

# INFLUENZA VIRUSES C AND D



*The mission of the Swine Health Information Center is to protect and enhance the health of the United States swine herd through coordinated global disease monitoring, targeted research investments that minimize the impact of future disease threats, and analysis of swine health data.*

August 2015 | Updated September 2021

## SUMMARY

### IMPORTANCE

- Influenza C virus (ICV) and influenza D virus (IDV) are potential emerging pathogens of pigs, although swine are not the primary host for either virus species. Swine can be naturally and experimentally infected with both ICV and IDV, but clinical illness occurs rarely, if at all.
- Currently, influenza A virus (IAV) is the only species of routine clinical significance in swine.

### PUBLIC HEALTH

- ICV is mainly a pathogen of humans. Transmission of ICV between humans and pigs is strongly suggested, but the direction of transmission is unclear.
- Whether IDV is zoonotic is not definitely known. Human infections have not been reported, but people can develop antibodies to the virus, particularly those with cattle exposure.
- Humans are rarely infected with IAVs that originate in swine (IAV-S).

### INFECTION IN SWINE

- Serosurveys show that pigs can be infected with ICV, although clinical illness has not been associated with natural infection in pigs. Experimentally, some pigs intranasally inoculated with ICV developed mild clinical signs (slight dyspnea, increased nasal secretion after intranasal inoculation), while others remained asymptomatic. No postmortem lesions have been associated with ICV in pigs.
- IDV was first isolated from a pig with influenza-like illness in 2011. Since then, additional studies have detected viral RNA or anti-IDV antibodies in pigs. An experimental study found that intranasal inoculation of 10-week-old pigs with IDV failed to produce clinical disease. In feral swine inoculated intranasally with IDV, pigs developed viremia, but none became clinically ill. No postmortem lesions have been associated with IDV in pigs.

### TREATMENT

- There is no treatment for influenza virus infection in swine.

### CLEANING AND DISINFECTION

- Influenza viruses are generally susceptible to heat, pH extremes, and drying. ICV replicates at lower temperatures than IDV (33°C vs. 37°C).
- In the presence of organic matter, formaldehyde, glutaraldehyde, beta propio-lactone, and binary ethylenimine are the most effective disinfectants for influenza. On clean surfaces, other products can be used, including phenolics, quaternary ammonium compounds, 5.25% sodium hypochlorite, 2% sodium hydroxide, 4% sodium carbonate, dilute acids, and hydroxylamine.

## PREVENTION AND CONTROL

- There are no recommendations for prevention of ICV or IDV in swine. Vaccination is an important component of IAV-S control, but there are no swine vaccines for other influenza species.

## TRANSMISSION

- Influenza viruses spread through direct or indirect contact with respiratory droplets or by inhalation of infectious aerosols.

## PATHOGENESIS

- Influenza viruses infect epithelial cells lining the respiratory tract. In cattle, IDV has been detected in the upper respiratory tract vs. the middle and lower respiratory tract in swine.

## DIAGNOSIS

- ICV is more difficult to culture than IDV. Reverse transcriptase polymerase chain reaction (RT-PCR) assays based on the highly conserved PB1 region have been used to detect IDV in swine samples. There are a few multiplex RT-PCR assays that detect all influenza species. Immunohistochemistry has also been used to detect IDV antigen in swine tissues.
- Anti-ICV and -IDV antibodies in swine can be detected by serological methods including serum neutralization, radioimmunoprecipitation, single radial hemolysis, enzyme-linked immunosorbent assays (ELISAs), microneutralization, and hemagglutination inhibition.
- Nasal swabs, pharyngeal swabs, tracheal swabs/washes, oral fluids, and lung have been used with RT-PCR. Lung or trachea are useful for IHC. Blood/serum is needed for antibody testing.

## EPIDEMIOLOGY

- Although ICV is predominantly a human pathogen, pigs, cattle, horses, camelids, and dogs can also be infected. IDV is primarily found in cattle. However, it has been detected in domestic and feral swine, as well as other species.
- Both ICV and IDV seem to be widely distributed. In swine, however, ICV has been described only in China, Japan, and Great Britain. IDV has been identified in swine in France, Ireland, Italy, Luxembourg, China, and the United States.
- Most studies have found that seroprevalence of ICV and IDV in swine is relatively low. One report from China found that up to 40% of tested pigs were seropositive for IDV.

## ETIOLOGY

- Influenza viruses are enveloped, single-stranded RNA viruses belonging to the family *Orthomyxoviridae*. There are currently four influenza species, A–D.
- Important differences between ICV and IDV vs. other influenza viruses include:
  - Presence of seven genome segments instead of eight,
  - Presence of a single hemagglutinin-esterase-fusion (HEF) glycoprotein instead of hemagglutinin (HA) and neuraminidase (NA) glycoproteins (related to virus attachment and release), and
  - Binding of 9-O-acetyl-N-acetylneuraminic acid (an acetylated sialic acid derivative) on target cells instead of sialic acid.
- There are six co-circulating ICV lineages and two co-circulating IDV lineages based on HEF sequencing.

## HISTORY IN SWINE

- ICV was first isolated from healthy pigs in China in 1981. IDV was initially detected in U.S. swine with influenza-like illness in 2011. ICV and IDV have since been detected in other swine populations.

## IMMUNITY

- Little is known about immunity to ICV and IDV in swine. Pigs experimentally infected with human ICV can remain infectious for 25 days. Feral swine experimentally infected with IDV shed virus for 3–5 days post-infection (dpi) and seroconverted at 21 dpi.
- There are no swine vaccines for ICV or IDV. Cross-protection does not occur between influenza species, including ICV and IDV

## GAPS IN PREPAREDNESS

- Knowledge of ICV and IDV in swine lags far behind IAV-S. There is no long-term surveillance for these species, and standard laboratory tests for influenza do not detect emerging strains.
- Non-specific cross-reactivity occurs in human HI testing, complicating the interpretation of laboratory data. More information is needed on both ICV and IDV to develop diagnostic tests and vaccines.
- Many questions remain regarding pathogenicity, temporal distribution, and zoonotic potential of emerging influenza viruses.

## LITERATURE REVIEW: INFLUENZA VIRUSES C AND D

### IMPORTANCE

Currently, influenza A virus (IAV) is the only species of routine clinical significance in swine.<sup>1</sup> However, influenza C virus (ICV) and influenza D virus (IDV) are potential emerging pathogens of pigs. Swine are not the primary hosts for either influenza species. Although they can be naturally and experimentally infected with both ICV and IDV, clinical illness rarely occurs, if at all. More information is needed on emerging influenza species to determine their potential impact on the swine industry and public health.

### PUBLIC HEALTH

Pigs can be infected with all four known influenza virus species (A–D),<sup>2</sup> although they are primary hosts only for IAV. Reassortment of swine, avian, and human IAVs can result in novel strains that are a serious public health concern. However, humans are rarely infected with IAVs that originate in swine (IAV-S).<sup>3</sup>

ICV causes sporadic outbreaks of mild respiratory disease in humans, the primary reservoir and host.<sup>4,5</sup> The virus occurs worldwide, and most people seem to develop antibodies against the virus by age ten.<sup>5,6</sup> In the 1990s, human ICV strains from Japan were found to be antigenically related to swine isolates from China. However, whether swine-to-human or human-to-swine transmission occurred was unknown.<sup>7</sup> Human ICV isolates are replication competent in experimentally infected pigs.<sup>8,9</sup>

The zoonotic potential of IDV remains unclear. Cattle are the primary host.<sup>10</sup> Although human infections have not been reported, people with<sup>11</sup> and without<sup>12,13</sup> occupational exposure to cattle can become seropositive. Additionally, IDV replicates in ferret<sup>13</sup> and guinea pig models,<sup>14</sup> as well as human airway epithelial cells.<sup>15</sup>

### INFECTION IN SWINE

#### CLINICAL SIGNS

Serosurveys have found evidence of ICV infection in pigs (see *Morbidity and Mortality*).<sup>9,16,17</sup> However, clinical illness has not been associated with natural infection in swine. Experimentally, some pigs intranasally inoculated with ICV developed mild clinical signs (slight dyspnea, increased nasal secretion after intranasal inoculation), while others remained asymptomatic.<sup>17</sup> No postmortem lesions have been associated with ICV in pigs.

IDV was first isolated from a pig with influenza-like illness in 2011.<sup>13</sup> Since then, additional studies have detected viral RNA or anti-IDV antibodies in pigs.<sup>18-22</sup> An experimental study found that intranasal inoculation of 10-week-old pigs with IDV failed to produce clinical disease.<sup>13</sup> In feral swine inoculated intranasally with IDV, pigs

developed viremia but none became clinically ill.<sup>23</sup> No postmortem lesions have been associated with IDV in pigs.

## **TREATMENT**

There is no treatment for influenza virus infection in swine. Antimicrobials may be used to treat secondary infections. In humans, neuraminidase inhibitors used to treat IAV and IBV are ineffective for ICV.<sup>24</sup>

## **CLEANING AND DISINFECTION**

### **SURVIVAL**

In general, influenza viruses are susceptible to heat, pH extremes, and drying, and they are unstable in the environment.<sup>25</sup> ICV replicates at lower temperatures than IDV (33°C vs. 37°C).<sup>26,27</sup> Variation in the HEF protein (responsible for virus-cell fusion) is thought to play a role in differences in thermostability.<sup>28</sup>

### **DISINFECTION**

Influenza viruses are inactivated by sunlight, disinfectants, and detergents.<sup>29</sup> In the presence of organic matter, formaldehyde, glutaraldehyde, beta propio-lactone, and binary ethylenimine are the most effective. On clean surfaces, additional disinfectants can be used, including phenolics, quaternary ammonium compounds, 5.25% sodium hypochlorite, 2% sodium hydroxide, 4% sodium carbonate, dilute acids, and hydroxylamine.<sup>25</sup>

## **PREVENTION AND CONTROL**

### **DISEASE REPORTING**

ICV and IDV are not OIE-listed diseases. There are no restrictions for importation of animals from countries or zones affected by ICV or IDV. Any suspicious clinical or necropsy findings should always be reported to the USDA and your State Animal Health Official.

### **DISEASE PREVENTION AND CONTROL**

There are no recommendations for prevention of ICV or IDV in swine. Vaccination is an important component of IAV-S control, but there are no swine vaccines for other influenza species.

## **TRANSMISSION**

Generally, influenza viruses spread through direct or indirect contact with respiratory droplets or by inhalation of infectious aerosols. Experimentally, human and swine ICVs can be transmitted to naïve pigs through direct contact with infected animals.<sup>9,17</sup> There is no evidence that ICV spreads through ingestion of meat from infected animals.<sup>29</sup> Studies on IAV (H1N1pdm09) have confirmed the absence of virus in pork and muscle tissue.<sup>1</sup> IDV seems to be less transmissible in pigs compared to bovids.<sup>23</sup>

## **PATHOGENESIS**

Generally, influenza viruses infect epithelial cells lining the respiratory tract. ICV and IDV are thought to enter cells via the endocytic pathway, fusing with the membrane of the endosome. HEF is involved in the process, following proteolytic cleavage into two subunits (HEF<sub>1</sub> and HEF<sub>2</sub>).<sup>5</sup> Although the HEF fusion mechanism is unknown, it is likely related to conformational changes that catalyze membrane fusion (similar to the role of HA in IAV entry).<sup>5</sup> Differential cleavage of HEF in ICV vs. IDV may be related to differences in host range. The esterase activity of HEF results in the destruction of receptors and release of virus particles from infected cells, though cleavage of acetyl from the C9 position of terminal 9-O-Ac-Neu5Ac.<sup>5</sup>

Tissue tropism for IDV can differ by host species. The virus has been detected in the upper respiratory tract of cattle vs. the middle and lower respiratory tract of swine.<sup>23,30,31</sup> Lesions associated with IAV are not seen in pigs infected with ICV or IDV.

## **DIAGNOSIS**

### **TESTS TO DETECT NUCLEIC ACIDS, VIRUS, OR ANTIGENS**

ICV is relatively difficult to culture.<sup>13</sup> Virus has been propagated in 8-10 day-old embryonated chicken eggs<sup>9,16,32-35</sup> Madin-Darby canine kidney (MDCK) cells,<sup>34-40</sup> human hepatocarcinoma (HuH7) cells,<sup>41</sup> human malignant melanoma (HMV-II) cells,<sup>35</sup> and human rectal tumor cells (HRT-18G).<sup>19,21,23</sup> In contrast, IDV can be readily isolated and cultured in swine testicle (ST) cells.<sup>13</sup>

Reverse transcriptase polymerase chain reaction (RT-PCR) assays based on the highly conserved PB1 region have been used to detect IDV in swine samples.<sup>13,18-21</sup> Multiplex qRT-PCR assays that detect all influenza species have also been described.<sup>42,43</sup> Immunohistochemistry (IHC) has been used to detect IDV antigen in swine tissues.<sup>23</sup>

### **TESTS TO DETECT ANTIBODY**

Anti-ICV and -IDV antibodies in swine can be detected by serological methods including serum neutralization,<sup>9,16</sup> radioimmunoprecipitation,<sup>9</sup> single radial hemolysis,<sup>17</sup> enzyme-linked immunosorbent assays (ELISAs),<sup>9</sup> microneutralization, and hemagglutination inhibition.<sup>9,13,16-20,44</sup> ICV and IDV agglutinate chicken or turkey erythrocytes, but not guinea pig erythrocytes.<sup>17,23,38,45,46</sup>

### **SAMPLES**

Nasal swabs, pharyngeal swabs, tracheal swabs/washes, oral fluids, and lung have been used with RT-PCR (for ICV and IDV).<sup>47</sup> Lung or trachea are useful for IHC. Blood or serum is needed for antibody testing.

## **EPIDEMIOLOGY**

### **SPECIES AFFECTED**

IAV infects birds, mammals, and bats. IBV is mainly a pathogen of humans, but seals and pigs are also susceptible.<sup>1</sup> ICV occurs mainly in humans, although pigs,<sup>9,16,17</sup> cattle,<sup>48</sup> horses,<sup>49</sup> camelids,<sup>50</sup> and dogs<sup>33,51,52</sup> can also be infected. Experimental infection in mice, hamsters, monkeys, and rats leads to seroconversion without clinical signs.<sup>33</sup>

IDV has a broad host range. It primarily affects cattle<sup>21,26,44,53-60</sup> and has been associated with bovine respiratory disease (BRD).<sup>44,61</sup> IDV has also been detected in swine,<sup>13,18-20</sup> feral swine,<sup>20,23</sup> small ruminants,<sup>44,56,62</sup> camelids,<sup>63,64</sup> horses,<sup>65</sup> and bioaerosols from a poultry farm.<sup>66</sup> Interestingly, a recent study of swine farm bioaerosols, swine feces, swine oral fluids, and farmworker nasal washes from Vietnam failed to detect IDV in any of the samples.<sup>67</sup>

### **GEOGRAPHIC DISTRIBUTION**

ICV has a wide distribution in humans. It co-circulates with IAV and IBV causing local epidemics, but ICV has never been implicated in a pandemic.<sup>13,68</sup> Serosurveys have identified ICV-positive swine in China,<sup>17</sup> Japan,<sup>9</sup> and Great Britain.<sup>16</sup> An early study of ICV in Chinese pigs identified a seasonal pattern that may be related to ICV in the human population.<sup>17</sup> However, a study from Japan found evidence of infection in pigs year-round.<sup>9</sup>

IDV has been detected in animals nearly worldwide.<sup>47</sup> In pigs, however, IDV has been identified only in France,<sup>20</sup> Ireland,<sup>44</sup> Italy,<sup>19,21</sup> Luxembourg,<sup>18</sup> China,<sup>22</sup> and the United States.<sup>13</sup>

### **MORBIDITY AND MORTALITY**

In Japan, 19% of swine samples from Hyogo Prefecture showed evidence of ICV infection,<sup>9</sup> although a study from Yamagata Prefecture found no anti-ICV antibodies in swine.<sup>52</sup> Prevalence of ICV in swine from China and Great Britain ranges from about 3–10%.<sup>16,17</sup>

Seroprevalence of IDV in swine appears to be generally low.<sup>13,18-20,44</sup> However, a study from Guangdong Province, China, found that up to 40% of tested pigs were seropositive.<sup>22</sup>

## ETIOLOGY

Influenza viruses are enveloped, single-stranded RNA viruses belonging to the family *Orthomyxoviridae*. They are known for the formation of novel variants. This occurs through mutation (antigenic drift) and reassortment and recombination of viral genetic material among strains within a genus (antigenic shift). Division of the influenza genome into segments facilitates reassortment between strains.<sup>8,28</sup>

As of 2020, there were seven genera and nine species in the family *Orthomyxoviridae* (see Table 1).<sup>2</sup> Influenza species are recognized by their similar lineage and ability to reassort with each other. IBV, ICV, and IDV share only 20–30% homology with IAV.<sup>13</sup> Phylogenetic studies suggest that ICV diverged from IAV and IBV prior to their separation and that IDV later diverged from ICV. Initially, IDV was thought to be an ICV variant since they are 50% homologous.<sup>13</sup>

Genus	Species
<i>Alphainfluenzavirus</i>	<b>Influenza A virus</b>
<i>Betainfluenzavirus</i>	<b>Influenza B virus</b>
<i>Deltainfluenzavirus</i>	<b>Influenza D virus</b>
<i>Gammainfluenzavirus</i>	<b>Influenza C virus</b>
<i>Isavirus</i>	Salmon isavirus (formerly infectious salmon anemia virus)
<i>Quarantavirus</i>	Johnson Atoll quarantavirus, Quarantil quarantavirus
<i>Thogotovirus</i>	Dhori thogotovirus, Thogoto thogotovirus

**Bold** indicates species known to infect pigs.

IAV and IBV have eight genome segments.<sup>2</sup> The hemagglutinin (HA) and neuraminidase (NA) spike glycoproteins are responsible for attachment to the host receptor and release of viral progeny into the host cell, respectively. IAVs are grouped according to HA and NA properties; there are currently 19 known HA and 11 known NA subtypes.<sup>47</sup> H1N1, H1N2, and H3N2 are IAV subtypes that have caused illness in both humans and pigs.<sup>47</sup> IBVs are grouped into two lineages, Victoria/B and Yamagata/B.<sup>69</sup> Both IAV and IBV bind sialic acid receptors ( $\alpha$ -2,3 and  $\alpha$ -2,6) on host cells in the upper respiratory system.

ICV and IDV have seven genome segments<sup>2</sup> that are packaged into eight ribonucleoprotein complexes.<sup>70</sup> They do not contain the HA and NA glycoproteins. Instead, they have a single hemagglutinin-esterase-fusion (HEF) glycoprotein that fulfills the roles of both HA and NA in virus attachment and release.<sup>47</sup> ICV and IDV bind 9-O-acetyl-N-acetylneuraminic acid (9-O-Ac-Neu5Ac, an acetylated sialic acid derivative) on target cells instead of sialic acid.

Both ICV and IDV encode for three polymerase proteins (PB2, PB1, and P3), HEF glycoprotein, nucleoprotein (N), matrix protein (M1), CM2 protein (an integral membrane protein), and two nonstructural proteins (NS1 and NS2).<sup>2</sup> Based on HEF sequencing, there are six co-circulating lineages of ICV, and two co-circulating lineages of IDV.<sup>5</sup> Reassortant ICV strains frequently emerge both *in vitro*<sup>32</sup> and *in nature*.<sup>27,45,71,72</sup> Reassortant IDVs also occur.<sup>73,74</sup> ICVs are more antigenically stable and slower to evolve than other influenza viruses, including IAV and IDV.<sup>5,13,27,28</sup>

## HISTORY IN SWINE

Although influenza-like illness occurred in pigs during the 1918 pandemic, influenza was first documented in pigs in 1930. Until the 1990s, H1N1 was the only influenza virus that was swine-adapted.<sup>1</sup> Then, influenza viruses emerged containing a reassortment of human, swine, and avian strains. ICV was first isolated from healthy pigs in China in 1981.<sup>17</sup> IDV was initially detected in U.S. swine with influenza-like illness in 2011.<sup>26</sup> ICV and IDV have since been detected in other swine populations throughout the world (see *Epidemiology*).

## IMMUNITY

### POST-EXPOSURE

Pigs experimentally infected with human ICV can remain infectious for 25 days. However, fewer pigs were seropositive when infected with human ICV compared to those infected with swine strains.<sup>17</sup> ICV reinfection in humans, especially within short periods of time, suggests that infection does not induce protective immunity in all individuals.<sup>45</sup> A similar phenomenon has been observed in cattle infected with IDV.<sup>75</sup>

Little is known about immunity to IDV in swine. Feral swine experimentally infected with IDV shed virus for 3–5 days post-infection (dpi) and seroconverted at 21 dpi.<sup>23</sup>

### VACCINES

There are no swine vaccines for ICV or IDV. Even human influenza vaccines protect only against IAV and IBV.<sup>8</sup>

### CROSS-PROTECTION

Cross-protection does not occur between influenza species, including ICV and IDV.<sup>13,73</sup>

### GAPS IN PREPAREDNESS

Knowledge of ICV and IDV in swine lags far behind IAV-S. There is no long-term surveillance for these species, and standard laboratory tests for influenza do not detect emerging strains. Non-specific cross-reactivity has been observed in human HI testing, complicating the interpretation of laboratory data. More information is needed on both ICV and IDV to develop diagnostic tests and vaccines. Additionally, many questions remain regarding pathogenicity, temporal distribution, and zoonotic potential of emerging influenza viruses.<sup>53</sup>

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