



## Swine health: history, challenges and prospects



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### **Abstract:**

In swine production systems, one of the critical points that must be strictly attended to is the health of the pigs. Health is a structural component of animal welfare and reflects an optimal state of the animals, which has a direct impact on a higher productive performance and better development conditions. Infectious diseases are one of the greatest threats to the health of pigs and can cause losses of up to 100 % of production; therefore, it requires constant attention and continuous monitoring by the veterinarian and producers, in perfect coordination with the official health authorities. Currently, the implementation of best practices in the production chain is of interest to both producers and consumers. The control of infectious diseases requires collaboration between the various actors in the environment

and must be considered a public good, since their negative repercussions can range from the local to the global level. This review will address the main infectious diseases that endanger swine health, their impact, the main contributions made by the National Institute for Research in Forestry, Agriculture and Livestock (INIFAP) in its 35 years of life, mainly at the National Center for Disciplinary Research in Animal Health and Safety (CENID-SAI), formerly known as the emblematic CENID-Microbiología or Palo Alto.

**Key words:** Pig Farming, Infectious Diseases, Technology, Innovation.

Received: 24/11/2020

Accepted: 11/03/2021

## Introduction

### Pig farming in Mexico and its global context

It is estimated that there are close to 100 million head of pigs in the world, with China, the United States and Brazil being the countries with the largest inventories. In 2018, FAO estimated that the per capita consumption of pork worldwide was 12.3 kg per year, making it the second most consumed meat<sup>(1)</sup>. In Mexico, the states of Jalisco, Sonora and Puebla are the largest producers. In 2020, the Agri-food and Fisheries Information Service (SIAP) reported an estimated production of 134,953 t, and the FAO recorded a *per capita* consumption of 12.8 kg in Mexico (2018), for which pork is considered the third livestock commodity with the highest economic participation in the country<sup>(2)</sup>. Swine production units in Mexico have been classified by their level of technification and by their production objective; with respect to the level of technification, there are technified, semi-technified and low-scale production units, commonly known as backyard units<sup>(3)</sup>. Technified production units account for 40 to 50 % of the national inventory and 75 % of national pork production<sup>(4)</sup>. Semi-technified production units have a 20 % national share and are production systems that are decreasing. Finally, there are low-scale or backyard production units; this type of production has a 30 % distribution at the national level<sup>(3,4)</sup>. In these three types of swine production, it is important to highlight that, in order for the species to be produced efficiently, it is necessary to comply with animal welfare standards during production, quality parameters during transport and, above all, to control the main critical points during slaughter, in order to obtain the best quality meat to be offered to the final consumer.

Pig farming worldwide has been constantly challenged by several direct and indirect factors. Currently, the Covid-19 global pandemic, generated by the SARS-CoV-2 virus, which is responsible for more than 45 million confirmed cases, including more than one million deaths, as of October 2020, has been identified as the most serious pandemic in the world<sup>(5)</sup>. It has been confirmed that pigs are not susceptible to SARS-CoV-2 infection. However, the pork industry has been affected, as the export and import of pigs has been restricted, and infection is common among workers on farms and in processing plants, decreasing pork production capacity<sup>(6)</sup>. There has been a low consumption of meat products during this period; for this reason, there were farms that had to eliminate the inventory that was destined for the market, due to the lack of sales. In addition, the price of live pigs in Mexico was affected, reaching extremely low prices (15 to 16 pesos per kilo), causing producers to forego some of the health programs used on farms. The global Covid-19 pandemic has altered consumer behavior, distribution, production and market prices. Production setbacks were one of the biggest challenges faced by the meat industry, but the sector's capacity has largely returned to normal in recent months.

Another factor affecting pig farming is infectious agents that cause high morbidity and mortality rates. A recent example is African swine fever (ASF), which is a viral disease that causes high mortality rates in domestic pigs. In 2018, outbreaks of this disease were reported in different provinces of China and currently causes outbreaks in Europe and Asia; the implementation of strict biosecurity measures are the tool to prevent the entry of this viral agent and depopulation is the control protocol, until the development of an effective biologic is achieved<sup>(7,8)</sup>. Fortunately, the American continent is still free of this infectious agent and this makes it one of the potential exporters of pork to China. Within this context, Mexican pork exports to China, reported a 929 % growth during January 2020, totaling 4,076 t of meat, *versus* the 396 t reported in January 2019. At the end of 2019, Mexico exported 30,072 tons of pork to China, which placed the Asian country as the second largest buyer of this type of Mexican meat<sup>(9)</sup>.

## **Swine health, infectious agents and their repercussions**

Today, the stability of human society around the world has been affected by various aspects, such as population growth, food security, the need for more efficient and sustainable production methods, and climate change. Population growth is expected to require 70 % more food production than today by 2050<sup>(10)</sup>. This requires more intensive production systems, with larger animal populations, leading to the emergence of emerging and re-emerging diseases, which are a continuous challenge in animal health. The following is a description of the main diseases that must be treated, some of which are exotic, while others are endemic;

however, all of them have a negative impact on swine production in economic and productive terms.

## **Bacterial agents**

### **Respiratory diseases**

Since 1960, respiratory diseases in pigs<sup>(11)</sup> have been described, and several investigations have been carried out with the aim of identifying the etiological agents involved in them. Different studies in pigs have shown that co-infections between bacteria and viruses lead to an exacerbation of pulmonary lesions, due to an increased immunological reaction characterized by an increase in the production of proinflammatory cytokines<sup>(12)</sup>. Porcine respiratory complex (PRC) related agents can be divided into primary and secondary or opportunistic pathogens<sup>(13)</sup>. Among the primary agents, there are some bacteria with certain serotypes of high virulence of *Actinobacillus pleuropneumoniae* (App), *Mycoplasma hyopneumoniae*, and *Bordetella bronchiseptica*. Among the bacteria included as secondary or opportunistic pathogens are low-virulence strains of App, *Glaesserella parasuis* (formerly *Haemophilus parasuis*), *Pasteurella multocida*, and *Streptococcus suis*<sup>(13)</sup>.

*Actinobacillus pleuropneumoniae* (App), Gram-negative bacteria causing fibrinous pleuritis, hemorrhagic and necrotic bronchopneumonia, which can lead to increased mortality<sup>(14)</sup>. The most virulent strains of App have tropism for the lower respiratory tract (bronchioles and pneumocytes), their main damage is caused by exotoxins (ApX I, II, III and IV) that produce cell lysis, which results in characteristic lesions<sup>(14)</sup>.

*Mycoplasma hyopneumoniae*, is the cause of enzootic pneumonia<sup>(15)</sup>. Two mechanisms are derived from *M. hyopneumoniae* and its participation in CRP: i) alteration in the ciliated epithelium cells, with loss of cilia and, therefore, permissiveness to the invasion of secondary pathogens, and ii) alteration of the immune response<sup>(15)</sup>. Infection with *M. hyopneumoniae* inhibits the phagocytic activity of some cells of the innate immune response, such as macrophages, favoring infections by other pathogens<sup>(15,16)</sup>. An established *M. hyopneumoniae* infection contributes to the potentiation of viral infections<sup>(12,17)</sup>. In recent years, several efforts have been made to eliminate *M. hyopneumoniae*, mainly in breeding females<sup>(18)</sup>. The probability that the herd will remain negative for at least one year after culling is 83 %<sup>(19)</sup>.

*Bordetella bronchiseptica*, Gram-negative bacteria, which can be considered as primary or secondary pathogens, depending on the time of infection. As a primary pathogen, it can cause necrotic and hemorrhagic bronchopneumonia in piglets. Clinical signs can range from a transient cold to atrophic rhinitis, when associated with another pathogen such as *Pasteurella multocida*. Most studies on the interactions of CRP pathogens focus on the evaluation of clinical signs and the impact of the disease; however, the mechanisms involved at the molecular level have been little studied<sup>(12)</sup>, which opens up a field of research in this area.

*Glässerella parasuis*, (formerly *Haemophilus parasuis*), a Gram-negative bacterium causing Glässer's disease, which produces fibrinous polyserositis and septicemia with localization in the brain, joints and/or lungs<sup>(13)</sup>. Mortality can be high, mainly in populations with no previous exposure<sup>(18)</sup>.

*Streptococcus suis*, is an encapsulated Gram-positive coccus<sup>(20)</sup> that mainly affects pigs from 5 to 10 wk of age. It causes acute death by septicemia, meningitis, polyarthritis, polyserositis, valvular endocarditis, and can also cause damage to the digestive and genital tract; occasionally, pigs may present dyspnea and cyanosis. In healthy pigs, it is commonly found in the tonsils and the upper respiratory tract. It is a zoonotic microorganism that has increased its importance in the last 10 years, of which serotype 2 is the most important for public health<sup>(21)</sup>. *S. suis* has been classified into 35 serotypes<sup>(22)</sup>, and its distribution depends on the geographical location<sup>(23)</sup>. In the USA and Canada, serotypes 2 and 3 are the most abundant; in the case of Mexico, no data are available, but it can be suggested that they are similar.

At CENID-SAI, studies have been conducted to identify the presence of these infectious agents; in 1997, a serological survey was carried out which detected a significant association between bacterial infection with *M. hyopneumoniae*, App, and infection with Aujeszky's disease (AD) virus<sup>(24)</sup>. In 2008, an end-point PCR test was evaluated and standardized, which identified different strains of App<sup>(25)</sup>. In 2011, *M. hyopneumoniae* was identified by PCR in early infected pigs with or without the presence of clinical signs<sup>(26)</sup>.

## Digestive diseases

In intensive production farms, enteric diseases in pigs cause economic losses due to increased medication costs and stunted growth.

*Brachyspira hyodysenteriae* is considered to be an anaerobic intestinal spirochete, which causes a mucohemorrhagic colitis known as swine dysentery. Swine dysentery affects pigs in the growing and finishing stage, which manifest moderate mucoid diarrhea without

affecting body condition or, in some cases, hemorrhagic diarrhea with mortality rates of 50 to 90 %<sup>(27)</sup>. In affected herds, swine dysentery causes economic losses due to mortality, decreased growth rates, lower feed conversion, and treatment costs<sup>(28)</sup>.

*Lawsonia intracellularis* is a Gram-negative obligate intracellular bacterium that causes proliferative enteropathy or ileitis. The disease is characterized by a thickening of the intestinal mucosa due to a proliferation of the intestinal crypt epithelium, located mainly in the ileum<sup>(29)</sup>. The disease manifests itself in acute and chronic forms. The acute presentation causes hemorrhagic proliferative enteropathy, with high mortality and bloody diarrhea, affecting pigs in the finishing stage and replacement females. Intestinal adenomatosis is the chronic manifestation of the disease, subclinical and self-limiting in young pigs, although complication by opportunistic bacteria is possible, resulting in necrotic enteritis with presence of fibrinous exudate and necrosis<sup>(30)</sup>. It has a wide distribution in pig farms. Its economic impact is due to the fact that clinical cases result in lower finishing weight and poor feed conversion<sup>(31)</sup>.

*Salmonella spp.* is a ubiquitous bacterium. In the case of pigs, *S. typhimurium* has an enteric presentation with diarrhea, a consequence of enterocolitis, while *S. cholerasuis* has a septicemic presentation<sup>(32)</sup>. It is most frequent in animals during the weaning stage up to five months of age. In the superacute form, septicemia causes sudden death, mainly in pigs from two to three months of age, with diffuse hemorrhage in different organs; the acute form presents yellowish diarrhea, fever and emaciation, with ulcers, which can lead to a chronic form, with the presence of botulinous ulcers, intestinal necrosis, and stenosis. Infected animals remain carriers for months and excrete the bacteria intermittently, via feces<sup>(33)</sup>.

*Escherichia coli*, a Gram-negative, facultative anaerobic bacillus, classified within the family *Enterobacteriaceae*, normally colonizes the intestinal microbiota of domestic animals. However, it causes neonatal diarrhea in piglets and edema disease in the post-weaning stage, commonly associated with enterotoxigenic strains, which produce, as a virulence factor, enterotoxins that cause secretion of water and electrolytes into the intestinal lumen, causing diarrhea, dehydration, acidosis, and edema<sup>(34)</sup>. Other virulence factors, related to adherence and infection of epithelial cells, are fimbria and pili, which are identified for a more accurate diagnosis of the type of strain involved in the clinical picture. There are other strains that produce the Shiga toxin (Stx2e) that causes edema disease<sup>(35)</sup>. Colibacillosis has economic implications resulting from mortality rates of 50-90 %, low growth rates, weight loss, treatment costs due to the use of antibiotics, antisecretory or probiotic drugs, and vaccination<sup>(36)</sup>.

In 1998, the PCR test was established for the first time in Mexico at the CENID-SAI of INIFAP, with the objective of detecting *L. intracellularis*<sup>(37)</sup>. The advantages of this methodology are its versatility, speed, high sensitivity and specificity. In 2005, a study was



conducted to determine the frequency of herds infected with *L. intracellularis*, and it detected 37 % of positive farms<sup>(38)</sup>. With the establishment of this methodology, diagnostic services were provided to private companies, and several studies were carried out on the excretion patterns of *L. intracellularis*. In 2004, microbial resistance-causing phages were identified in strains of *Salmonella* spp<sup>(39)</sup>. In recent studies, *L. intracellularis*, *B. hyodysenteriae* and *Salmonella* spp. were detected in 26 %, 11 % and 4 %, respectively. At CENID-SAI, technology was generated and validated based on the simultaneous detection of *B. hyodysenteriae*, *L. intracellularis*, and *Salmonella* spp. by PCR from a single stool sample. Clinical and laboratory diagnosis for these three diseases was difficult, laborious and costly. This technology was transferred to private laboratories, which were able to offer the service to producers in order to confirm the presence of these agents in their herds. This was reflected in a difference in net income of 650 % for users of INIFAP technology compared to a control technology, and an economic benefit of \$936,000.00 MXN, derived from the analysis of 900 samples from pigs<sup>(40)</sup>.

## **Viral agents**

### **Endemic diseases**

#### **Infection by porcine circovirus type 2 (PCV2)**

Porcine circovirus (PCV) belongs to the genus *Circovirus* of the family Circoviridae, viruses with a single-stranded circular DNA genome. To date, four types of porcine circoviruses (PCV1-4) have been reported<sup>(41,42)</sup>. There is a high genetic diversity of PCV2 and eight genotypes (PCV2a-h) have been identified. PCV2 genotypes cannot be identified by conventional serology, as they have high cross antigenicity; this characteristic has maintained the use of available PCV2 vaccines. However, there is no cross antigenicity between PCV2 and PCV3<sup>(41,42)</sup>. To date, PCV1 (contaminant of the PK-15 cell line) is considered non-pathogenic in swine<sup>(43,44)</sup>. In 1997, PCV was associated with a disease affecting weaning pigs known as postweaning multisystemic wasting syndrome (PMWS)<sup>(45,46)</sup>. PMWS is distributed worldwide and is commonly described in pigs at weaning or early fattening in unvaccinated farms. PCV2 seroprevalence within farms ranges from 15 % to 100 %, regardless of the existence of PMWS<sup>(46,47)</sup>. In 2003, the first isolation and detection of antibodies against PCV2 was performed in Mexico. A retrospective study demonstrated the presence of antibodies against PCV2 in Mexico since 1973. This study showed that PCV2 infection has been enzootic in Mexico for many years prior to the first description of PMWS<sup>(48)</sup>. Epidemiological studies have detected up to 98 % seroprevalence in backyard pigs<sup>(49)</sup>.



Current studies have demonstrated the existence of PCV2a (12.5 %), PCV2b (87.5 %)<sup>(50)</sup>, PCV2d and, recently, PCV3<sup>(51)</sup>. In 2018, 49 % of PCV2-positive cases were identified and co-infection with PRRS virus was confirmed, these results were obtained from standardized and validated molecular tests at CENID-SAI<sup>(52)</sup>.

### **Porcine circovirus type 3 infection (PCV3)**

In 2015, reproductive problems and swine nephropathy syndrome, pneumonia and swine dermatitis were identified in swine production units in the United States. When molecular diagnosis was performed for the identification of PCV2, the results were negative, so it was decided to perform metagenomic studies, identifying the presence of a new porcine circovirus genogroup, which was named porcine circovirus type 3 (PCV3)<sup>(53)</sup>. In subsequent years it has been identified in Japan<sup>(54)</sup>, China<sup>(55,56)</sup>, the United Kingdom (since 1992)<sup>(57)</sup>, Italy<sup>(58)</sup>, Germany<sup>(59)</sup>, and Sweden<sup>(60)</sup>. As for Latin America, the first report of identification of specific antibodies against PCV3 was in samples obtained from swine production units in Mexico and the U.S.A.; these results were reported in 2016<sup>(53)</sup>. In 2017, the presence of PCV3 was reported in Brazil<sup>(61)</sup>, and the presence of PCV3 was confirmed in the Americas, Europe and Asia. The main clinical signs associated with the infection were post-weaning multisystemic wasting syndrome, nephropathy syndrome, dermatitis and reproductive failure. In Mexico, in 2018, the presence of PCV2a and PCV2b<sup>(50)</sup> was confirmed, and, therefore, vaccination strategies were implemented that have allowed the control of these clinical signs and of the economic and productive impact. These control strategies had been efficient; however, as of 2013, the appearance of some associated clinical signs was reported, and, upon diagnosis, the presence of PCV2 was ruled out, but the presence of PCV3 was confirmed. In 2017, at CENID-SAI, INIFAP, the complete genome of PCV3, detected in a production unit with reproductive failure and in pigs with clinical signs associated with post-weaning multisystemic wasting syndrome, dermatitis and nephropathy, was detected and amplified; the sequences were reported in the global gene bank (GenBank: MH192340.1 and MH192341.1)<sup>(51)</sup>. CENID-SAI has continued studying this disease; in 2019, serum samples obtained between 2012 and 2017 were analyzed; in the states of Guanajuato and Jalisco, the presence of PCV3 was identified since 2012 in both states, with a frequency of 31 %; co-infection PRRSV and PCV2 was also detected. Sequencing, genetic characterization and phylogenetic analysis were performed on the positive samples. In 2020, PCV3 whole genome sequences from serum samples of pigs from the states of Jalisco and Guanajuato were reported; these sequences were submitted to GenBank and are currently under review. These studies confirmed the presence of PCV3 in Mexico and established genetic homologies between strains; however, it is necessary to increase the number of representative sequences from different swine production units in order to establish such control strategies as the design of biologics for vaccination.

## Porcine Reproductive and Respiratory Syndrome (PRRS)

Porcine Reproductive and Respiratory Syndrome (PRRS) is a disease caused by a virus that belongs to the family *Arteriviridae*, genus *Arterivirus*. It is an enveloped virus with a 15 kb RNA genome containing nine open reading frames<sup>(62)</sup>. PRRS affects pigs of all ages, but the greatest problems occur in pregnant sows and piglets. In females, the clinical picture is characterized by decreased fertility, late abortions, increased repetitions, and a high incidence of stillbirths, mummifications, and weak births. In piglets, it causes mainly respiratory problems. PRRS was first described in 1987 in North Carolina, USA<sup>(63)</sup>. The PRRS virus (PRRSV) was first isolated in 1991 in Lelystad, The Netherlands<sup>(64)</sup>. In the U.S.A. it was isolated in 1992 (strain VR-2332)<sup>(65)</sup>. The PRRSV has a high mutation rate, generating the emergence of various viral strains grouped into two genogroups —the European strains (EU-PRRS1) and the American strains (NA-PRRS2)—, which have a homology of 63%, indicating a high genetic variability<sup>(66)</sup>. Despite the great productive and economic impact, no vaccines have been developed to serve as prevention and control tools for the clinical signs caused by this viral agent<sup>(67)</sup>. PRRSV is one of the most important infectious problems of viral origin, due to the economic impact it causes to the national and international swine industry. Worldwide, annual losses of up to \$664 million dollars have been reported. In 2016, the economic expense associated with this virus was estimated in more than 40 farms in Mexico, identifying losses of more than \$3,000.00 pesos per year per sow<sup>(68)</sup>. The economic losses in Mexican swine farming due to this disease are estimated at 400 million pesos per year, making it one of the most important diseases in Mexico. For pigs in the production line, the estimated cost is \$130 to \$260 pesos per animal per year. In Mexico, the first study showing positive serology for PRRSV was carried out in pigs imported from Canada and the United States, and a prevalence of between 2.7 and 13% was identified in the states of Sonora, Jalisco, Guanajuato, and Aguascalientes<sup>(69)</sup>. In 1997, it was reported that 78-84 % of swine production units were positive for the presence of PRRS<sup>(70)</sup>. In 2000, the first viral isolation was performed in Mexico<sup>(71)</sup>. In recent years, epidemiological studies carried out by CENID-SAI have shown that the proportion of farms that have animals with antibodies is high, reaching up to 70 % in the central part of the country. In 2007, a molecular diagnostic test for the detection of PRRSV was developed at CENID-SAI and adopted by the National Animal Health Diagnostic Center (Centro Nacional de Servicios de Diagnóstico en Salud Animal, CENASA) of the General Directorate of Animal Health (Dirección General de Salud Animal, DGSA). Currently in Mexico, antigenic and genetic characterization studies have been carried out with the strains circulating in Mexico, and it has been reported that PRRS strains present antigenic and genetic variations in the same production unit<sup>(72)</sup>. Various groups of researchers are working on the study of the antigenic regions of PRRSV<sup>(73)</sup>, with the aim of identifying prototype strains for the development of diagnostic tools and vaccines, as potential tools for prevention and control in Mexico.

## Blue eye disease

Porcine rubulavirus (PRV), the causative agent of swine blue eye disease, was discovered in the early 1980s<sup>(74-76)</sup>. PRV is currently classified as *Porcine orthorubulavirus*, within the family *Paramyxoviridae*<sup>(77)</sup>. PRV has been described only in Mexico<sup>(78)</sup>. The disease is characterized by neurological, respiratory and reproductive alterations accompanied by corneal opacity in pigs of different ages<sup>(75,79-83)</sup>. Serological diagnosis can be performed with hemagglutination inhibition, viral neutralization, immunoperoxidase and ELISA tests. The hemagglutination inhibition test is the most commonly used test, although it can frequently give false positives if it is not correctly standardized<sup>(84)</sup>. Detection and quantification of PRV by real-time RT-PCR has been reported<sup>(85,86)</sup>; these tests can be costly if applied to large populations. Therefore, there are areas of opportunity for the development of rapid tests applicable in the field. Control of the disease has not yet been achieved, mainly due to the fact that animals may present subclinical and persistent infections<sup>(82)</sup>. Sequencing of neurovirulent strains that affected the states of Jalisco and Mexico in 2015, as well as other studies, indicate that there are genetic variations from earlier outbreaks<sup>(87)</sup>. These changes in viral proteins can generate antigenic diversity, which would cause antibodies produced against one variant to lose the ability to recognize other variants<sup>(88)</sup>. From the point of view of human health, no zoonoses due to PRV have been reported, although the presence of antibodies against the virus has been demonstrated in veterinary staff<sup>(89)</sup>. It has been suggested that PRV has the potential to cause zoonosis, due to the widespread contact between humans and pigs, as has occurred with other paramyxoviruses infecting animals<sup>(90)</sup>. There are two commercial inactivated virus vaccines on the market. The results of studies suggest that the use of an outdated vaccine strain may generate little protection against circulating PRV strains<sup>(88)</sup>, due to the accumulation of mutations. Therefore, further options have been investigated, e.g., the possibility of using recombinant PVR proteins as antigens to produce a protective response. The use of HN protein expressed in *E. coli* and *Pichia pastoris*, which induce the formation of antibodies, has been studied<sup>(91,92)</sup>. Structural and antigenic prediction studies show that, in addition to the HN protein, the F, NP and M proteins potentially induce an immune response. It should be considered that the F protein of paramyxoviruses is widely conserved; in most of the predicted epitopes for PRV, very little or no variation was identified<sup>(93)</sup>. PRV has been circulating in Mexico for at least 40 yr, and the challenge is to eradicate the disease; therefore, it is important to focus on three important issues: first, the development of an effective, rapid and inexpensive diagnostic method that will allow wide use; second, the development of an effective vaccine against different variants of the virus that normally circulate, and third, a molecular epizootiological surveillance program that will allow the updating of both the diagnosis and the vaccine. These points will make an important contribution to the control and eradication of PRV in pig farms in Mexico and, thus, focus efforts on other important conditions in pigs.

## Coronavirus disease

Within the family *Coronaviridae*, there are two subfamilies: on one hand, *Coronavirinae*, with the genera *Alphacoronavirus*, *Betacoronavirus*, *Gammacoronavirus*, and on the other, *Deltacoronavirus*, and the subfamily *Torovirinae*<sup>(94)</sup>. Five coronaviruses have been identified in swine: four belong to the genus *Alphacoronavirus*, transmissible gastroenteritis virus (TGEV), described in 1946; porcine respiratory coronavirus (PRCoV), originated by mutation of the TGEV, isolated in 1984, and porcine epidemic diarrhea virus (PEDV), identified in 1977 and the recently discovered porcine enteric coronavirus (PEC), resulting from the recombination of the S gene of PEDV CV777 and TGEV. Porcine hemagglutinating encephalomyelitis virus (PHEV), isolated in 1962, which belongs to the genus *Betacoronavirus*; porcine deltacoronavirus (PDCov), of the genus *Deltacoronavirus*, detected in 2012<sup>(95-97)</sup>. TGEV was described in 1946, in the USA, and was highly prevalent during the 1970s and 1980s. PRCoV is the consequence of a natural deletion in the S protein of TGEV that turns its enteric tropism into a respiratory one, causing a subclinical disease in pigs. The emergence and spread of PRCoV resulted in a decrease in the impact of TGE in the U.S. and Europe, as PRCoV-seropositive farms reduced TGE-attributed mortality through cross-immunity. In contrast to Europe, outbreaks with both TGEV and PEDV were frequently observed in Asian countries, leading to co-infections and the need for differential diagnosis<sup>(98)</sup>. Infection with TGEV, PEDV, PDCov, and SECov affects the gastrointestinal tract of pigs, causing severe clinical signs of diarrhea and vomiting, with high mortality rates attributed to dehydration, especially in newborn piglets<sup>(95,98)</sup>. These pathogens are present in the main swine producing countries because they are highly contagious and because of the international trade of live animals or by-products, which is spreading in countries like China, USA, Canada, South Korea, and Mexico.

Immunological pressure and the high passage of the virus between animals generated mutations in the virus, giving rise to highly pathogenic variant strains of PEDV, responsible for the epidemic outbreaks in 2010. In 2013, the first outbreak of PED in the USA (phylogenetically related to strain AH2012) was described with 90-95 % mortality in piglets. Subsequently, strains with lower virulence have been identified that register insertions and deletions (INDELs) in the S<sup>(99)</sup>. According to the sequence of the spike or S protein, PEDV strains have been classified into genogroups G1a, G1b, G2a, and G2b. Group G1a includes the prototype strain CV777 and the attenuated strains historically distributed in Europe and Asia; G1b includes the S-INDEL strains, located in Europe, Asia, and North America. Strains in genogroup G2a are exclusive to the Asian continent, and in G2b is the 2013 U.S. prototype strain. PDCov was identified in 2014 during outbreaks of porcine epidemic diarrhea (PED) co-infection with PEDV in the USA. A retrospective study using samples collected prior to 2014 showed that PDCov was circulating prior to its isolation. The signs are similar to those caused by PEDV; however, the mortality rate is significantly lower<sup>(96)</sup>. The first cases of PED in Mexico occurred in the Bajío region, in Jalisco and Michoacán, in 2013. INIFAP

researchers and collaborators were pioneers in attending to producers concerned about the sanitary situation. In the first cases, diarrhea, vomiting and anorexia were observed in pregnant sows and growing pigs; in piglets, profuse yellowish diarrhea, vomiting and 100% mortality were observed<sup>(100)</sup>. By 2014, the disease was widespread in the states of Jalisco, Michoacán, Guanajuato, Querétaro, Hidalgo, Mexico, Aguascalientes, Puebla, Veracruz, Nuevo León, Tamaulipas, Sinaloa, and Sonora, causing severe economic losses. The presence of the disease was proven by the clinical and epidemiological characteristics of the outbreaks that occurred in 2013 and early 2014<sup>(101)</sup>. In that year, the National Service of Health, Safety and Food Quality (SENASICA) officially recognized the PED in our country<sup>(102)</sup>.

The first sequences of the circulating strains in Mexico, in 2013, were reported by INIFAP, in the GenBank global repository. The economic impact analysis revealed a decrease in the swine herd from 16.2 million in 2013 to 16.1 million head in 2014. On the other hand, the annual rate of pork production reported a growth of 1.9 % between 2005 and 2013; however, only 0.5 % growth was registered in 2014. Finally, 8.7 % fewer pigs were processed in 2014 than in 2013<sup>(103)</sup>. In 2016, the 2014 disease report was released, with mortality rates of 100 % in piglets<sup>(104)</sup>. According to the latest report sent to the OIE on February 11, 2016, cases of PED continue, and it is currently considered an endemic disease in Mexico<sup>(102,105)</sup>. In the Virology Laboratory of CENID-SAI, the genetic characterization of PEDV circulating in six states of the Mexican Republic in the period 2013-2016 was carried out, identifying the presence of G2 and INDEL genotypes<sup>(106)</sup>. From the identification of PEDV and PDCov in various states<sup>(101)</sup>, INIFAP has developed technologies to support producers, and two diagnostic methods have been made available: ELISA for antibody detection<sup>(107)</sup> and real-time RT-PCR for quantification of viral RNA. Research has been carried out to isolate, identify tropism, cell susceptibility and, as part of the innovation process, the development of a recombinant biologic has been proposed which has shown satisfactory results in a second phase of evaluation<sup>(108,109)</sup>. It is currently working on obtaining greater antigenic mass through scaling processes<sup>(110)</sup> in order to perform tests under farm conditions and seek registration of the product for transfer to interested laboratories in the area.

## Swine flu

Influenza is an emerging and re-emerging acute respiratory disease that affects a wide range of birds and mammals, including humans. Influenza A viruses belong to the family *Orthomyxoviridae*, have an envelope made up of the glycoproteins hemagglutinin (HA) and neuraminidase (NA), which correspond to surface antigens. These proteins participate in pathogenesis, determine viral subtypes and play a crucial role in the interaction between the virus, the host cell and the pig immune system. Currently, 18 types of HA and 11 types of NA are recognized<sup>(111-115)</sup>. The mechanism of transmission is by air via aerosols or by direct contact with nasal secretions or contaminated objects (fomites). When the virus enters the



mucosa of the upper respiratory tract, NA evades the defensive action of cilia and mucus, and the initiation of virus replication is mediated by HA binding to sialic acid (SA) receptors in respiratory tract epithelial cells. These receptors are primarily linked galactose by an  $\alpha$ -2,6-linkage (SA  $\alpha$ -2,6), present in human tracheal epithelial cells, and by SA  $\alpha$ -2,3, present in epithelial cells of the intestinal tract of birds. However, its presence has been demonstrated in respiratory tract cells in humans<sup>(116)</sup>.

The pig expresses receptors for human and avian viruses, giving rise to the possibility of generating new viral subtypes<sup>(117,118)</sup>. H1N1, H3N2, and H1N2 subtypes of swine influenza viruses are the most frequently reported<sup>(114,119,120)</sup>. Disease outbreaks are generally observed in the winter season with a morbidity of almost 100 % and mortality close to 1 %<sup>(121,122)</sup>. Because this disease is a zoonosis and therefore has public health importance, early and timely diagnosis of swine influenza virus should be considered<sup>(123)</sup>. Diagnosis should be made by laboratory tests, including viral isolation, RT-PCR, and serological tests. In addition, differential diagnosis should be performed<sup>(122)</sup>. In 2009, the first influenza pandemic of the century occurred, caused by subtype pH1N1<sup>(124)</sup>. It was shown that pigs are susceptible to this subtype<sup>(125)</sup>; in retrospective studies, seropositivity has been recorded since 2009<sup>(126)</sup>. The origin and genetic and antigenic characteristics of these viruses differ according to the continent or region where they are isolated, due to two phenomena: recombination and genetic drift<sup>(115,127)</sup>. Currently, the disease is widely distributed in all swine-producing countries, and is endemic in Mexico<sup>(120)</sup>. In 2004, a study was conducted to determine the association of PRRS with other viral and bacterial agents, including swine influenza<sup>(128)</sup>. In 2016, an experimental study found that co-infection of H1N1 influenza A virus in conjunction with Porcine rubulavirus exacerbates respiratory disease in growing pigs<sup>(129)</sup>. CENID-SAI is currently working on the validation of molecular and serological diagnostic tests and on the development of a universal biologic that will confer immunity, regardless of the subtype circulating on the farm.

## **Parvovirosis**

Porcine parvovirus (PPV, recently named Ungulate Protoparvovirus 1) causes reproductive disorders in sows<sup>(130)</sup>. Due to the absence of the immune response in the embryo or fetus in early stages, the virus can replicate, resulting in the death of the products<sup>(131)</sup>. PPV is present in the areas with the highest swine production, being widely described in the United States of America, China, Germany, Europe, Hungary, Mexico, Colombia, and Cuba. A large proportion of first-time females are naturally infected with PPV before entering the breeding herd<sup>(131,132)</sup>. Despite the continued use of vaccines, new strains have recently been described. PPV was considered to have a more conserved genome than other parvoviruses and ssDNA viruses. The first evolutionary analysis was performed in 2011, studying viruses affecting pigs in intensive production<sup>(133)</sup> and wild boar<sup>(134)</sup>, and high mutation rates (approximately 3-5 x 10<sup>-4</sup>) in the VP gene were found. The main divergences have been introduced in the last

10 to 30 yr. This evolutionary history is similar to that of carnivorous and human parvoviruses, suggesting that high mutation rates may be typical of porcine parvoviruses. Studies with strains from clinical events in various countries, including Austria, China, Rumania, and Switzerland, have reported the existence of six genotypes, with new profiles and clusters (A, B and E), exhibiting a predominance of domestic pig strains in Clusters C and D in Europe and Cluster F in China<sup>(133-136)</sup>.

Molecular profiles of new capsids with different antigenic properties have been described, including viruses used in commercial vaccines<sup>(137)</sup>. These findings have led to the hypothesis that the emergence of new capsid profiles may be due to viral adaptation to the most commonly used vaccines and, therefore, may represent "escape mutants" in a partially immune population<sup>(133,134)</sup>. The fact that novel porcine parvoviruses have been found in domestic pigs and wild boar suggests active interspecies gene flow<sup>(132)</sup>. As PPV is able to replicate in cells of bovine and human origin, its host range may be broader than commonly thought. In 1991, specific antibodies against porcine parvovirus were identified in sows and rats<sup>(138)</sup>. In 1996, CENID-SAI researchers identified that there is no statistical difference between the immunity conferred by vaccination and the immunity conferred by natural infection and that the use of vaccination does not completely prevent the reproductive problems associated with infection by this virus<sup>(139)</sup>. In 2004, they also conducted a study based on the identification of the association between PRRS virus and other infectious agents and stated that no statistical association was found with parvovirus, since all sows exhibited antibodies against this virus<sup>(128)</sup>. In the CDMX, seroprevalence has been described in backyard pigs during 2000-2009<sup>(140)</sup>. It is necessary to continue monitoring PPV in the various swine producing regions of the country in order to determine the epidemiology of the virus and to have a picture of its distribution at the national level. Actions such as the establishment of efficient diagnostic methods and updating of vaccine strains for PPV will help to strengthen disease control strategies.

## **Exotic diseases**

### **Classical swine fever**

One of the biggest sanitary problems in Mexican swine farming in the past decades was classical swine fever (CSF). In 2018, the eradication of this disease was internationally recognized, and the disease-free status has been maintained throughout the national territory. Classical swine fever is caused by a *Pestivirus* of the family *Flaviviridae*. It is a highly contagious disease, which causes, as main signs, fever, poor appetite, general weakness, neurological deterioration and hemorrhages. Morbidity and mortality in acute cases can reach



100 %<sup>(141)</sup>. In 1975, the efforts made by INIP (now INIFAP) through the work carried out by Dr. Pablo Correa in coordination with scientists from Cornell University, U.S.A., resulted in an excellent vaccine, PAV-250 (*porcine attenuated virus-passage 250*), which proved to have superior characteristics to existing commercial vaccines. Studies identified that the vaccine was safe, had satisfactory potency and did not spread. The technology developed was made available to the National Veterinary Biologics Producer (Productora Nacional de Biológicos Veterinarios, PRONABIVE), and to the private industry (SANFER and Litton Laboratories), which contributed to the success of the National CSF Eradication Campaign. Studies were conducted with the PAV-250 vaccine for the purpose of analyzing the stability of the biological product<sup>(142)</sup> and potency in the face of challenge with highly virulent strains<sup>(143)</sup>. In the same way, the safety of the vaccine was tested at different stages of production<sup>(144,145)</sup>. With the validation of PAV-250 in field conditions, it was concluded that, when applied in areas with frequent outbreaks of the disease, it was effective and safe. All the work carried out at INIFAP on the PAV-250 vaccine contributed significantly to the eradication of CSF<sup>(146)</sup>. As part of the process, it was of vital importance to have methods and techniques for the diagnosis of the disease. For virus detection, various batches of conjugate were prepared, which proved to be highly specific, of excellent quality, and with a satisfactory titer. This was verified by CENASA, as it was used routinely. It was also marketed to private industry and provided through the UN's FAO to several Latin American countries. On the other hand, the RT-PCR technique for the detection of the CSF virus was established for the first time in 2003. The test was compared with the official diagnostic tests established by the disease control and eradication campaign, direct immunofluorescence and viral isolation. It was comparable with both techniques, and, therefore, it was recommended for use as a confirmatory test for the disease<sup>(147)</sup>. With the established technology, it was possible to determine the kinetics of the vaccine virus and the characterization of field strains<sup>(148)</sup>. The widespread use of the PAV-250 vaccine led to the eradication of CSF in the country in 2009. It is estimated that the use of this vaccine prevented losses of at least 26 billion pesos during the most critical stages of the campaign to control and eradicate this disease.

### **Aujeszky's disease**

Aujeszky's disease (AD) was the second swine disease that required the implementation of a national campaign for its control and eradication. At present, it is considered eradicated in Mexico. The etiological agent is porcine alphaherpesvirus 1, which mainly causes severe neurological disease in young pigs; in adult animals, manifestations include respiratory symptoms and reproductive failure<sup>(149)</sup>. In countries where AD is endemic, it causes high economic losses and constitutes a barrier to trade in pigs and their by-products. AD still affects some countries in Europe, Asia and South America. In Mexico, AD was diagnosed for the first time in cattle in 1945<sup>(150)</sup>, and later it was isolated and typed<sup>(151)</sup>. Outbreaks in pigs were observed in the late 1970s. In the early 1990s, epidemiological studies focused on

the sanitary evaluation of pig farm animals and backyard pigs<sup>(152-154)</sup>. These studies helped the animal health authorities to make decisions in the campaign for the benefit of the national pig industry. With the generation of knowledge based on epidemiological studies, evaluation of vaccines, the use of a deleted vaccine, and the ELISA test for the detection of animals infected with the field virus, the country was declared free of AD on June 24, 2015. The vaccine used in the National Campaign against Aujeszky's disease (NOM-007-ZOO-1994), which was the key to this enormous effort, was developed from a strain with gE gene deletion. Previously, different vaccine strains used in Mexico were evaluated in order to identify which strains conferred greater protection<sup>(155)</sup>. In 1997, INIFAP developed and evaluated a dot enzyme-linked immunosorbent assay (Dot-ELISA) proposed as an alternative screening test for the detection of antibodies against AD virus. The study reported a high degree of agreement with the serum neutralization test<sup>(156)</sup>. At the request of CENASA authorities, the polymerase chain reaction (PCR) test for the detection of the AD virus was established in 2012. The test showed high sensitivity and specificity and was recommended as a complementary test to those established in the disease control and eradication campaign<sup>(157)</sup>. Subsequently, the simultaneous molecular diagnostic test for AD and enzootic pneumonia in pigs was generated. This was adopted by Laboratorio de Investigación y Patología S.A. de C.V., located in the municipality of Tepatitlán, Jalisco. The technology adopted allowed producers to detect the infectious agent early, reducing their medication costs by up to 15 % in the development and completion stages, and stunting by 10 %. On the other hand, this technology contributed to the Aujeszky's disease control and eradication campaign through its use as a complementary diagnostic test in the epidemiological surveillance of the region.

### **African swine fever**

The African swine fever virus (ASFV) is an arbovirus responsible for producing the disease of the same name (ASF) and currently represents one of the main economic threats to swine farming in the world, due to its high morbidity and mortality rate in domestic and wild pigs<sup>(158)</sup>. ASFV is a double-stranded DNA virus and is the only member of the family *Asfarviridae*<sup>(159)</sup>. The B646L gene sequence has been used to characterize ASFV in 22 genotypes (I-XXII), however, it is not predictive of virulence<sup>(160)</sup>. In terms of virulence, the various strains of ASFV can show contrasting clinical characteristics ranging from acute presentations, associated with hemorrhagic fever and death within a few days of infection, to chronic presentations with a subclinical presentation, the biological mechanisms related to the differences in virulence between strains being currently unknown<sup>(161)</sup>. ASFV was first described in Kenya in 1921; since then, it has remained endemic in a sylvatic cycle among ticks and wild boars, the latter being able to produce viraemia during infection, without developing clinical signs<sup>(158)</sup>. The first reports of ASFV (genotype I) outside the African continent were described between the 1950s and 1980s in Russia, Spain, Italy, France, Sardinia, Malta, Belgium, the Netherlands, Brazil, Cuba, and the Caribbean islands<sup>(158)</sup>. The last outbreaks in the American continent were recorded in 1984, while ASFV was eradicated

in the mid-1990s in countries outside the African continent, with the exception of Portugal where an isolated outbreak was recorded in 1999, and the island of Sardinia, where the virus has been endemically established until the present day<sup>(162,163)</sup>. In 2007, the ASFV related to genotype II was reported to have emerged in the Republic of Georgia and spread to several countries in Europe and Asia<sup>(164)</sup>. According to the OIE, it was recently reported in wild pigs in Germany, in September 10, 2020. In Europe, 67 % of the outbreaks associated with this genotype were reported between the years 2016 and 2020, mainly in wild pigs. On the other hand, in terms of mortality, Asia represents 82 %, with a total of 6,733,791 dead domestic pigs. The high virulence of strains associated with genotype II has been experimentally demonstrated in domestic pigs and wild boars, and a mortality rate of infected animals of up to 100 % has been identified within 7 to 10 d after infection<sup>(165-168)</sup>.

Undoubtedly, one of the most important challenges in terms of ASF control and prevention is the development of an effective vaccine, which does not exist commercially at present. Different strategies have been employed with the aim of obtaining a vaccine against ASF<sup>(169)</sup>, with attenuated vaccines being the most promising candidates<sup>(170)</sup>. In this sense, the development of attenuated vaccine candidates has been based on the selective deletion of ASFV genes<sup>(166,167,171-174)</sup>. One of the most promising vaccine candidates at present is the recombinant ASFV-G/ $\Delta$ I77L virus<sup>(167)</sup>. This recombinant was developed by deletion of the I77L gene of the highly virulent Georgia (genotype II) strain of ASFV. In initial tests, none of the pigs inoculated with different doses (1x10<sup>2</sup>- 1x10<sup>6</sup> HAD) of the recombinant ASFV-G/ $\Delta$ I77L developed clinical signs. Interestingly, 28 days after inoculation, 100% of the animals survived the challenge with the parental strain, producing sterile-type immunity in these animals. The results are promising; however, further research is still needed. Another interesting question, previously raised by other authors<sup>(170)</sup>, is associated with the ability of attenuated ASFVs to become endemically established in regions where this type of vaccine is used, due to the presence of a viraemia phase produced by viruses such as ASFV-G/ $\Delta$ I77L, which could represent a source of virus for ticks, with the potential to produce sylvatic cycles.

All these questions reflect the complexity of ASF control and the need for multiple research efforts in the short, medium and long term. Although ASF is a disease not found in the Mexican territory, it is essential to have a diagnostic and prevention system against it. The National Service for Agri-Food Safety and Quality (Servicio Nacional de Seguridad, Inocuidad y Calidad Agroalimentaria, SENASICA), in addition to having a high security level 3 laboratory, also has a network of laboratories throughout Mexico, all of which are managed by the U.S.-Mexico Commission for the Prevention of Foot and Mouth Disease and Other Diseases (CPA). Based on this infrastructure, it is considered that one of the greatest challenges for Mexico is to remain at the forefront in terms of diagnosis and training of those involved in the laboratory and in the field. In this sense, it is possible to suggest inter-institutional collaboration agreements with important laboratories in the region, such as the Plum Island Animal Disease Center, in the United States, and the National Center for Foreign

Animal Disease, in Canada, which are dedicated to the diagnosis and research of multiple viral diseases with economic impact on domestic animals. The creation of a group to harmonize the diagnosis of ASF among the three countries may also be proposed. Finally, it is important to note that the National Producer of Veterinary Biologicals (Productora Nacional de Biológicos Veterinarios, PRONABIVE) has a proactive participation in regard to the possibility of obtaining licenses for the use of different ASFV vaccine candidates and preparedness to provide a rapid response in case of the arrival of this disease in Mexico.

## **Challenges and perspectives**

The increasing pressure of pig production, the wide network of imports-exports, the constant evolution of pathogens that allow them to develop new adaptation and diversification mechanisms, and climate change, are some of the challenges faced by the global pork industry. Control protocols based on herd depopulation and restocking have historically been used to curb the damage caused by high impact diseases. At present, the great technological advances in the development of effective biologics, diagnostic tools, and in the development and implementation of biosecurity measures, among others, have contributed positively to the resolution of these challenges, reducing the transmission of diseases and preventing, in some cases, the use of aggressive control methods. It is important that more complete studies on the predominant strains and serotypes be carried out in our country, and that the diagnostic techniques be improved in order to be able to evaluate them using molecular methods with a genetic profile, which will make it possible to determine the properties and virulence of the infectious agents. Infection models require optimization and have the potential to improve knowledge about the pathogenicity of the disease; these models will contribute significantly to the development of new vaccines. In the coming years, when antibiotic restrictions and pork consumption will increase, the use of effective vaccines will be an important factor. Today, autogenous vaccines have shown high effectiveness, and in Europe and the United States their use is being regulated with good manufacturing practices (GMP), although validation through efficacy studies is required.

INIFAP will continue to do research focused on the generation of diagnostic tests and vaccines based on biotechnology and molecular biology. The adoption of these technologies will contribute to complement a set of tools aimed at preserving animal health and, consequently, improving the productivity of swine production units. Thus, it will be possible to implement a support program for small and medium-sized producers, aimed at strengthening herd health and, therefore, improving herd productivity in the short and medium term. An important point to consider during the upcoming years is the increase of pork consumption, not only at the national level, but also at the international level. For this

purpose, must be consider the health in pig farms, since proper management and control of the various pathogens will allow both a higher production and a reduction of costs.

## Conclusions

Control and eradication strategies should be developed, under the premise that many of the diseases are controllable through good animal husbandry practices. Timely and effective diagnosis should be proposed as a method of control and prevention in the production units, as well as vaccination, encouraging the updating and use of the strains that circulate at the national level. Biosafety measures should be strengthened, and the technification of production units should be encouraged through the dissemination of information and technology transfer to small and medium-sized producers. The application of diagnostic tests in production units to identify the circulation of infectious agents should be promoted in order to establish the prevalence of these in different regions of the country and define control programs. To develop validated, easy to apply diagnostic methods with adequate sensitivity and specificity, using samples collected through non-invasive procedures. Studies should be designed to demonstrate the efficacy of commercially available vaccines in the target population (pregnant sows or their litters). In the innovation process, national biologics should be developed using different strategies and formulations (inactivated and attenuated viruses, subunit vaccines, replicating particles, DNA vaccines, vectored vaccines, etc.) should be promoted, along with the evaluation of safety, efficacy and the best cost-benefit ratio. All these technologies, developed by INIFAP, will benefit producers, allowing them to achieve better yields and profits.

## Acknowledgments

The authors are grateful to all CENID-Microbiología researchers who have devoted their professional life to research in swine diseases –especially to Pablo Correa Girón<sup>†</sup>, MA; Atalo Martínez Lara<sup>†</sup>, MSc; María Antonia Coba Ayala, MSc; Laura Zapata Salinas, MSc; Antonio Morilla González, PhD–, and to all the technical and support personnel who worked in the past and are currently working at INIFAP, as well as to the project FONSEC SADER-CONACYT 2017-06-292826.

### Conflict of interests

The authors declare that they have no conflict of interests with the information presented herein.

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