP	Non-Cytopathogenic
MAB	Monoclonal antibody
DFA	Direct fluorescent antibody test
MDBK	Madin Darby bovine kidney

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General Introduction

Prior to 1991, pestiviruses were allocated to the family *Togaviridae*. In 1991, they were transferred to the family *Flaviviridae* (Ridpath et al. 2010). Pestiviruses infect both domestic and feral even-toed ungulates (Artiodactyla), including cattle, sheep, pigs, and old- and new- world camelids, as well as deer, chamois, and antelopes (Ridpath et al. 2010; Hilbe et al. 2012). Pestiviruses are highly contagious and can be spread through direct or indirect contact with infected animals, as well as through contaminated feed, water, or equipment.

The members of Flaviviridae show various degrees of similarity in their geneticorganization. The genome of the encapsulated pestivirus is a positive-polarity, single-strandedRNA with a single big open reading frame. Contaminations of cell lines, fetal bovine sera madein diverse locations of the world, and even animal or human vaccines may likely lead to the discovery of additional pestiviral strains in the future (Studer et al 2002; Kosaza et al 2011). New strains of pestiviruses and novel host species have developed on a regular basis includingin Switzerland (Bachofen et al., 2008); at least a portion of this growth can be attributed to a greater knowledge of the prevalence of these viruses. Pestiviruses, such as classical swine fever virus (CSFV), bovine viral diarrhea virus (BVDV), and atypical porcine pestivirus (APPV) are important pathogens in pigs. BVDV and CSFV are classified as reportable diseases by the Office International des Épizooties (OIE). These viruses cause significant economic losses to the livestock industry, due to reduced productivity, increased mortality, and trade restrictions.BVDV, including the two species (formerly called genotypes BVDV-1 and BVDV-2), is one of the most virulent infectious agents in cattle, with a global distribution and a high incidence rate (Rümenapf et al. 2008). Notably, it may be one of the most prevalent terrestrial viral representatives. BVDV and Border disease virus (BDV) are sometimes known as ruminant pestiviruses, they have minimal virulence and rely on a unique form of persistence in a small proportion of hosts. Unlike other viruses that cause persistent infection (PI), BVDV and BDVdo not elicit an immune response in their hosts but are immunologically tolerated (Peterhans et al. 2013). BVDV and BDV are capable of either transitory or permanent infection of their hosts(Barkema et al. 2001).

In many regions of the world, classical swine fever is endemic in both domestic pigs and wild boars. CSF, previously known as hog cholera, is caused by the CSF virus (CSFV) andcan result in severe morbidity and mortality in pigs despite eradication attempts. Identificationof the virus can significantly reduce pig exports (Paton et al. 2003).

Bungowannah virus, initially isolated from pigs in an Australian piggery (Kirkland et al. 2007), Linda virus atypical (HoBi-like) pestiviruses found in Europe, Asia, and South America (Stahl et al. 2010; Mao et al 2012; Decaro et al 2013), atypical porcine pestivirus (Hause et al. 2015) and the pronghorn antelope pestivirus (Vilcek et al. 2005) are only a few instances of such newly developing pestivirus strains.

The Atypical Porcine Pestivirus (APPV), which is ubiquitous in pig herds worldwide, was originally identified in the United States in 2015 and has subsequently been detected in Asia, Europe, North America, and South America (Mosena et al. 2017; Gatto et al., 2019,). APPV is not a new virus (Kauffman et al., 2019), but a newly found virus that is well- established in numerous pig populations and likely disseminated globally (Postel, et al., 2017). It is suspected that the virus can elicit type A-II congenital tremors (CT) in newborn piglets and can lead to significant losses in production as a result of death of the piglets due to starvation. This syndrome is comparable to that produced by CSFV which is highly contagious and is characterized by body tremors and varying brain and spinal cord hypomyelination (Bradley et al., 1983).

The symptoms of pestivirus infections vary depending on the strain of the virus and thespecies of the animal, but common symptoms include fever, diarrhea, vomiting, and weight loss. Effective control and prevention measures for pestiviruses include vaccination, biosecurity measures, and early detection and eradication of infected animals. In severe cases, pestivirus infections can result in high mortality rates particularly in young animals, reduced productivity and increased mortality. The increasing prevalence of atypical porcine pestiviruses in the world and the incidence of bovine viral diarrhea virus in pigs which causesserological cross reaction leading to false positive result during CSF diagnosis and can affect eradication programs.

CHAPTER 1: A systematic review on the prevalence of pestivirus K in pigs (Atypicalporcine pestivirus)

Abstract

Pestiviruses are worldwide in distribution, causing a significant economic loss in livestock, and have been reported to have a high prevalence in most regions. Atypical porcine pestivirus (APPV) is one of the pestiviruses that affect pigs and causes high mortality in piglets as a result of the neurological signs that make them unable to suckle. The atypical porcine pestivirus (APPV) is an RNA virus from the Flaviviridae family that is associated with congenital tremor (CT) type A-II in newborn piglets. APPV has been in circulation as far as 1986 but was first discovered in2015 in the United States of America. Due to the reported global outbreaks of CT in several continents, its capacity to coinfect, and its effect on pig productivity, more attention should be placed on atypical porcine pestivirus (APPV) research. This study collects as many relevant citations as possible regarding different countries that have reported the prevalence of APPV from 2015 to 2022. From the selected articles, data from the year of sample collection, the diagnostic technique, the samples used, the animal stage sampled, and the clinical signs were extracted. We discovered that APPV prevalence had been documented in different countries that represent the continents; Europe, South America, North America, and Asia. The results showed that APPV is worldwide in distribution and its prevalence may be under-reported by different countries. China had a greater number of articles published on the prevalence of APPV. Europe as a continent has more articles published reporting the prevalence of APPV. We observed that real-time RT-PCR was used by most studies to detect the APPV genome in serum and/or tissue samples and that APPV infection can occur in herds with no obvious clinical disease. Different reports from the articles in this study relate the prevalence of APPV to congenital tremor (CT) in piglets and also, boar/ wild boars revealed the presence of APPV in their serum sample which may suggest that persistent infection can occur and boars can play a critical role in APPV epidemiology. Due to the reported global outbreaks of CT in several continents, we encourage researchers in different continents/countries to conduct research on also healthy pigs, CT piglets, and boars to avail information on the prevalence of APPV which will enhance knowledge on APPV epidemiology.

Keywords: virus, swine, congenital tremor

1.0 Introduction

In the last few decades, numerous novel pestiviruses have been discovered in domesticand wild ruminant species (Kirkland et al., 2007; Wu et al., 2012; Firth et al., 2014). Congenital tremor (CT) was initially documented about a century ago. Previous research has revealed thatthere are numerous causes of CT, including diet, genetics, and viral infections (Knox et al., 1978; Bradley et al., 1983). CT, popularly known as "dancing piglet," was originally described in neonatal piglets (Kinsley, 1922). CT is classified as types A and B. Type A is characterizedby obvious histological lesions in the central nervous system (CNS), whereas type B lacks such lesions (Done, 1968). However, the sickness was traced primarily to an unnamed virus (Arruda et al., 2016). In 2015, technological advancements in sequencing led to the first description of Atypical porcine pestivirus (APPV) from pig serum using metagenomic sequencing (Hause et al., 2015). Pestsivirus are known to impact a wide variety of ruminants, both domestic and wild, including pigs. A wide variety of species, including cattle, pigs, goats, antelope, Norway rats, and bats, have been shown to harbor atypical strains of the virus. There has not been any mention of a zoonotic pestivirus in the literature. Pestivirus is known to cause a variety of clinical symptoms based on the virus species and strain, as well as the age and immune status of the host (Bielefeldt 1988).

The APPV of the *Flaviviridae* family and *Pestivirus* genus is made up of a positive-sense single-stranded RNA (+ssRNA) with a size of roughly 11.5 kb [Hause et al 2015]. It has an open reading frame (ORF) of 3665 amino acids, and two sections flanked by 5’ untranslated regions (5-UTR) and 3’ untranslated regions (3-UTR). According to several studies (Stark et al. 1993, Lamp et al. 2013, Hause et al. 2015, Tautz et al. 2015, Pan et al. 2019,) the polyprotein that this RNA encodes is likely processed into four structural proteins (C, Erns, E1, and E2) and eight non-structural proteins (Npro, P7, NS2, NS3, NS4A, NS4B, and NS5A and NS5B. The E2 protein is regarded as the main antigenic component of the pestivirus family, which can trigger the generation of protective neutralizing antibodies. It is the main target of subunit vaccines that are being developed for the treatment of diseases (Zhang et al 2018, Li et al 2020 Liu et al 2021,). The E2 protein is a significant component of the envelope and is involved in interaction with cellular receptors. On the other hand, the E1 and the E2 can combine to form heterodimers (Wang et al., 2015). E^{rns} glycoprotein is known to mediate the neutralizing effect on antibodies. NS5b is an example of an RNA-dependent polymerase that is responsible for the initiation of replication (Li et al., 2020). NS5a is involved in the replication of viral RNA, the assembly of viral particles, and interactions between viruses and their hosts (Tellinghuisen

et al., 2006). NS2 has cysteine-auto protease activity, while NS3 is a multifunctional protein with serine protease, helicase, and NTPase activity (Klemens et al., 2015). High genetic variety and variability are characteristics of the APPV strains, according to (Postel et al., 2017).

The Atypical Porcine Pestivirus (APPV), which is prevalent in pig herds all over the world, was first identified in the United States in 2015 and has since been reported in different continents, including Asia, Europe, and North and South America (Mosena et al. 2017; Gatto et al., 2019,). It is believed that the virus can induce type A-II congenital tremors in newborn piglets (CT). This syndrome is similar to the condition caused by classical swine fever virus and is characterized by body tremors and variable hypomyelination in the brain and spinal cord (Bradley et al., 1983). The primary clinical signs are rhythmic head, abdominal, and limb tremors (spaying legs). These tremors eventually become increasingly incapacitating, causing difficulties standing or complete incapacity to move, ultimately resulting in starvation of affected piglets as they are unable to stand to eat (de Groof et al., 2016; Schwarz et al., 2017; Yuan et al., 2017; Gatto et al., 2018). In addition to demyelination, which is diagnostic of an APPV infection, (Possatti et al., 2018) observed neuronal necrosis in the cerebrum and cerebellum, neuronophagia, and gliosis in all piglets. Their findings suggest that neuronal necrosis of the brain, neuronophagia with satellitosis, and gliosis, in addition to the characteristic white matter demyelination and/or hypomyelination, ballooning degeneration of the uroepithelium and respiratory epithelium, should be considered as neuropathologic features of APPV infection and that these lesions are more necrotic than inflammatory. Atypical porcine Pestivirus targets the CNS (cerebellum) and lymphoid organs (thymus, lymph node, tonsil, and spleen), and has been detected in the feces, boar preputial swabs, preputial fluid, semen, digestive tract (small and large intestines, pancreas and liver), lungs, hearts, and semen (Postel et al 2016; Arruda et al., 2016; Gatto et al 2018; Houston et al., 2022).

In addition to horizontal transmission via the oronasal route, APPV could also be transmitted vertically via transplacental infection (Arruda et al., 2016; de Groof et al., 2016). In the Flaviviridae family, there are 11 identified species that are not related to one another [Smith et al. 2017., King et al. 2018]. APPV classifications might aid swine-related molecular epidemiology research. Although the economic significance of the losses brought on by the APPV outbreak is still unknown, it has been estimated that pigs' reproductive efficiency on farms infected with the virus declined by 10% (Schwarz et al., 2017). Preweaning mortality rises in APPV-infected newborn pigs who develop clinical sickness of CT, which impairs their ability to suckle milk (Pan et al., 2019). Early research from Germany and the United States showed that a significant fraction (22.4%, and 2.4%, respectively) of the virus' genome was

found in pigs that appeared to be in good health (Hause et al., 2015; Postel et al., 2016; Beer et al., 2016).

A phylogenic study of the genomes of APPV strain samples from pigs and wild boars in a variety of countries and regions uncovered a high level of genetic diversity among the strains, with some strains subdividing into numerous clusters. This was the case with some of the strains (Shen et al., 2018; Sozzi et al., 2019). According to the findings of numerous scientific investigations, the various strains of the APPV virus can be divided into three distinctclasses on the basis of the genomic sequences that they carry: types I, II, and III (Yan et al., 2019; Guo et al., 2020). Additionally, type A-E was discovered based on the Npro or E2 lineages (Zhang et al., 2018).

Multiple studies have reported both conventional polymerase chain reaction (RT-PCR) and quantitative RT-PCR for virus detection (Arruda et al., 2016; Possatti et al., 2018). Metagenomic sequencing is a potent method for identifying novel virus pathogens, and it was successfully used to identify the APPV for the first time in swine in the United States (Hause et al., 2015). With the availability of the APPV genome sequence, several RT-PCR protocols that target conserved regions within NS3, NS4B, or NS5 have been developed to detect the APPV and quantify the viral genome copies in clinical samples (Arruda et al., 2016; Mosena et al., 2017; Postel et al 2017). The virus has also been identified by polymerase chain reaction (PCR) from peripheral nerves, heart, lungs, liver, kidney, bladder, pancreas, small intestine, central nervous system (brain, cerebellum, brainstem, spinal cord, and trigeminal ganglia) and the lymphoid organs (thymus, tonsils, spleen, and mandibular, tracheal, inguinal, or mesenteric lymph nodes) (Arruda et al 2016, de Groof et al 2016, Schwarz et al 2017, Yuan et al 2017). Serological assays are another important diagnostic tool used to identify infectious diseases. Assays for APPV infection include indirect immunofluorescence test (IFI), virus neutralization, and an indirect enzyme-linked immunosorbent assay (ELISA) to the NS3, E2, and E^{rns} proteins (Schwarz et al., 2017; Cagatay et al., 2019; Michelitsch et al., 2019). These tests can be used for a variety of important objectives, such as population-based epidemiological investigations and the monitoring of sickness within the herd. They are also straightforward and inexpensive.

The results of a serological investigation suggested that APPVs may be prevalent throughout the pig population in the United States (Hause et al., 2015). Congenital tremors (type AII), a disorder that was just recently related to APPV, can also be discovered randomly in a number of nations that produce pigs, and it is likely to be present everywhere in the world (Arruda et

al., 2016). The earliest year that the APPV isolate can now be linked to is 1986, indicating that the virus has been present in Switzerland (Kauffman et al., 2019).

With the increasing number of APPV outbreaks, this study aims at gathering several published articles on the prevalence of APPV in different countries to give a better insight and report on different prevalence recorded, the year of sample collection and detection technique used which can help us confirm if APPV is worldwide and also give us information to enhance epidemiological survey.

2.0 Methods

We reviewed all studies on Atypical porcine Pestivirus. Our strategy can be divided into three components: plan review, conduct review, and report review. Planning the review was the primary step, during this phase, research questions were chosen to include the population of study, the intervention, the outcome/results and the context. A protocol was devised, and the procedure was ultimately validated to see whether the approach was practical. When all of this information was been defined, the protocol was reevaluated to ensure that the review protocol is appropriate. When conducting the review, the publications were chosen by searching each database. The data was extracted, which means that their details regarding authors, and year of publication were recorded. After accurately extracting the necessary data, the data were synthesized to offer an overview of the relevant publications published to date (2015 - December, 2022). The review report was completed by documenting the results.

To collect as many pertinent citations as possible, a comprehensive search of medical, agricultural, and scientific databases was conducted to identify primary studies in this field.

The literature was obtained from online databases including Science Direct (https://www.sciencedirect.com/), Web of Science (http://accesss.webofknowledge.com/), Pub med (https://pubmed.ncbi.nlm.nih.gov/), and Scopus (https://www.scopus.com/search/). Data extracted were considered because of their high ability to appropriate filters and retrieve relevant information in this field of study.

This keywords for this study were: “atypical porcine pestivirus”, AND “pestivirus K” AND “prevalence” AND “swine”. The following aspects were taken into consideration in each study: the country where the samples were taken, the year of sample collection for each study, the category of animal sampled (piglet, boar/wild boar, sow/gilt), clinical symptoms, detection method, samples used, and the prevalence recorded.

In the initial phase, the titles of all articles in the database were thoroughly analyzed to determine their relevance to this systematic review. Articles that did not meet all of the aforementioned inclusion criteria were excluded. Articles that were not in English or is a review paper was excluded, also articles that did not record the prevalence of atypical porcine pestivirus in swine was screened out. To reduce the likelihood of personal errors and/or bias, two independent reviewers conducted the screening in parallel and double-checked disagreements.

3.0 Results and Discussion

A total, of 328 references were collated in a bibliographic management system software (Zotero). From the 328 articles selected from the databases using the keywords at the conclusion of the search in December 2022. We had 67 articles in Science Direct, 64 in Pub Med, 99 in Scopus, and 67 in Web of Science (98). 159 articles were removed due to duplicate elimination, 91 records were eliminated based on the title not relating to the aim of the study, and 37 records were screened out based on the abstract not containing the desired information for this study. We were left with 41 articles of which 1 article was screened out from the 41 based on duplicate publications revealing the same prevalence and other different associated research in different journals. A review of the remaining 40 articles was conducted as seen in Figure 1 which revealed the number of initially retrieved papers and the number of papers after selection criteria were applied.

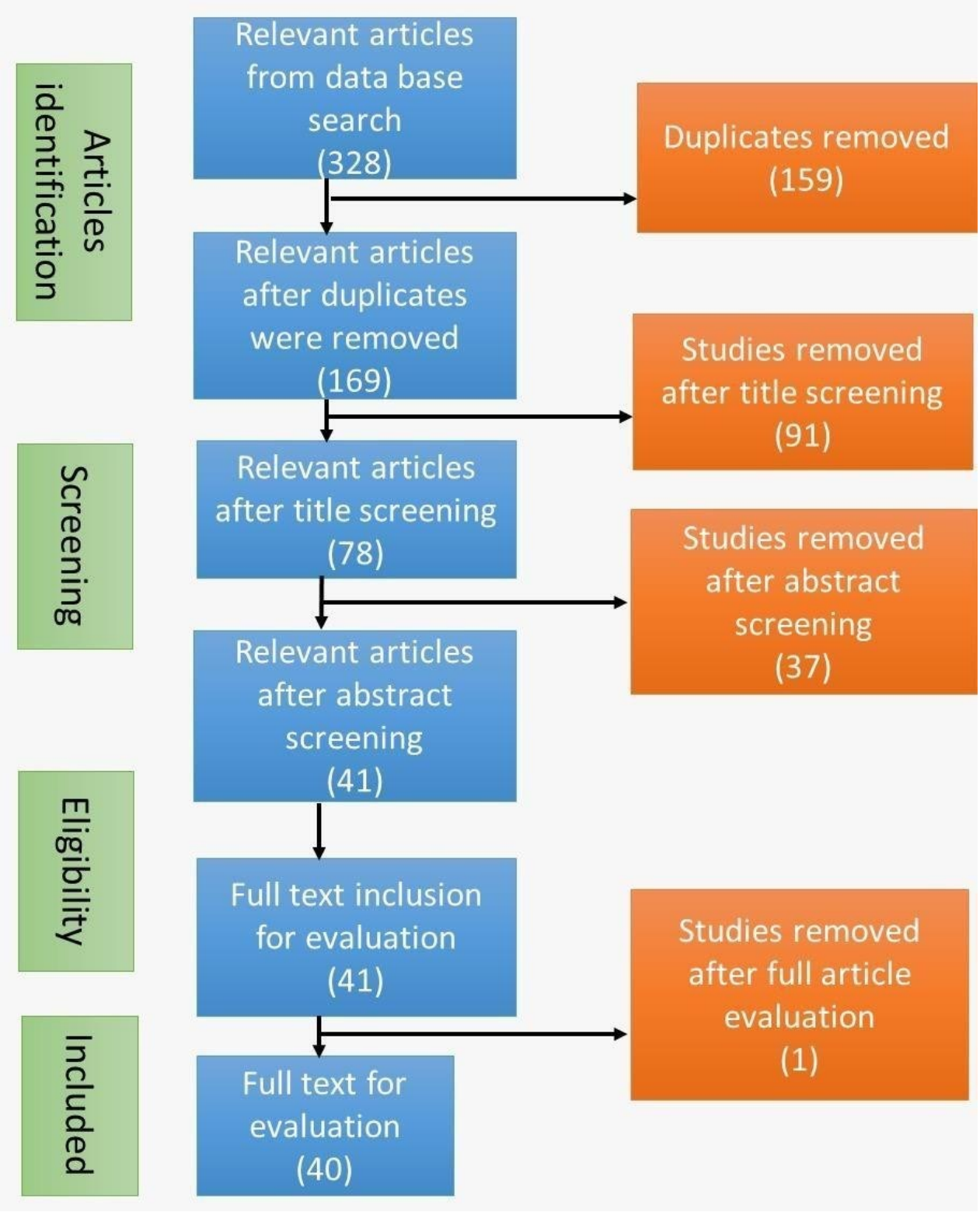

Figure 1. **Flow diagram of the selection of articles for the review.**

From the 40 articles reviewed in this research, 2019 was the year with the most publications following the trend from 2015-2022 (Figure 2) which was when the first case of the virus was reported by Hause et al 2015.

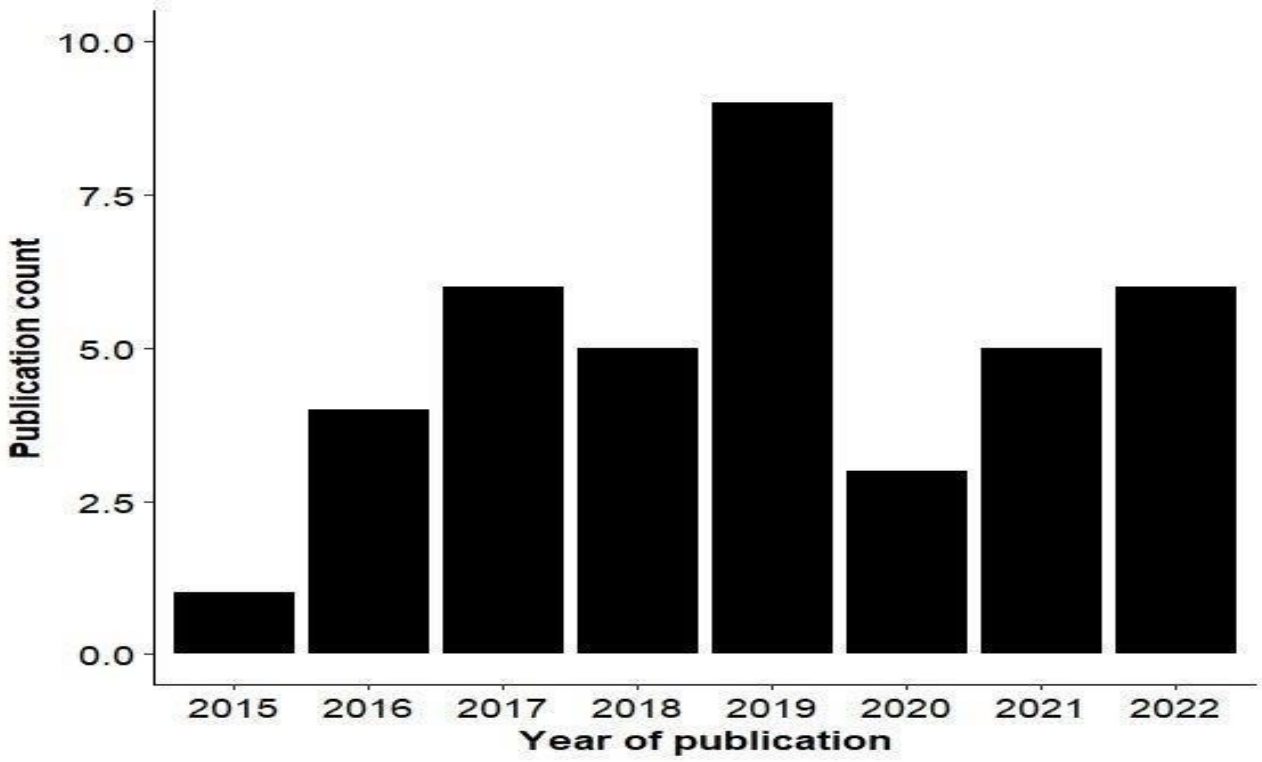

Figure 2. Publication count of articles revealing the prevalence of atypical porcine pestivirus published per year from 2015-2022.

Many countries including Austria, China, Germany, Great Britain, Italy, the Netherlands, Serbia, Spain, Switzerland, the United States, Japan, Denmark, Hungary, South Korea, Brazil, and Taiwan, have reported the prevalence of APPV in pig herds from different samples including serum and tissues indicating that the disease is widespread.

From the 40 articles used in this study, China has the most published studies relating to the prevalence of APPV which maybe as a result of their large swine population and the endemicity of the virus totaling 26% followed by the USA (17%), Germany (12%), Sweden, Spain, Netherlands, Switzerland, Brazil, Italy (5%), Austria, Japan, South Korea, Hungary, Denmark, Taiwan and Austria (2%) Figure 3.

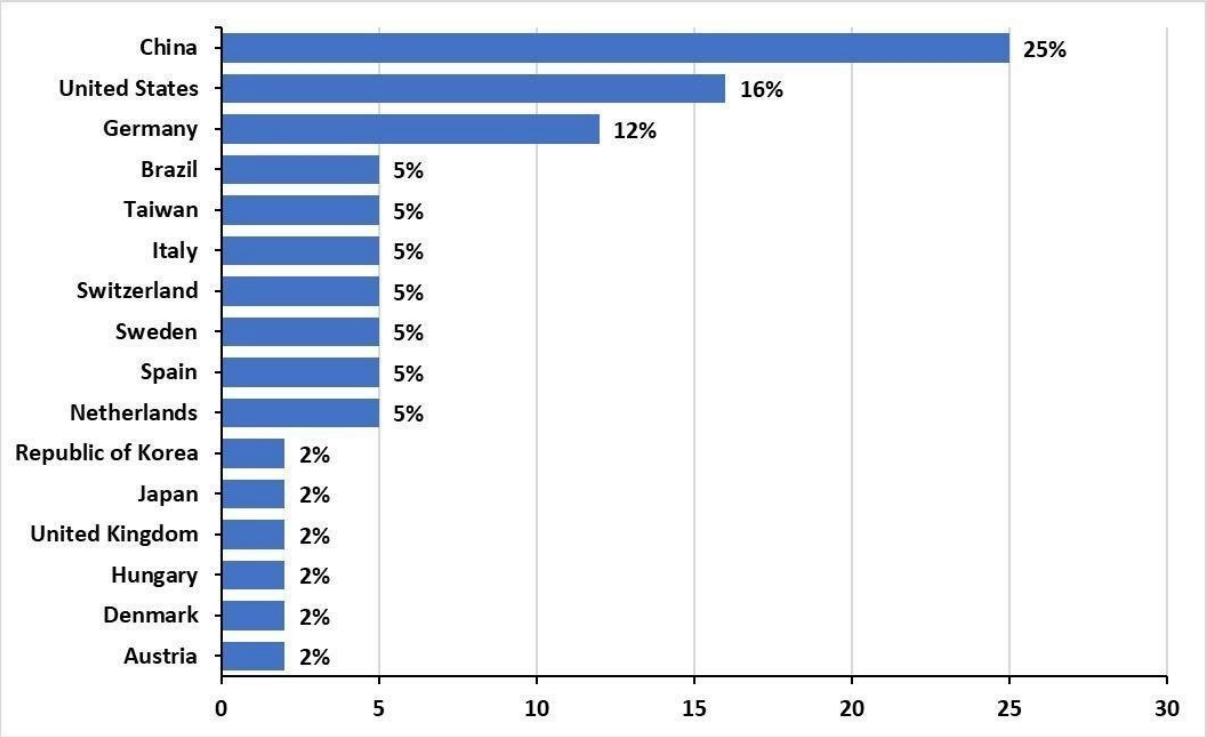

Figure 3. Percentage of the publication count of the different countries that atypical porcine Pestivirus prevalence have been published from 2015-2022.

China

The 1960s saw the first reports of congenital tremor (CT) in China. Throughout the ensuing decades, CT was documented in the majority of the nation's regions. The pig industry suffered significant financial losses as a result of the complete eradication of diseased piglets (Yuan et al., 2017).

Yuan et al., (2017) collected 135 sow and fattening pig serums from 10 farms. PCR-based testing confirmed the presence of APPV in five of the ten examined farms, with a prevalence of approximately 5.2%.

According to Postel et al., (2017) PCR was used to detect APPV from 1,460 serum samples collected from seemingly healthy pigs in different countries, 5% (11/219 samples) of serum samples from mainland China were positive for APPV.

The prevalence of APPV in 165 sera from piglets in southwest China. Viral RNA was found by qRT-PCR in 43.6 % (17/39, 95 % CI 27.8–60.4%) of piglets with CT, while none ofthe sera from healthy piglets contained viral RNA (Zhou et al., 2018).

In 27 swine farms from different regions, APPV prevalence was at 63.4% (Yan et al., 2019), and there were 29 cases of complicated co-infection between APPV and the other 12 swine viruses. Guangdong samples exhibited the highest APPV positivity rate, at 70.2%

(179/255), followed by Guangxi and Anhui, at 54.8% (63/115) and 52.9% (37/70), respectively. All 166 of the Anhui 15 piglets tested positive for APPV. The detection rate was 79.8% (67/84) and 69.7% (23/33) for the 84 piglets from Guangdong and the 33 piglets from Guangxi, respectively. RT-PCR was used to determine the presence of APPV in 14 different types of samples, including tissues and serum from CT piglets.

From May 2017 to March 2018, Xie et al., (2019), reported that 83 piglet samples (brain, lung, heart, kidney, spleen, and lymph nodes) with significant CT clinical indications were collected from 12 commercial swine farms in three southern Chinese provinces. RT-PCR revealed that the prevalence of APPV was as high as one hundred percent (12/12) in swine farms and 90.4% (75/83) in samples.

Shi et al., (2020) collected 53 tissue samples (brain, liver, spleen, and lymph node) from neonatal piglets with CT from October 2017 to May 2019, using RT-PCR for APPV identification, 41 of 53 samples (77.36%) were positive for APPV.

Liu et al., (2021) reported collecting 384 tissues from piglets suspected of being affected in Guangxi Province, Southern China, in 2018-2019. The samples were examined using the standard mRT-PCR technique. The data indicated that the APPV positive rate was 4.17 percent.

Liu et al., 2022 employed qRT–PCR to detect ASFV, CSFV, and APPV in 509 clinical samples from Guangxi Province, southern China. The positive frequencies of ASFV, CSFV, and APPV were 45.58, 12.57, and 3.54 percent, respectively, showing that these viruses were still common in pig herds throughout southern China.

Xia et al., (2022) examined 219 clinical samples for CSFV, ASFV, and APPV utilizing a quadruple PCR-based gene microarray assay for simultaneous comparison with established procedures (RT-PCR), the results showed 100% coincidence with 54 (24.7%) samples being positive for CSFV-W, 40 (18.7%) samples were positive for CSFV-V, 9 (4.1%) samples were positive for ASFV, 10 (4.6%) samples were positive for APPV, and 106 were negative.

Multiplex real-time PCR was used to evaluate 451 clinical serum and tissue samples (246 samples from Xinjiang, 198 from Tianjin, 6 from Hebei, and 1 from Hunan provinces in China); the results revealed positive rates for CSFV (0.22%, 1/451), ASFV (1.3%, 6/451), and APPV (0%, 0/451), respectively (Song et al., 2022).

Fifty-two (52) serum and tissue samples (heart, liver, spleen, lung, kidney, cerebrum, cerebellum, brainstem, spinal cord, submaxillary lymph nodes, tonsil, and serum) were taken as reported by Ren et al., (2022) at two distinct times (2020 and 2021) from 8 CT-affected newborn piglets on the same Hubei province pig farm. qRT-PCR was used to detect APPV in CT-affected piglets, and all samples were positive for APPV

United States of America

As part of a PRRSV ongoing metagenomic sequencing, Hause et al. (2015) performed metagenomic sequencing on 182 serum samples that were qRT-PCR positive for porcine reproductive and respiratory syndrome virus (PRRSV) and detected four additional APPV-positive samples. Positive results originated from five states, and ELISAs employing recombinant APPV Erns detected cross-reactive antibodies in 94% of a collection of pig serum samples, indicating extensive dispersion of APPV in the US swine population. The genetic and serological results imply that APPV is a novel, highly divergent, and widely dispersed porcine pestivirus in the United States.

Pestivirus RNA closely related to the atypical porcine pestivirus was found by RT- qPCR in 6% (21 of 362) of samples from herds exhibiting diverse clinical symptoms indicatingthe virus is present in tissues (Arruda et al., 2016)

Gatto et al. (2017) report that 597 samples of semen, preputial fluid, and preputial swabs, each representing a separate pig, were collected from four commercial boar studs in three different U.S. states. qRT-PCR found APPV viral RNA in 90 samples (15.08%; 90/597), with the highest percentage of positive samples coming from preputial swabs (23.81 percent; 5/21) followed by preputial fluid (22.81 percent; 26/114) and sperm (12.91 percent; 59/457).

Sutton et al., (2019) used a qPCR technique to profile the presence of APPV in serum samples from affected (CT) and unaffected pigs, revealing a 69.6% positivity for APPV.

Using A duplex semi-quantitative RT-PCR (sqRT-PCR) to investigate 67 clinical cases, Chen et al. (2019) revealed that 17 (25.0%) were positive for PPgV, APPV, or co- infection with PPgV and APPV. Seven of the twelve serum cases (58.3%) were positive for APPV, while only four of the eleven tissue cases were positive (7.2%).

Yuan et al. (2021) found a 19% prevalence of APPV in U.S. swine herds after examining 1785 clinical samples (2016-2018) with multiplex RT-qPCR, with oral fluids being the most reliable specimen for viral identification.

In another study by Houston et al. (2022) where five randomly selected barrows were bled and their serum was submitted for APPV qRT-PCR analysis 24 hours after being placed in an isolation nursery, as part of a study conducted on a commercial farm where pigs are raisedfrom birth to slaughter. Two of the five barrows were positive for APPV RNA (Ct values of 27.5 and 30.4). Finisher barn oral fluid and serum samples were submitted for APPV qRT-PCR testing. During the study period, the prevalence of atypical porcine pestivirus in the isolation nursery increased from 15 of 40 pens (37.5%) to 31 of 40 pens (77.5%). The

proportion of randomly selected pigs rose from 4 of 20 pigs (20%) to 6 of 13 pigs (46%). One of the five submitted pen swab samples contained detectable APPV RNA (Ct = 35.70), while another contained suspicious APPV RNA (Ct = 37.64). During the finishing stage, eight of thirty (30%) randomly selected pigs (27%) exhibited detectable serum APPV RNA on the initial sample date, but none on the second sampling date. During the finishing phase, all oral fluid samples contained detectable APPV RNA on both sampling dates. The prevalence of clinical APPV cases was indistinguishable between sows (prevalence of affected litters within batch farrowing group ranging from 4 to 17%) and gilts (prevalence of affected litters within batch farrowing group ranging from 4 to 14%). The prevalence of atypical porcine pestivirus in the isolation nursery increased from 15 of 40 pens (37.5%) to 31 of 40 pens (77.5%). The prevalence of randomly selected pigs increased from 4 of 20 pigs (20%) to 6 of 13 pigs (46%). One of the five submitted pen swab samples contained detectable APPV RNA (Ct = 35.70), while another contained suspicious APPV RNA (Ct = 37.64). During the finishing stage, eight of thirty (30%) randomly selected pigs (27%) exhibited detectable serum APPV RNA on the initial sample date, but none on the second sampling date. During the finishing phase, all oral fluid samples contained detectable APPV RNA on both sampling dates.

Germany

Early studies from the United States and Germany indicated a rather high prevalence (2.4%-22%) of APPV genomes in ostensibly healthy pigs (Hause et al., 2015; Postel et al., 2016; Beer et al., 2016), which likely play a significant epidemiologic role as viral carriers.

Postel et al., (2016) used RT-PCRs to examine 369 blood samples from clinically unsuspicious sows and fattening piglets from South (20 farms, 200 sera) and North Germany (9 farms, 169 sera). In all, six finishing pigs (one herd) from Lower Saxony and three sows (two herds) from Bavaria had APPV genomes identified by both assays, yielding an individual genome prevalence of 2.4% and an overall prevalence of 10% at the farm level. For pathological and virological research, eight piglets (six affected and two unaffected animals) from three affected litters were slaughtered. The two SYBR-Green-based APPV-specific PCRs were used because the generic Pan-Pestivirus RT-PCR was unable to detect the viral genomes of CSFV and other recognized pestivirus species. While both piglets without tremors tested negative, all six clinically affected piglets had APPV genomes in their serum, cerebrospinal fluid, and pooled central nervous system samples (which included the cerebrum, cerebellum, and spinal cord). APPV genomes were not found in sera (n=23) taken from sows on the affected

farm with and without impacted litters. This suggests that the presence of APPV genomes in newborn piglets correlates with CT.

In another study in Mecklenburg-Western Pomerania by Beer et al. (2016), a total of 367 tonsil samples were collected from a rendering plant where pigs from various farms were processed. At a slaughterhouse, 12 tonsils from an organic farm were also taken. Additionally, 63 serum samples were taken from breeding and young fattening pigs in Schleswig-Holstein, and 63 ovine sera were used as control samples. Unexpectedly, the tailored RT- qPCR revealed that 9% of the tonsils from the rendering plant were positive, with Cq-values ranging from 22 to 39. All of the tonsils from the organic farm were positive, with Cq-values above 30. Furthermore, 22% of the porcine sera from different herds in Schleswig-Holstein had Cq-values above 30. In contrast, none of the sheep samples tested positive.

A report by Catagay et al., (2018) stated that during the 2015/2016 and 2016/2017 hunting seasons in northern Germany, 456 serum samples from wild boars were analyzed for the presence of APPV genomes and virus-specific antibodies. Real-time RT-PCR analyses revealed a 19% detection rate for genomes.

A total of 1115 porcine serum samples were taken from 122 farms located in seven German states (BY, BW, NW, NI, ST, BB, and MV). 600 of the samples were collected in 2009 or 2010, while the other 515 were taken in 2018. Out of the 1115 samples, 16.3% (182) tested positive for antibodies against APPV, and these sera originated from 51 of the 122 farms (41.8%) using RT-PCR (Michelitsch et al., 2019).

Catagay et al., 2019 stated that RT-PCR analysis of serum samples from 15 pigs obtained during a CT outbreak in 2016 revealed that 93% (14/15) were positive for the presenceof the APPV genome.

Switzerland

Research has shown that APPV has been present in Switzerland for a long time, mainly in young animals, with the earliest known report of it being in 1986. Kauffman et al. (2019) conducted qRT-PCR on 1080 serum samples from adult pigs in fattening farms with an unknown history of congenital tremor. In 1986 7%, in 2006, 18%, in 2011, 12%, in 2015, 9%, and in 2018 0.3% prevalence was recorded. The results showed that the prevalence of APPV genomes ranged from 0.3% in 2018 in breeding farms to 7-18% in fattening farms in the years 1986 to 2015, with a total of 96 (9%) of 1080 tested samples being APPV genome positive.

Grahoefer et al., (2020) reported that out of the 131 serum samples collected from 125 sows aged 180 days or older and six boars, 93.1% tested positive for APPV when examined

using an indirect APPV-specific ELISA and an APPV RT-PCR targeting the NS3 encoding regions.

Netherland

In 2016, Folguerias et al., (2020) reported that serum was collected from 196 breedinggilts as part of routine farm surveillance for APPV and other porcine viruses. qRT-PCR was used to quantitatively detect APPV in samples, during which 15% of 196 gilts tested positive for APPV.

Eleven piglets from March and April 2012 serum samples were analyzed by PCR for the presence of APPV, and all samples were positive. From March 2012 to February 2016, 99 serum samples collected from CT piglets for the presence and distribution of ATPPV tested positive for the virus (100%) (De Groof et al., 2016).

Spain

In a retrospective study conducted by Munoz et al., (2017) using serum samples collected from 1997 to 2016, high and moderate levels of APPV genome RNA were detected in various tissues of pigs affected by CT. The highest levels of APPV RNA were found in lymphoid organs, suggesting that they are a target for APPV replication. Out of the 642 samples evaluated, 89 (13.9%) tested positive for the APPV genome. Additionally, CT cases were associated with APPV infection in viraemic piglets, indicating that APPV has been present in pigs from northeastern Spain since at least 1997.

A study by Colom et al., (2018) using sera from 437 wild boars collected during the hunting season from 2012 to 2016 in the Catalunya region (north-eastern Spain) identified thepresence of APPV genome in one (male) animal serum (0.23%, CI 95: 0.01–1.28%). There was an abundance of RNA in the kidney, tonsil, and submandibular lymph nodes.

Sweden

From June 2017 to June 2018, a retrospective study was conducted on the brain tissue sample from 15 piglets from four Swedish farms with congenital tremors and 14 piglets with spay legs collected in 2004 (n = 11) and 2011/2012 (n = 3). Two RT-qPCR methods specific to APPV were used, targeting the NS3 and NS5B regions, respectively. Of the 29 piglets exhibiting CT symptoms, 27 (93%) were PCR-positive for APPV (Stenberg et al., 2020).

Analysis of 595 serum samples from wild boar in 13 counties in Sweden between 2000and 2018 by Stenberg et al., (2021), revealed that APPV is widespread in the wild boar population; 12% of the samples tested positive for the APPV genome and 72% had antibodies

against the APPV-glycoprotein Erns, indicating that APPV has been present in the Swedish wild boar population since 2000.

Italy

Postel et al., (2017) examined 1,460 serum samples from apparently healthy pigs in Germany, the United Kingdom, Italy, Serbia, Switzerland, mainland China, and Taiwan using an APPV-specific PCR and an indirect APPV ELISA; 17.5% (35/200 samples from Italy) were positive for APPV.

Sozzi et al., (2019) reported that, between 2016 and 2018, RT-PCR was used to examine 360 swine fetuses and 430 hunted wild boar serum samples, which identified two strains (0.6%) from the former and three strains (0.69%) from the latter in the area.

Austria

The reactivity rate was 35.3% based on research conducted by Schwarz et al. (2017) in five Austrian piglet-producing farms with CT issues, one of which had several farrowing with clinically overt CT in APPV-positive piglets, but no APPV RT-PCR-positive adults.

Great Britain

Postel et al. (2017) conducted a study with 1,460 serum samples from apparently healthy pigs in Germany, the United Kingdom, Italy, Serbia, Switzerland, mainland China, andTaiwan using an APPV-specific PCR and an indirect APPV ELISA. Genome detection rates ranged from 2.3% (2/86 samples from the United Kingdom)

Taiwan

Postel et al. (2017) conducted a study using 1,460 serum samples from apparently healthy pigs in Germany, the United Kingdom, Italy, Serbia, Switzerland, mainland China, and Taiwan. Using an APPV-specific PCR and an indirect APPV ELISA, the APPV genome was detected in 11% of Taiwanese samples.

South Korea

Choe et al., (2020) conducted a study in nine provinces of South Korea using serum samples from 2,297 wild boars collected in 2019. Using RT-PCR to detect APPV in the samples (1126 males, 1045 females, and 126 unknown), 18 APPV strains were detected in the samples, indicating a prevalence of 0.78%. 15 of the 18 wild boars that tested positive for APPV were male (83.3%), two were female (11.1%), and one was of unknown sex (5.6%).

Japan

In Japan, from 2005 to 2020, a retrospective study was conducted to detect APPV using a total of 399 porcine samples consisting of the supernatants of homogenized organ tissues or sera collected from 1-day-old to 3-year-old pigs. Three samples were positive for APPV using qRT-PCR; these samples were collected from pigs without CT who were 20 days, 68 days, and 12 weeks old (Kasahara et al.,2021).

Hungary

RT-PCR performed on tissue samples of 25 cases of CT in piglets from 2005, 2007, 2010, and 2016–2018 originating from six different farms. revealed that all of the affected piglets were positive for APPV (Denes et al., 2018).

Denmark

In an observational case study of 16 Danish sow herds from June 2019 to April 2020, Pedersen et al., (2021) collected serum from 55 CT-affected piglets in ten case herds, 25 healthy piglets from five herds without a history of CT-affected piglets, and five piglets without CT (intermediate herd) APPV was detected in all 55 piglets (100%) with CT, but only one out of 25 healthy piglets (4%) tested positive for the virus.

Brazil

Mosena et al., (2017) revealed that RT-PCR analysis of nine blood samples from CT piglets from two farrow-to-wean farms in Brazil and three tissue samples from necropsied piglets was positive for APPV.

qRT-PCR detected APPV in all piglets with CT from four farms in two Brazilian states. All Cerebellum samples (n = 11) were consistently positive, followed by spleen and lymph node, cerebrum, and brain stem respectively (Gatto et al., 2018).

The selected publications shown in Table 1 reveals the country where the sample was collected, the year of sample collection for the study, the category of the pig in which the sample was collected, the clinical signs observed before sample collection, the detection

technique, the prevalence, and the references. The clinical signs not recorded are from samples collected from apparently healthy animals or those not recorded by the author.

The prevalence of APPV from this study are more in piglets. The prevalence within countries and provinces varies and can be high in piglets when CT signs are present. Many studies confirmed the correlation between APPV and CT. The economic costs of CT clinical disease induced by APPV are unknown; nevertheless, seriously affected piglets with CT may die due to colostrum intake difficulties and hunger, resulting in a greater pre-weaning mortality rate (Stenberg et al., 2020). Most CT-infected piglets were born to gilts (female pigs in their first pregnancy), viremia in adult animals is not always associated with the disease. This suggests that the immunological status of the dam is probably the most important factor in the development of disease in piglets, as indicated by reports showing that APPV-associated CT is more common in gilt litters than in sow litters although piglets born from higher parity sowscan also be affected (de Groof et al. 2016, Gatto et al. 2018). Given that gilt litters are the most susceptible to APPV infection (Possatti et al., 2018), it is probable that the entry of naive gilts into the herd poses a serious threat to the disease's epidemiology (Cagatay et al., 2019).

One important observation in this study is the high prevalence of APPV infection in herds or individuals with no obvious clinical disease. Apart from the CNS and lymphoid organs, the virus was detected in peripheral nerves, heart, lungs, liver, kidney, bladder, pancreas, small intestine, colon, salivary glands, skeletal muscle, umbilical blood, serum, cerebrospinal fluid, saliva, nasal swabs, rectal swabs, and even semen. The frequency of detection and the amount of the virus present varies from sample to sample and from study to study, with the lymphoid organs being the most commonly positive samples. The exact pathogenesis is still unknown, but the presence of APPV in the central nervous system may explain neurological symptoms (Arruda et al., 2016; Postel et al., 2016; de Groof et al 2016; Schwarz et al., 2017; Yuan et al., 2017; Gatto et al., 2017; Munoz et al., 2017). Additionally, the virus has been detected in the serum samples of wild boars in Germany (Cagatay et al., 2018), Italy (Sozzi et al., 2019), South Korea (Choe et al., 2020), and Sweden (Stenberg et al., 2021) suggesting that wild boars may be an APPV reservoir worthy of epidemiological investigation at local and transboundary levels. The clinical manifestation of APPV infection in piglets was defined by CT type A-II, but APPV infection in adult pigs could lead to the animal becoming a persistent carrier and shedder (de Groof et al., 2016; Schwarz et al., 2017).

Both farmed pigs and wild boar populations have been found to carry the APPV virus (Cagatay et al., 2018; Gatto et al., 2018; Colom et al., 2018; Sozzi et al., 2019, Grahofer et al., 2020, Choe et al., 2020; Stenberg et al., 2021).

Molecular methods using RT-PCR are said to be the commonly used detection method with a high positivity rate and APPV-specific antibodies can be detected using a unique NS3-based blocking ELISA. RT-PCR was used by most researchers to identify high levels of APPV in the sperm, serum, fluids, swabs, and various tissue samples of infected pigs. CSF and APPV are both capable of inducing CT in newborn piglets, which is typically difficult to diagnose based on clinical symptoms. (Dall et al. 2020). Therefore, laboratory differential diagnosis is essential. Due to a lack of understanding regarding the prevention of this disease, concurrent infections (coinfections) and secondary infections caused by two or more viral pathogens are prevalent.

The detection of APPV genomes at a relatively high prevalence in clinically healthy animals may indicate long-term persistence in postnatally infected animals without clinical manifestations.

Table 1: Presents different articles that have reported the prevalence of atypical porcine Pestivirus from 2015-2022 revealing the countrythe sample was collected, year of sample collection, category of animal sampled, clinical sign present at the time of sampling, the detection technique used, the samples collected for the test, the prevalence recorded and the corresponding references.

Country	Year	Category	Clinical signs	Detection Tech	Samples	Prevalence (%)	References
China	2016	sow/fattening pigs	CT	qRT-PCR	serum and tissue samples	5.2	Yuan et al., (2017)
China	2017	pigs	-	PCR	Serum	5	Postel et al., (2017)
China	2018	piglets	CT	qRT-PCR	Serum	43.6	Zhou et al., (2018)
China	2016-2018	piglets	CT	RT-PCR	serum and Tissue samples	63.4	Yan et al., (2019)
China	2017-2018	piglets	CT	RT-PCR	Tissue samples	90.4	Xie et al., (2019)
China	2019	piglets	CT	RT-PCR	Tissue samples	77.36	Shi et al., (2020)
China	2019	piglets	-	mRt-PCR	Tissue samples	4.17	Liu et al., (2021)
China	2018-2020	piglets	-	qRT-PCR	Tissue samples	3.54	Liu et al., (2022)
China	2022	piglets	-	RT-PCR	serum and tissue samples	4.6	Xia et al., (2022)
China	2022	pigs	-	mRt-PCR	blood and tissue samples	0	Song et al., (2022)
China	2020-2021	piglets	CT	RT-PCR	Tissue samples	100	Ren et at., (2022)
Japan	2020	piglets	CT	RT-PCR	Tissue samples	0.75	Kasaharakam et al., (2021)
South Korea	2020	wild boar	-	RT-PCR	serum	0.78	(Choe et al., 2022)
Taiwan	2017	pigs	-	PCR	serum	11	Postel et al., (2017)

USA	2015	pigs	-	ELISA	serum	94	Hause et al., (2015)
USA		piglets	CT	qRT-PCR	Tissue samples	6	Arruda et al., (2016)
USA	2017	boar	-	qRT-PCR	semen, preputial fluids	15.08	Gatto et al., (2017)
USA	2017	piglets	CT	qRT-PCR	serum	69.6	Sutton et al., (2019)
USA	2019	pigs	-	sqRT-PCR	oral fluids, tissue	25	Chen et al., (2019)
USA	2016-2018	piglets, sows, gilt,	CT	RT-PCR	oral fluids, serum, feces and tissue samples	19	Yuan et al., (2021)
USA	2019-2020	pigs	CT	qRT-PCR	serum and semen	30.3	Houston et al., (2022)
Germany	2016	sow	-	RT-PCR	serum	2.4	Postel et al., (2016)
Germany	2016	pigs	-	qRT-PCR	serum and Tissue samples	9%	Beer et al., (2016)
Germany	2018	wild boar	-	RT-PCR	serum	19	Catagay et al., (2018)
Germany	2018		-	RT-PCR	serum	16.3	Michelitsch et al, (2019)
Germany	2016	piglets	CT	RT-PCR/ELISA	serum	93	Catagay et al., (2019)
Spain	1997-2016	piglets	CT	qRT-PCR	serum and tissue samples	13.9	Munoz et al., (2017)
Spain	2012-2016	wild boar	-	RT-PCR	serum	0.23	Colom et al., (2018)
Sweden	2018	piglets	CT	qRT-PCR	Tissue samples	93%	Stenberg et al., (2020)
Sweden	2000-2018	wild boar	-	RT-PCR/ELISA	serum	72	Stenberg et al., (2021)
Netherland	2016	gilts/piglets	CT	qRT-PCR	serum and fecal samples	15	Folguerias et al., (2020)
Netherland	2012-2016	piglets	CT	qRT-PCR	serum, tissue, and fecal samples	100	De Groof et al., (2016)

Switzerland	1986-2018	slaughter pigs	CT	qRT-PCR	serum	7,18,12,9,0.3	Kauffman et al., (2019)
Switzerland	2020	boar	-	ELISA	serum	93.1	Grahofer et al., (2020)
Italy	2017	pigs	-	PCR	serum	17.5	Postel et al., (2017)
Italy	2018	wild boar	-	RT-PCR	serum	0.69	Sozzi et al., (2019)
Austria	2015-2016	piglets	CT	RT-PCR	Tissue samples	35.3	Schwarz et al., (2017)
Great Britain	2017	pigs	-	PCR	serum	2.3	Postel et al., (2017)
Hungary	2005-2018	piglet	CT	RT-PCR	Tissue samples	100	Denes et al., (2018)
Denmark	2019	piglets	CT	qRT-PCR	Serum	100	Pedersen et al., (2021)
Brazil	2017	piglets	CT	RT-PCR	serum and tissue samples	100	Mosena et al (2017)
Brazil	2017	piglets	CT	qRT-PCR	tissue samples	100	Gatto et al., (2018)

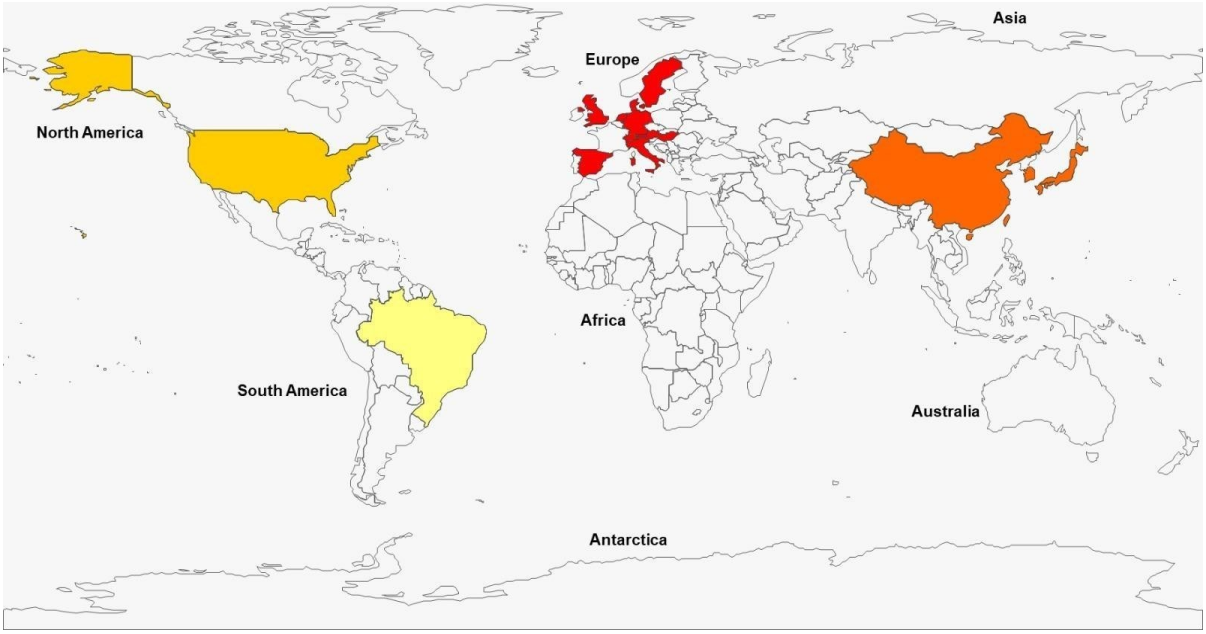

Figure 4. Map of the world revealing the different continents where the prevalence of atypical porcine pestivirus was published from 2015-2022.

This map reveals that APPV has been reported in several countries of the world and continents (worldwide distribution). Countries in Europe have more articles that reported prevalence of APPV followed by Asia, North America, and South America Figure 4.

APPV is not a new virus (Kauffman et al., 2019), but a newly found virus that is well established in numerous pig populations and likely disseminated globally (Postel, et al., 2017). APPV may be the recent and most distantly related novel pestivirus, and it appears to be at least one cause of congenital tremor type A-II, which has been considered a transmissible disease with an unknown viral etiology for a long time. Data on the prevalence of this disease was not found in most countries but the disease has been reported in some.

4.0 Conclusion

APPV is worldwide in distribution and its prevalence has been reported by 16 countries (China, Japan, South Korea, Taiwan, USA, Germany, Spain, Sweden, Netherland, Switzerland, Italy, Austria, Great Britain, Hungary, Denmark, and Brazil) countries within 4 continents namely; Asia, North America, Europe and South America. Europe has had more data on the prevalence of APPV. China has a greater number of published articles revealing the prevalence of APPV. RT-PCR is the most used diagnostic technique to detect APPV. Tissue and serum samples are the frequently used sample for the detection of APPV. The presence of APPV in wild boar

population and adult pigs could lead to the animal becoming a persistent carrier/shedder and reservoir worthy of epidemiological investigation. A high prevalence of APPV infection observed in herds with no obvious clinical disease and piglets with CT can lead to reduced performance, increased mortality, and decreased growth rates. There is a need for researchers in different countries to conduct studies in healthy pigs and CT piglets to inform the globe on this disease because of its ability to coinfect with other diseases and its devastating impact on the swine economy, also studies on the presence in semen should be carried out to better understand the function of semen in the transmission and clinical manifestation of APPV.

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CHAPTER 2: Occurrence and distribution of antibodies against *Pestivirus a* (BVDV-1) and *Pestivirus b* (BVDV-2) in swine farms from the micro-region of Ponte Nova, Minas Gerais state, Brazil.

Uzoka Ugonna Henry

ABSTRACT

Pestiviruses are worldwide in distribution, causing a significant economic loss in livestock, and have been reported to have a high prevalence in most regions. The pestivirus genus contains one of the significant pathogens known as the Bovine Viral Diarrhea Virus (BVDV) that affects cattle and pigs. Cattle are said to be the reservoir host of this virus and as such possible connections between bovine and swine herds farmed in close proximity have been incriminated in the transmission of BVDV from cattle to pigs, and the prevalence of BVDV in pigs can lead to problems in the diagnosis and epidemiological surveillance of Classical swine fever (CSF) because of serological cross-reaction. In recent years, the prevalence of BVDV in pigs has increased, and its effects have had a severe economic impact on swine breeding. This study surveys to determine the occurrence and distribution of antibodies against pestivirus A (BVDV-1) and pestivirus B (BVDV-2) using virus neutralization test in nine swine farms in the micro-region of Ponte Nova, Minas Gerais State, Brazil to be informed on its prevalence in sows and finished pigs. Four hundred serum samples from sows and finished pigs (200 each) collected from nine farms in six municipalities; Jequeri, Rio Casca, Urucania, Ponte Nova, Teixeras, and Coimbra all in the microregion of Ponte Nova revealed low antibody titers (between 2 - 3.32 in log2). A prevalence of 4.75% (19/400; C.I.95% 2.88-7.32) was recorded and 44.4% (4/9; C.I 95%, 13.7-78.8) of the farms presented at least one positive animal. BVDV-1 had a 0.25% (1/400; C.I 95%, 0.01-1.39) and BVDV-2 had a 4.5% (18/400; C.I 95%, 2.69-7.02) prevalence. No prevalence was recorded in the farms from Rio Casca and Ponte Nova, BVDV-1 was only recorded in the Coimbra region and BVDV-2 prevalence was recorded in Jequeri, Urucania, and Teixeras with finished pigs recording more prevalence. In conclusion, the farms that presented BVDV antibodies were sited in close proximity to a cattle herd which may be a source of transmission. The positive municipals being recorded are classical swine fever free and as such should be under close observation by the veterinary inspection services department as the presence of BVDV antibodies in swine serum can lead to false positive results of classical swine fever because of serological cross-reaction which can hamper CSF eradication programs.

Keywords: bovine viral diarrhea virus, Swine, Pestivirus, Virus neutralization.

1.0 Introduction

Pestivirus infections are detrimental to animal health. They infect wild and domestic ruminants, as well as pigs. Virus infections, such as those caused by this genus are among the illnesses that inflict serious economic and productivity losses around the world in the livestock industry (Tadesse et al., 2019). Infections with Bovine viral diarrhea virus (BVDV) or border disease virus (BDV) have resulted in significant financial losses for producers. Most pestivirus infections' financial costs are attributable to reproductive disorders, following calving and lambing losses, and immunosuppressive side effects that may lead to subpar growth, decreased productivity and increased susceptibility to other illnesses (Gatto et al., 2018). In 2012, BVDV was classified as a listed disease by the International Epizootic Office (OIE). Epidemiological studies have incriminated cattle as the natural host, in addition, infection with ruminant pestiviruses may affect an animal's immunity, increasing its exposure to and severity of various illnesses such as mastitis, diarrhea, and respiratory sickness (Firaol and Abdissa, 2021; Laureyns et al., 2010). More emphasis is laid on the ruminant pestivirus that affects swine because of the similarities in clinical signs between BVDV and classical swine fever virus (CSFV). Cross reaction between BVDV and CSFV, and the antigenic similarities between both pestiviruses has increased doubts about the laboratory test used in the control of CSFV and its ability to differentiate infections caused by the different viruses and adequate diagnosis. The virulence of the viral strain and the immune system of the pig dictate the course of the BVDV infection in that animal. However, the virus detection in the secretions of ill animals shows that pigs may act as a source of infection, furthering the virus' spread across the herd. (Pereira et al., 2018). While these illnesses are diagnosed and treated, the underlying BVDV or BDV infection often remains undiagnosed and untreated (Laureyns et al., 2010), resulting in increased losses related to ruminant Pestivirus infections. Although BVDV does not pose the same threat to pig herds as it does to ruminants, it can cause a variety of clinical signs and eventually result in a serological cross-reaction with classical swine fever monitoring and surveillance programs, leading to disease misdiagnosis, prompting many countries to pay attention to porcine BVDV infections (Gomez et al.,2019). While the vertical transmission of the bovine diarrhea virus (BVDV) and its various clinical and pathological manifestations in cattle have been extensively described, studies with swine farms as a focus in literature are limited. The serological cross reaction between BVDV and CSF in swine can lead to false results in CSF diagnosis therefore the presence of BVDV antibodies in swine should call for a close observation of the swine herd.

2.0 The study's historical backdrop

Minas Gerais state, which has the most milked cows and accounts for 26.6 percent of production and 20.0 percent of total milking animals, reasonably accounts for the possibility of pestivirus A (BVDV-1) and pestivirus B (BVDV-2) occurrence and distribution among pigs from the microregion of Ponte Nova, Minas Gerais State. Worldwide studies on BVDV have revealed a prevalence ranging from 18 to 93%. (Talafha et al., 2009; Guarino et al., 2008).

2.1 Occurrence of Bovine viral diarrhea virus

Several reports indicate that Brazil has been infected with BVDV since the late 1960s (Correa et al., 1968). Numerous serological studies have shown that infection is widespread in Brazilian cattle, affecting between 22 and 67% of animals and 43 to 90% of herds (Quincozes et al., 2007; Almeida et al., 2013). The percentage of seropositive animals varies according to location and type of exploration. Due to a lack of reports, the role of other animal species in BVDV epidemiology in Brazil is unknown. In Brazil, BVDV-1 (pestivirus A), BVDV-2 (pestivirus B), and 'HoBi'-like viruses have been frequently documented in terms of genetic diversity (Weber et al., 2014a; Weber et al., 2014b). According to Weber et al. (2014b), BVDV-1 and BVDV-2 are nearly equally prevalent, with BVDV-1 occurring 40-57 percent of the time and BVDV-2 occurring 42-45 percent of the time. These BVDV-2 prevalence rates appear to be comparable to those found in Chile (Pizarro-Lucero et al., 2006), but higher than those found in North America and Europe (Ridpath et al., 2010). The genetic diversity of Brazilian pestiviruses, combined with the high prevalence of BVDV-2, emphasizes the importance of understanding circulating pestiviruses, as evidenced by reports of molecular detection techniques failing (Schirrmeier et al., 2004; Weber et al., 2014a) and significant antigenic changes at the specie and subtype level, as revealed by cross-neutralization (Ridpath et al., 2010; Bianchi et al., 2011).

2.2 Taxonomy of Bovine viral diarrhea virus

Bovine viral diarrhea (BVD) is an infection caused by the bovine viral diarrhea virus (BVDV), a single-stranded positive polarity RNA virus belonging to the Pestivirus genus and the Flaviviridae family (Khodakaram et al., 2017). Pestivirus species have been renamed from A to K, with the major swine-related viral species being Pestivirus A (BVDV-1), Pestivirus B (BVDV-2), Pestivirus C (Classical Swine Fever Virus), and Pestivirus K (atypical porcine

pestivirus) (King et al.,2018). Figure 5 shows that pestiviruses have a single-stranded positive-sense RNA genome with one open reading frame bordered on both ends by non-coding regions (NCR) that encodes a polyprotein that is processed into 12 polypeptides (Simmonds et al., 2011). Pestiviruses are 40 to 60 nm in diameter, with an icosahedral nucleocapsid enclosed in a lipid envelope derived from the host cell's membrane cytoplasm (Ridpathet al., 2007). It has four structural proteins: a capsid protein (C) and three glycoproteins (Erns, E1, and E2) inserted into the envelope (Thiel et al., 1991). E2 envelope glycoprotein is primarily responsible for pestivirus antigenic similarity and difference, and it is also the dominant glycoprotein in which antibodies produced against it are important for diagnostic tests and vaccination-induced immunity (Jelsma et al., 2013). The 5' and 3' ends of the genome contain two untranslated regions (UTR). 5′NCR and N terminal auto protease (Npro) are frequently used in phylogenetic techniques (Moening and Becher,2015). Additionally, there are twenty-one subtypes of BVDV-1 (1a-1u) and four subtypes of BVDV-2 (2a-2d) (Yeşilbag et al., 2014; Kuca et al., 2020). Antigenic differences within each genotype exist and are dependent on their effects on tissue cell culture biotypes of BVDV and can either be cytopathogenic (CP) {that causes vacuolization and cell death}, or non-cytopathogenic (NCP) strains (Walz et al., 2015).

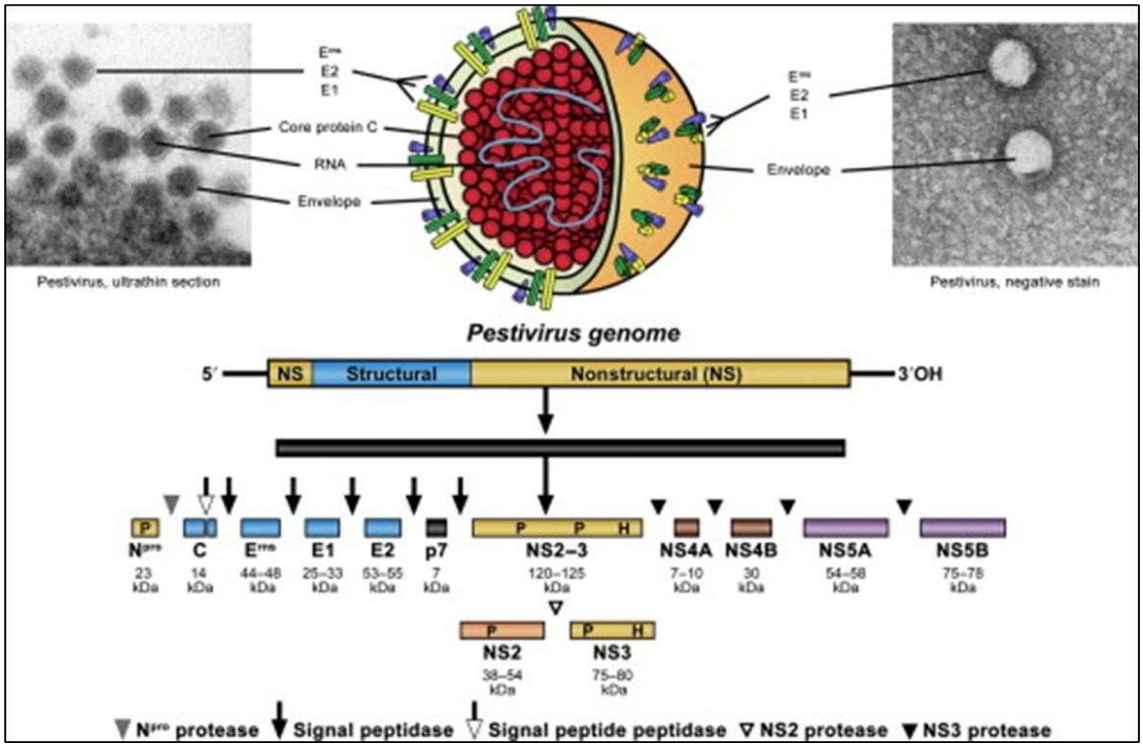

Figure 5. Schematic description of the structure of a virion of the genus Pestivirus.

Source: Tautz et al. (2015)

Epidemiology of BVDV in swine farms

The first report of ruminant pestivirus isolation in naturally infected pigs is said to have happened in 1973. Almost 50 years later, the prevalence of these pestiviruses among animals is still widespread in many countries worldwide (Almeida et al., 2017). Infections caused by BVDV have been reported in countries such as China, the Netherlands, Brazil, and others, according to De Oliveira et al., (2020). Furthermore, Almeida et al. (2017) states that the prevalence of BVDV infection in pigs varies by region, with 11 Chinese provinces reporting a prevalence of infected sows ranging from 20-30% having been infected with BVDV-1. Positivecases were found in 0.42% of finishing pigs, 2.5% of sows, and 11% of swine herds in the Netherlands. The prevalence in Ireland was reported to be very low, at 0.14%. (Graham et al.,2001). In another study by Lipowski. (2014), 14,608 pig sera collected between 2008 and 2011in Poland were tested with ELISA, and BVDV was found in 11 (68.75%) of the 16 provinces.Seroprevalence ranged from 0.1% to 1.04% (average: 0.31%).

Focusing on Brazil, which has one of the world's largest cattle populations, a study was conducted in 2015 to detect anti-BVDV antibodies in finishing pigs slaughtered in the state ofSao Paulo, with 817 swine blood samples collected from animals in several Brazilian states. The virus neutralization tests were performed, and the seroprevalence of anti-BVDV-1-Singerneutralizing antibodies was 2.32%. (Gatto et al., 2015).

Gatto et al., (2016) reported a 4% prevalence of BVDV-1 antibodies at the animal levelin swine herds in Mossoro, Rio Grande do Norte, Brazil. A total of 412 blood samples were collected from slaughterhouse animals and tested using virus neutralization.

Rio Grande do Sul in its southernmost region, which has more than 13 million cattle, according to the State Veterinary Office (SEAPA-RS) and serologic testing. Performed on cattle from this region found BVDV seroprevalences of 56–68% (Mósena et al., 2020). As such, the prevalence of BVDV in pig herds may be speculated to be closely linked with the prevalence of the disease in cattle herds.

Almeida et al., (2017) conducted a cross-sectional study of Bovine Viral Diarrhea Virus (BVDV) infections in swine in the Brazilian state of Sao Paulo. According to their findings, 4.72% (17/360; IC 95 %: 2.97-7.43%) of 360 swine serum samples tested positive for virus neutralization (VN). Geometric meant titer (GMT) values ranged from 10 to 640 for the BVDV-1 strain Singer sample and from 10 to 80 for the BVDV-2 strain VS253 sample. In both cases, the titers obtained were generally low. Regarding herd prevalence, at least one positive animal was found in 26.79% (15/56, 95% CI: 15.19-38.38 %) of the herds. Mosena et al. (2020)

in a study involving pestivirus genomes and BVDV antibodies in serum from backyard pig herds in Southern Brazil. Antibodies against ruminant pestiviruses were found in 27 (4.2%) of 639 serum samples from 18 households (5.6%). The use of the BVDV-1a, -1b, and -2b strains to neutralize 27 VN-positive samples from 639 examined samples revealed the following: Three (11.1%) of the twenty-seven had significantly higher titers against BVDV-1a than against the other subtypes; nine (33.3%) had significantly higher titers against BVDV-1b, andnine (33.3%) also had significantly higher titers against BVDV-2.

2.2.1 Causative Agent and Transmission

In terms of transmission, epidemiological studies show that cattle are natural BVDV hosts and the primary source of infection for pigs and other ruminants (Almeida et al., 2017; Gatto et al., 2018) and that close contact with cattle farms or cattle in the same facilities were identified as risk factors for BVDV infection in swine. This transmission can occur through feeding infected cattle milk and other dairy products to pigs, using infected cattle milk and other dairy products to pigs, using contaminated CSF vaccines, and fomites (Mósena et al., 2020).

BVDV spread from infected to vulnerable animals has been themed through various mechanisms through which BVDV might be infected on vulnerable animals. Direct contact with a chronically infected animal is the most effective mechanism of virus transmission in natural settings (Peterhans, 2010; Mahmoud. 2015). Primarily acquired or gained from chronically infected animals, the pestivirus BVDV is typically known to harm pigs that have contact with these animals, with cattle being the primary source in perspective since it is recognized as a natural reservoir for the illness. Meanwhile, inclinations of this relationship exist anytime both animals are reared close to one another. Therefore, while the incidence among pigs has been associated with interaction with cattle, BVDV infection in pigs with no evidence of viral transmission from cattle has also been observed (Peterhans, 2010; Mahmoud,2015; Khodakaram et al.,2017; Firaol and Abdissa, 2021).

According to Asmare et al., (2018), BVDV can be found in ruminants and other wild animals.Hence, integrated farming systems encouraging small livestock co-habitation may be considered an area where pestivirus (BVDV) transmission could ensue. Several studies have evaluated viral shedding from acutely infected or persisitently infected (PI) heterologous hosts with regards to BVDV, where it was detected after acute experimental infection in nasal, oral,or rectal, or any combination, swab samples from alpacas, elk, mule deer, sheep, swine, and

white-tailed deer (Chakraborty et al., 2018; Walz et al., 2020). Although the viral loads from these samples were low or undetectable in experimentally infected swine, it still poses a threat for concern since the virus can be excreted in the semen of these acutely infected animals.

According to (Choe et al., 2002; Firaol and Abdissa, 2021), another way of possibly transmitting the bovine viral diarrhea virus is the exposure of free animals to contaminated vaccines. Because BVDV can contaminate cell cultures and fetal calf serum, this is more clearly demonstrated in countries where CSFV vaccination is encouraged, the prevalence of BVDV in swine herds has been linked to the widespread use of live CSF vaccines made from bovine sera from positive Chinese cattle herds. Furthermore, while it is well known that biotechnological procedures have the potential to spread diseases to free herds, batches of live CSFV vaccines used in China have already confirmed BVDV-contaminated samples, confirming the high prevalence of BVDV within the region as opposed to the generally low prevalence of BVDV in pig herds in other parts of the world (De Oliveira et al., 2020). Thus, the need for proper management is very vital in any working environment.

2.2.2 Clinical Signs and pathology of the BVDV disease in Pigs

Infected pigs are frequently asymptomatic, allowing the virus to spread undetected. Natural pestivirus infection of pig herds other than CSFV has been linked to breeding issues such as low conception rates, abortion, and stillborn piglets in certain circumstances (Mahmoud, 2015). In addition, piglets had anemia, rough hair coats, growth retardation, congenital tremors, conjunctivitis, diarrhea, polyarthritis, petechiae on the skin, and blue ear tips (USDA, 2007).

To summarize, while hemorrhage and leukopenia are common in symptomatic CSF cases, leading investigators to misdiagnose BVDV infectious diseases in pigs as CSFV, it was also discovered that transient leukopenia, hyperemia, severe gastroenteritis, and septicemia with lymph node, epicardium, and kidney hemorrhages are the most common lesions following experimental infection of pigs with BVDV strains (Firaol and Abdissa, 2021; Tadesse et al., 2019). While bleeding and leukopenia are common in acute CSF cases, investigators frequently confuse pig BVDV infection with CSFV infection. Numerous reports of pigs, particularly pregnant sows, are experimentally inoculated with BVDV via oral, intranasal, intramuscular, or intrauterine routes (Brodersen. 2014). The results are inconclusive but primarily depend on the strain and stage of pregnancy used. This suggests that, while pigs rarely show clinical signs of infection when infected with the bovine viral diarrhea virus (BVDV), its presence damages many of the animal's vital organs.

2.2.3 BVDV diagnosis and prevention in swine farms

BVDV preferentially replicates in defense cells, particularly lymphocytes, but it can also infectmonocytes and dendritic cells. Dendritic cells, as antigen presenters, play an essential role in cellular immunity by initiating the nonspecific immune response to various diseases (Chase, 2013). Its infection causes monocyte lysis to evade the immune system, influencing the identification and subsequent formation of a distinct immunological-humoral response (Iwasaki, 2010). BVDV can infect cell cultures and fetal calf serum (Nuttall et al., 1977). Diagnostic techniques that detect BVDV antibodies in serum are effective, faster, and less expensive. techniques such as viral isolation, direct fluorescent antibody test (DFA), virus neutralization test (VNT), enzyme-linked immunosorbent assay (ELISA), and a monoclonal antibody test (MAB) can be used. VNT is the reference test for the diagnosis of bovine viral diarrhea (OIE, 2015), because it has several advantages, including the ability to detect and quantify antibodies, the ability to test sera from different animal species, and the flexibility to use different genotypes/sub-types (Dubovi, 2013).

In the serum of animals previously exposed to a Pestivirus member, neutralizing antibody titersare generally medium-high for homologous viral species and low (or non-reactive) for other species (Ridpath et al., 2007). Anti-BVDV antibodies were shown to protect pigs against CSFV infection and clinical symptoms, even when anti-CSFV antibody titers were low, potentially hampering CSFV outbreaks in herds with a high frequency of anti-BVDV antibodies (Paton and Done, 1994). A similar scenario could occur in the presence of anti-Border Disease Virus (BDV) antibodies because cross-reactions could impair CSFV transmission and should be investigated for an accurate diagnosis of a CSFV infection and for the adoption of specific surveillance methods in outbreaks (Loeffen et al., 2009).

2.3 Justification

BVDV-1 and BVDV-2 have become a concern for pigs, it is an animal disease that significantlyimpacts classical swine fever, the economy, and the pig industry. The antigenic similarity between BVDV and CSFV can cause cross-reactivity in diagnostic tests, making a definitive diagnosis very difficult, along with similar clinical symptoms. In addition, statistics on the incidence and prevalence of BVDV infection in pigs especially from the Micro-region of Ponte Nova, Minas Gerais, Brazil are sparse, thus the need to confirm the occurrence of pestivirus and the distribution of antibodies in the swine farms of micro-region against BVDV and its seroprevalence by providing data and knowledge to the existing literature for BVDV infectionin pigs.

2.4 Objectives

To identify the seroprevalence of BVDV-1 and BVDV-2 from swine sera in the micro-region of Ponte Nova Minas Gerais, Brazil using the Virus neutralization technique.

To determine the antibody titers of BVDV antibodies in positive swine serum samples in this micro-region.

3.0 Materials and methods

This study was approved by the Ethics Committee on the Use of Animals from the Federal University of Viçosa (CEUA-UFV) with number 15/2022.

3.1 Study area

The samples collected from different properties were processed in the laboratory of immunological and animal virology of the veterinary department, Federal University of Vicosa.

3.2 Sample collection and preparation

This work was carried out in the Zona da Mata region of the state of Minas Gerais, Brazil in the micro-region of Ponte Nova belonging to the municipalities of Jequeri, Coimbra, Ponte Nova, Urucania, Rio Casca, and Teixeras. Convenience sampling was done between March - July 2022 where 400 blood samples were collected; 200 were from sows and 200 from finishedpigs. A total of nine farms were sampled in these municipalities.

The estimated sample size was determined through the online platform “Epi Info”, using the formula: *n* = [EDFF*Np(1-p)]/ [(d2 / Z2 1-ÿ/2*(N-1) +p*(1- p)], with a Confidence Interval of95%.

Five mls (5ml) of whole blood was drawn from the jugular vein of healthy animals to obtain serum. The blood samples were collected in tubes without anticoagulant and stored in a box with ice until arrival at laboratory. Subsequently, centrifuged at 3000 RPM for 10 minutes andthe supernatants were collected and stored under refrigeration of -20°C until we analyzed them.

3.3 Cells (MDBK)

Cells of the Madin-Darby bovine kidney (MDBK) lineage were cultured in a modified (Dulbecco's Modified Eagle Medium (DMEM) medium containing 2 mM L-glutamine, 10 mM HEPES, 1 mM sodium pyruvate, 4500 mg/L glucose, and 1500 mg/L sodium bicarbonate and 10% equine serum, at 37°C, in a humidified oven, with 5% CO2 and 95% atmospheric air.

3.4 Virus (BVDV)

The BVDV1(singer strain) and BVDV-2 (VS323) strain were provided by the Virology Laboratory of the Department of Veterinary Medicine, Universidade Federal de Santa Maria, and was stored at -80°C in the Laboratory of Immunobiological and Virology Animal of the Veterinary Department of the Federal University of Viçosa.

3.5 Viral Titration

To carry out the serology, the viral strain used must have a known titer. Thus, MDBK monolayers were infected with a viral strain suspension subjected to a sequence of 8-based 10dilutions (10^{-1} to 10^{-8}). The procedure was performed in 96-well microtiter plates using the Tissue culture infective dose method ($TCID_{50}$). The titer was calculated according to the Reedand Muench method (1938) after 72 hours of incubating the plates in an incubator with 5% CO_2. Careful reading of the wells for cytopathic effect was done using the inverted optical microscope.

3.6 Virus Neutralization Test

The methodology adapted from (House and Baker., 1971), and 96 well microplates was used in which the first columns constituted the control wells of the cells not receiving the viral suspension. The first column was replaced by 100µl of media and constituted the serum cytotoxicity. This well received cell suspension and undiluted whey. An equal volume (50 µl) of a stock of cytopathic strain of BVDV containing $TCID_{50}$ (50%) added to each well. The dilution of the suspected serum was carried out between lines B and H of the microplate at base 2 in an ascending fashion of serum (1:2, 1:4, 1:8, 1:16, 1:32, 1:64, 1:128). In this way, each serum sample was equivalent to each of the plate columns, and each serum dilution occupied one row of the microplate in a decreasing order of concentration.

After the dilutions, each well received a dose of virus-containing 100$TCID_{50}$/50µl of the BVDV-1 and BVDV-2 strain, except Column 1 and lane 1. After incubation of the serum and virus mixture for 1 hour at 37°C in a CO_2 incubator, a 50µl MDBK cell suspension was added,followed by incubation in a CO_2 oven at 37°C. The reading of the tests was done after 72 hoursof incubation by monitoring the cytopathic effect and the neutralizing antibody titer was the reciprocal of the highest serum dilutions capable of inhibiting viral replication and, consequently, the production of cytopathic effect. Positive and negative reference samples wereused as a control. The results obtained in the VN were either positive or negative. The positivesamples were those which occurred neutralization of the 100 TCID50 in a dilution higher or equal to 1:4.

3.7 Statistical analysis

The statistical analysis was performed using the R studio environment (R Core Team, 2021). The frequency for either positive or negative results was obtained using descriptive statistics. The prevalence and confidence interval at 95% for frequency was determined using epiR package.

4.0 Results

A total of 4.75% (19/400; C.I.95% 2.88-7.32) BVDV prevalence was recorded within 9 farmsin the 6 municipalities tested.

Regarding BVDV-1, of the 400 samples tested in the nine farms, one sample was positive in the farm in Coimbra municipal, 0.25% (1/400; C.I 95%, 0.01-1.39).

Regarding BVDV-2, of the 400 samples tested in the nine farms, three farms from three different municipalities (Urucania, Teixeras and Jequeri) recorded a prevalence of 4.5% (18/400; C.I 95%, 2.69-7.02) (Table 2).

Table 1. Prevalence obtained in the virus neutralization test of swine serum samples using bovine BVDV-1 (Singer strain) and BVDV-2 (Vs253 strain) from 9 farms in the micro-region of Ponte Nova Minas Gerais, Brazil.

Municipalities (Farm)	**Stage**	**Prevalence of BVDV-1 (%)**	**CI (95%)**	**Prevalence of BVDV-2 (%)**	**CI (95%)**
Jequeri (A)	Sow	0 (0/20)	0-16.84	0 (0/20)	0-16.84
	Finished	0 (0/20)	0-16.84	5 (1/20)	0.13-24.87
Jequeri (B)	Finished	0 (0/40)	0-8.81	0 (0/40)	0-8.81
Ponte Nova (A)	Sow	0 (0/20)	0-16.84	0 (0/20)	0-16.84
	Finished	0 (0/20)	0-16.84	0 (0/20)	0-16.84
Ponte Nova (B)	Sow	0 (0/40)	0-8.81	0 (0/40)	0-8.81
	Finished	0 (0/30)	0-11.75	0 (0/30)	0-11.75
Rio Casca	Sow	0 (0/20)	0-16.84	0 (0/20)	0-16.84
	Finished	0 (0/20)	0-16.84	0 (0/20)	0-16.84
Teixeras	Sow	0 (0/20)	0-16.84	10 (2/20)	1.24-31.68
	Finished	0 (0/20)	0-16.84	50 (10/20)	27.20-72.80
Urucania (A)	Sow	0 (0/20)	0-16.84	5 (1/20)	0.13-24.87
	Finished	0 (0/20)	0-16.84	20 (4/20)	5.73-43.66
Urucania (B)	Sow	0 (0/10)	0-30.85	0 (0/10)	0-30.85
	Finished	0 (0/30)	0-11.75	0 (0/30)	0-11.75
Coimbra	Sow	2 (1/50)	0.05-10.65	0 (0/50)	0-7.11
Total		**0.25 (1/400)**	**0.01-1.39**	**4.5 (18/400)**	**2.69 -7.02**

Rio Casca and Ponte Nova municipal recorded no positive sample.

Teixeras municipal had a total of 12 positive BVDV-2 samples out of 40 samples with a relativeprevalence of 30%, and more positive samples were recorded in the finished stage.

Urucania municipal recorded five (5) positive BVDV-2 samples out of 80 samples from theregion with a relative prevalence of 6.25%, the finished stage had more positive samples

Jequeri municipal recorded one positive BVDV-2 sample out of 80 samples from the regionwith a relative prevalence of 1.25%, the positive sample was from the finished stage.

Coimbra municipal recorded one positive BVDV-1 sample out of 50 serum samples from the region with a relative prevalence of 2%, the positive sample was recorded in the sow stage. (Figure 6).

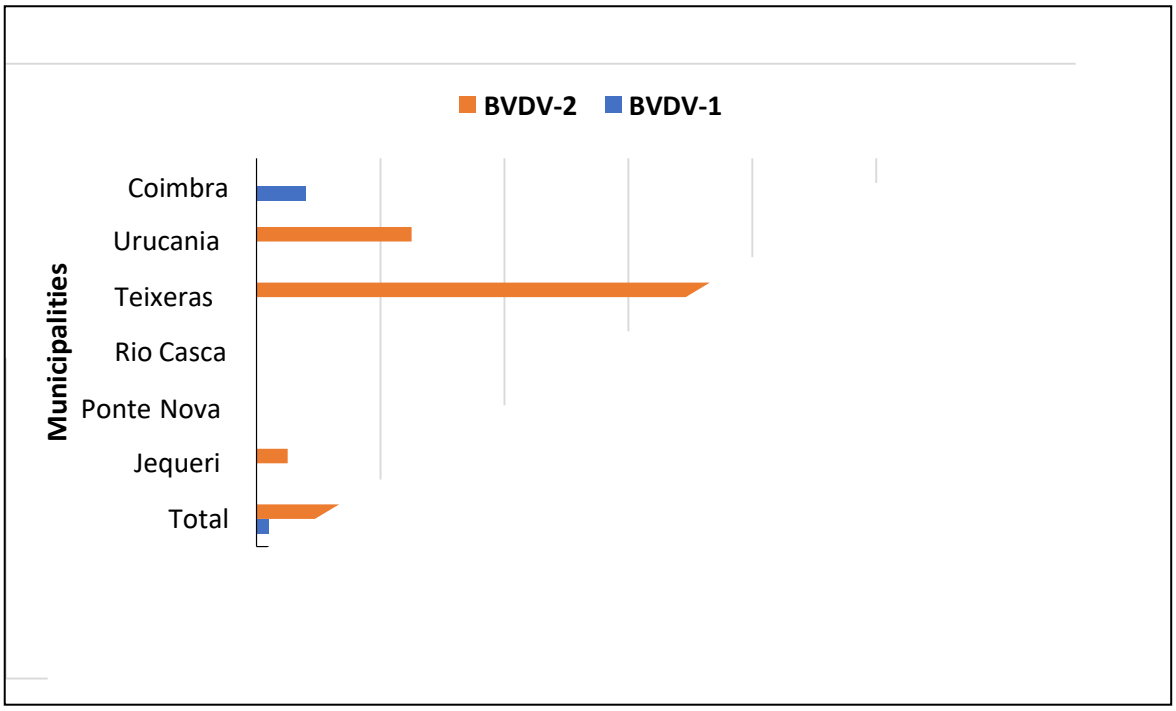

Figure 6. Prevalence of bovine viral diarrhea virus (BVDV-1 and BVDV-2) from swine serum samples from 9 farms of different municipalities in the micro-region of Ponte NovaMinas Gerais State, Brazil.

*The estimated prevalence corresponds to the number of positive samples divided by the totalnumber of samples collected from both stages in their farm.

4 municipalities recording at least one positive sample out of the 9 municipalities tested and with a herd prevalence of 44.4% (4/9; C.I 95%, 13.70-78.80).The mean antibody titers values from the BVDV positive farms were converted in logarithmic base 2 and it revealed that generally the antibody titers were low. Urucania had a mean antibodytiter of 3.32 which was the highest recorded and Teixeras had 2.83, Jequeri and Coimbra had 2 (Table 3).

Table 3: Antibody titer results of swine serum samples for bovine viral diarrhea virus from nine farms based in different municipalities in the micro-region of Ponte Nova Minas Gerais State, Brazil.

Municipalities/farm	Number of positive samples	Mean antibody titer (log2)
Jequeri	1	2 ± 0
Teixerias	12	2.83 ± 0.71
Coimbra	1	2 ± 0
Urucania	5	3.32 ± 0.71

5.0 Discussion

Pestivirus A (BVDV-1) and B (BVDV-2) are two distinct species of the genus Pestivirus, which is part of the family Flaviviridae. The two species are genetically and biologically distinct, with Pestivirus A being more closely related to the bovine viral diarrhea virus (BVDV) and Pestivirus B being more closely related to the classical swine fever virus (CSFV).Pestivirus A and B differ in their genomic organization and antigenic properties.

Pigs have been infected with BVDV in China (Deng et al., 2012), the Netherlands (Loeffen et al., 2009), Brazil (Almeida et al., 2017; Gatto et al., 2018; Mosena et al., 2020), Austria (Liessand Moening 1990), Germany (O'Connor et al., 1991), Norway (Loken et al., 1991), Ireland (Graham et al., 2001) and Denmark (Jensen, 1985), among other countries. Given that close contact with cattle farms or cattle in the same facilities was identified as a risk factor for BVDVinfection in swine (Mósena et al., 2020), epidemiological studies indicate that cattle are natural BVDV hosts and the main source of infection for pigs and other ruminants (Almeida et al., 2017; Gatto et al., 2018). Transmission can happen when tainted CSF vaccines are used, when infected milk from infected cows and other dairy products are provided to the pigs, introduction of newly acquired infected animal into the herd and also through fomites (Carbrey et al., 1976; Wensvoort et al., 1988). The transmission also occurs from one pig to another, although rarely (Wieringa-Jelsma et al., 2006).

We investigated the prevalence of BVDV-1 and BVDV-2 in 9 different farms from six different municipalities in the micro-region of Ponte Nova, Minas Gerais (CSF-free zone OIE, 2020) therefore the animals were not vaccinated against CSF.400 swine serum samples were used forthis study; 200 (sow) and 200 (finisher pigs). In this study, we observed that more positive samples were from the finished pig stage when compared to the Sow stage which differs fromstudies by Loeffen et al. 2009 who recorded a lower prevalence in finisher pigs.

The prevalence of BVDV-1 antibodies in this study revealed a prevalence of 0.25% (1/400; C.I.95%, 0.01-1.39) which is lower than the prevalence reported by Gatto et al 2016, Graham et al., 2001, Loeffen et al., 2009; Deng et al 2012; Almeida et al 2019).

We recorded BVDV-2 prevalence of 4.5% (18/400; CI 95%, 2.69-7.02).Five of the farms in this study did not provide any seroreagent samples, validating the findings of Gatto et al 2017 who didn't find any seropositive animal in four properties, and O'Sullivan et al. (2011), who found no seropositive animals for BVDV in the Canadian province of Ontario.

The BVDV prevalence value recorded in this study at 4.75% anti-BVDV antibodies and 44.4%herd prevalence is similar to the reports of Almeida et al 2017 who detected 4.72% anti-BVDV antibodies in the swine from non-technician herds in the northeastern regions of São Paulo state and Almeida et al 2019 who also recorded a 4.31% prevalence on anti-BVDV antibodies and 44.35% (51/115; CI 95%: 35.27-53.43%) of farms sampled from 3,084 swine serum samples of 115 swine herds of eight Brazilian States. Jensen, 1985 reported that in countries declared free of CSF, the prevalence of BVDV-infected pigs ranged from 1.6% to 43.5% which is withinthe range of the prevalence recorded in this study; also, findings from Gatto et al 2015 where the bleeding from pigs from 20 different small farms in the city of Mossoró - Rio Grande do Norte State, 412 samples of swine blood were collected in the city's slaughterhouse, revealingthat 9 out of 20 (45%) farms had at least one positive animal. Similar to the findings of this study where 4.13% of the animals (17 pigs) tested positive for virus neutralization. The 4.75% anti-BVDV antibodies detected in this study differ from Loeffen et al. (2009), who found 2.5%prevalence in sows, 0.42% in finisher pigs, and 11% in sow herds. The prevalence recorded inthis study can be attributed to the pig farm and bovine herd raised in close proximity which hasalso been reported by Mosena et al 2020 that there is increased seroprevalence in pigs when cattle are reared on the same farm.

The BVDV prevalence in this study can be compared to the increased BVDV cattle prevalencereported by Figueiredo et al. (1997) who recorded a 61.7% prevalence of BVDV in cattle herdsin the state of Minas Gerais; later studies carried out in this state found 57.56% of animals (bovine) reactive to the serum neutralization test (Samara et al. 2004). A study carried out in the State of Rio Grande do Sul a different state had a slightly higher prevalence

of BVDV-2 (Flores et al., 2000).

The BVDV mean antibody titers in swine serum were low ranging from 2- 3.32 regardless of the strain used when compared to previous reports by Almeida et al., 2019 who detected low antibody titers also Loeffen et al. (2009) detected eight positive swine samples with titers ranging from 15 to 3,840 using the BVDV-1 strain Osloss, and five positive samples with titersranging from 30 to 160 using the strain NADL which is higher than the titers recorded in this study. Due to the fact that pigs are atypical hosts for BVDV (Wensvoort et al., 1988), It is possible that the low number of positive samples and the low antibody titers detected are a result of the virus's difficulty in infecting the pig host. As a result, the animal's immune systemwould be better able to combat the virus, resulting in an increased frequency of low antibody titers (Almeida et al 2019). It is possible that the low number of positive samples and the low antibody titers detected are a result of the virus being highly variable and can mutate rapidly, it is often difficult for the immune system to recognize and respond to BVDV in swine. In addition, the virus can induce a persistent infection, which might impair the immune system and reduce antibody titer.

Vaccines that are contaminated due to the use of contaminated cell cultures to produce them can cause seroconversion or even disease in vaccinated animals (Levings & Wessman, 1991). The antibodies found in pig serum in this study may be the result of biosecurity or feeding of pigs with contaminated cattle products or acquisition of pigs in the past few months, though there was no information on the vaccination, history and feeding of the pigs. This can cause the pigs to seroconvert and exhibit low antibody titers. We did not detect any cross-reaction among both genotypes which is a common feature of Pestivirus. A low prevalence of BVDV antibodies was expected, given that animals were kept in intensive breeding, but genotype 2 was more prevalent in the herd than genotype 1 which is similar to the findings of Gatto et al., 2017, Gatto et al, 2018, Almeida et al, 2017. BVDV-2 infection can cause fetal abnormalities in cattle; nevertheless, if the fetus survives the infection long enough, non-specific changes inlymphoid tissue maturation may occur (Ohmann, 1982).

The increased prevalence of BVDV- 2 should also be of concern to cattle breeders in this region as it can have a severe economic impact on cattle production. Transmission of BVDV to pigs may have been influenced by the physical proximity to infectedcattle herds, and the transmission of BVDV through air although unlikely has been reported among animals kept physically close, particularly in the presence of persistently infected calves (PI) with BVDV (Niskanen et al., 2003). The high genotype prevalence in cattle herds may be connected to the high prevalence of anti-BVDV-2 antibodies in swine. Numerous isolated cases of BVDV-1 demonstrate the virus' substantial prevalence in Brazilian cattle herds (Flores et al., 2000; Flores et al., 2005). Therefore, it's probable that the greater BVDV2 prevalence in the municipalities where we conducted this research reflected the higher prevalence of BVDV-2

antibodies discovered in pigs. According to Lipowski (2014), the low prevalence of BVDV in swine herds can be attributed to the specialization of the swine industry which encourages areduction in interspecies contact. The frequency of BVDV in pigs is low because it is highly infectious and can spread rapidly between animals. Low prevalence of BVDV in swine can beas a result of the strain used for virus neutralization may differ with the strain in circulation andalso the strain used was from bovine. In addition to being difficult to detect, the virus can causea wide variety of symptoms, making it challenging to diagnose. The discovery of anti-BVDV antibodies in swine serum is alarming since the potential of a cross-reaction in CSF diagnostictests is increased by the antigenic similarities between the CSFV and BVDV. In a serological test for the diagnosis of Classical swine fever, the presence of BVDV antibodies in swine serum might result in false positive results, which can impede CSF eradication programs or even epidemiological surveys of the disease (De Smit et al., 1999; Loeffen et al., 2009; Tao et al., 2013).

6.0 Conclusion

We recorded a low antibody titer; the finished stage of the swine revealed more positivity of BVDV antibodies. BVDV antibodies were recorded in Jequeri, Teixeras, Urucania and Coimbra municipals in the micro-region of Ponte Nova Minas Gerais Brazil. The 4.75% BVDV prevalence in swine serum regions should serve as a warning to the veterinary services inspection department because of the cross-reaction that can occur between BVDV and CSFV.The antigenic similarities between both pestivirus have increased doubts about the laboratory tests used in the control of CSFV and its ability to differentiate infections caused by the different viruses.

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MINISTÉRIO DA EDUCAÇÃO
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Viçosa, 8 de junho de 2022

CERTIFICADO

A Comissão de Ética no Uso de Animais da Universidade Federal de Viçosa, CEUA/UFV, certifica que o Processo nº 15/2022, com o Projeto de Pesquisa intitulado**, "Ocorrência e distribuição de anticorpos contra pestivirus A (BVDV-1) e pestivirus B (BVDV-2) em suínos da micro-região de Ponte Nova, Minas Gerais"** coordenado pelo(a) professor(a) Ricardo Seiti Yamatogi do Departamento de Veterinária, está de acordo com a legislação vigente, Lei 11.794, de 08 de outubro de 2008, com as Resoluções Normativas editadas pelo Conselho Nacional de Controle da Experimentação Animal, CONCEA e, apresenta especificidade, caracterizando "*A não utilização de animais vivos*", portanto sendo aprovado por esta comissão em 08 de junho de 2022.

CERTIFICATE

The Ethics Committee in Use of Animals of the University of Federal de Viçosa, CEUA-UFV, certify that the 15/2022 Process, with the Research Project titled, "**Occurrence and distribution of antibodies against pestivirus A (BVDV-1) and pestivirus B (BVDV-2) in swine farms from micro-region of Ponte Nova, Minas Gerais"**, coordinated by Ricardo Seiti Yamatogi teacher of Department of Veterinary, is of according to current legislation, Law No. 11,794, of October 08, 2008, with the Normative Resolutions issued by the National Council for the Control of Animal Experimentation, CONCEA and, presents specificity, characterizing "Non-use of live animals", therefore being approved by this commission in June 08, 2022.

Prof. Fabrício Luciani Valente
Coordenador
Comissão de Ética no Uso de Animais – CEUA/UFV