

# Influenza A virus: the virus that reinvents itself



IDEXX Reference Laboratories responds to emerging influenza strains with specific RealPCR tests for rapid, accurate diagnosis

The 2015 Midwest H3N2 canine influenza outbreak is no longer confined to dogs in the greater Chicago region as cases have now been detected in 18 states, including an emerging outbreak in the Atlanta, Georgia, region. IDEXX Reference Laboratories has also confirmed infection in a cat from New York (see case study on page 6). The evolution and spread of canine influenza in the United States is described below, along with recommendations for diagnosis, prevention, and management of an outbreak in your clinic or neighborhood.

## Background: a changing virus

Influenza A virus is a single-stranded RNA virus in the Orthomyxoviridae family, which was first isolated in 1933,<sup>1</sup> was retrospectively confirmed to be responsible for the 1918 human pandemic,<sup>2</sup> and is suspected to be the cause of earlier outbreaks recorded through the centuries. There are many different strains and subtypes of influenza A, which vary in their infectivity for different species. Influenza subtypes (HxNx) are identified by variants in the two surface glycoproteins: hemagglutinin and neuraminidase. Within these subtypes, different strains may become adapted to a particular species (avian, human, swine, equine, or canine), and are named for their primary host species. Avians are considered the natural host for influenza A, and they can serve as a reservoir for all the subtypes. Endemic influenza strains have been found in humans, pigs, horses, and dogs. Rarely, other species can also be infected, including cats, ferrets, guinea pigs, camels, seals, and whales.

Although the influenza virus has been recognized for a long time, it is frequently reported in the news as an “emerging virus” when new outbreaks and even pandemics occur in humans, poultry, horses, or dogs. Influenza virus can mutate rapidly, resulting in both antigenic drift and antigenic shifts. In antigenic *drift*, one particular subtype of the virus changes just enough to either infect a new species or to evade the immune system of animals vaccinated for or previously infected by that particular subtype. It is these minor changes that are responsible for failure of vaccines to provide complete immunity. In antigenic *shift*, a complete change in the virus occurs—usually a new subtype produced by recombination when an animal is infected with two different subtypes of influenza at the same time. This can occur, for example, when poultry and swine are housed together in close quarters. The emergence of new subtypes or strains that can infect an immunologically naive group of animals has been the cause of outbreaks, and even pandemics, over the years. Recognizing the emergence of new strains is vital for control of local epidemics in an at-risk population of animals or people. Two such emergencies have occurred in dogs in the United States: H3N8 in 2004 and H3N2 in 2015. More recently, an avian H7N2 influenza virus with a high cat-to-cat transmission rate emerged in shelter cats in New York City in November 2016.

## H3N8 canine influenza virus emergence

The H3N8 influenza virus originated in horses but adapted to become infective for dogs, resulting in a canine influenza strain that is specific for dogs.<sup>3</sup> H3N8 canine influenza was first detected during a 2004 respiratory outbreak in racing greyhounds at a Florida track. Additional isolated outbreaks were reported through the summer of 2004 at 14 tracks representing 6 states (Florida, Texas, Alabama, Arkansas, West Virginia, and Kansas). The virus continued to spread through early 2005 to an additional 20 racing tracks representing 11 states total and from there to the general pet dog population. At this time, confirmed positives have been identified in 30 states.<sup>4</sup>

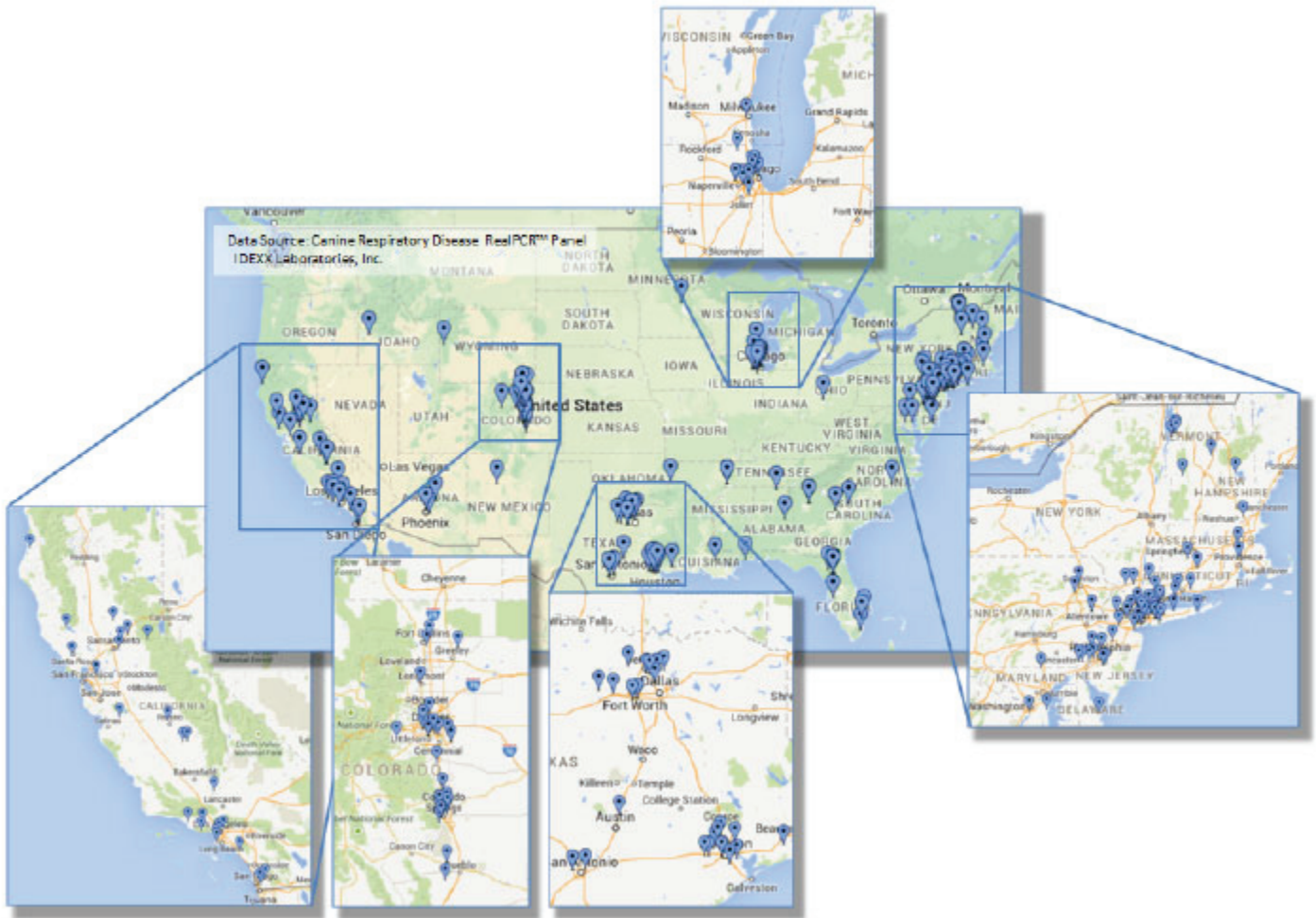
H3N8 canine influenza virus has been included in the IDEXX Canine Respiratory Disease RealPCR™ Panel since 2007. The yearly frequency of positive H3N8 Influenza Virus RealPCR™ results in this panel has been 1%–4% over the past 8 years with declining frequency noted in the last 4 years.<sup>5</sup> A cluster of infected dogs was seen in Chicago in 2008, with isolated positives in that region since then. Clusters of H3N8 canine influenza are currently active in Southern California, Texas, and New York. Map A shows the distribution of positive H3N8 RealPCR™ results identified at IDEXX Reference Laboratories from 2007–2015.<sup>6</sup>

## H3N2 canine influenza virus emergence

In early spring of 2015, veterinarians in the greater Chicago region began reporting unexpectedly high numbers of dogs presenting with respiratory illness, often accompanied by fever and cough. The signs were classic for influenza, yet the dogs tested negative on IDEXX canine respiratory disease RealPCR™ panels for the H3N8 canine influenza virus and for the H1N1 human influenza virus, which can infect dogs by reverse zoonosis. The majority of affected dogs were also negative for the other respiratory panel pathogens. Additionally, both unvaccinated dogs and dogs vaccinated for H3N8 canine influenza were affected. H3N8 serology performed at Cornell University Animal Health Diagnostic Center (AHDC) was also reported to be negative in most unvaccinated dogs affected by the outbreak. In March 2015, Cornell reported that these dogs, although negative for H3N8 canine influenza, were testing positive on a more general influenza A PCR test directed against the matrix protein genes (that are conserved across the influenza A subtypes). Subsequent neuraminidase typing by the Wisconsin Veterinary Diagnostic Laboratory, determined the infecting virus to be a strain new to North America, the H3N2 canine influenza virus.<sup>7,8</sup>

Although new to the United States, H3N2 canine influenza emerged in Asia around 2006.<sup>9</sup> Until now, this canine influenza strain has only been reported in Korea, China, and Thailand. Sequencing of the complete genome of an April 9, 2015, isolate from Chicago

**Map A.**  
**H3N8 Influenza Virus RealPCR™ Test positives**  
**reported by IDEXX Reference Laboratories (2007–2015)**



shows 99% similarity to strains previously isolated from South Korea.<sup>10</sup> This suggests that the virus currently infecting dogs in the United States may have originated in Asia.

In response to the Chicago H3N2 outbreak, IDEXX Reference Laboratories developed and validated the H3N2 Canine Influenza RealPCR™ Test in May 2015. This test was added to the IDEXX canine respiratory disease RealPCR™ panels at no additional charge. In addition, stored canine and feline respiratory PCR specimens dating from February–April 2015 were reanalyzed for the presence of H3N2 canine influenza RNA. The earliest detected H3N2-positive results were two dogs from Chicago and Michigan, respectively, on March 1, 2015. All February submissions tested negative for H3N2 influenza. During this reanalysis, a cat with respiratory signs was confirmed to have been infected with H3N2 canine influenza virus (see case study on page 6).

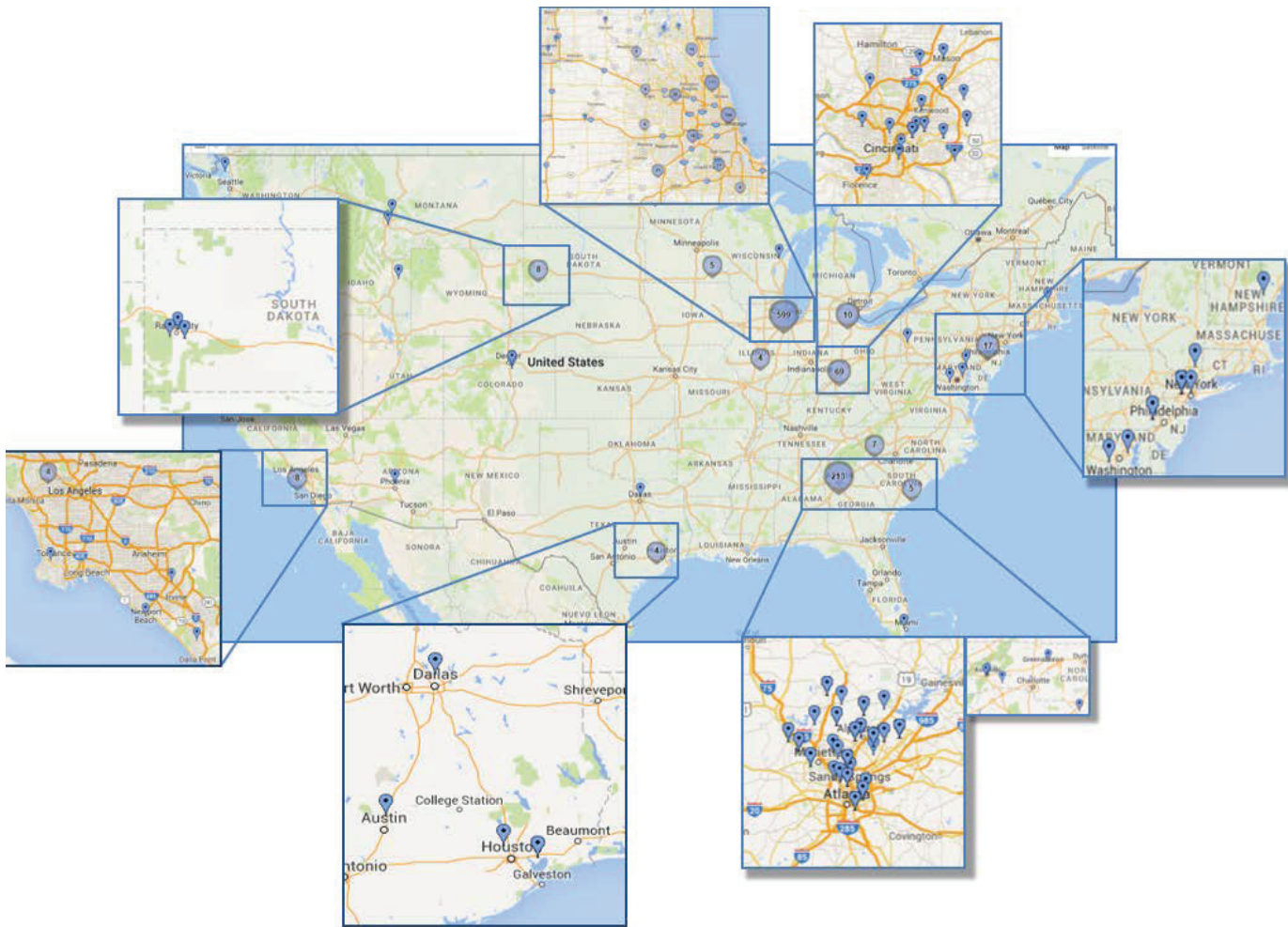
Initially, H3N2-positive cases were primarily restricted to the greater Chicago region. However, over the next 3 months, isolated cases were reported in other states, including Alabama, California, Georgia, Indiana, Maine, Maryland, Michigan, Minnesota, New

Jersey, New York, North Carolina, Ohio, South Carolina, South Dakota, Texas, Virginia, and Wisconsin. In June, a growing number of cases began to be reported in the greater Atlanta region, suggesting the development of a new regional epidemic. Map B shows the distribution of cases identified by IDEXX RealPCR™ testing from March 2015–November 2016.<sup>11</sup> It is expected that the virus will appear in other states within North America as dogs and humans increase travel this summer.

**Transmission and pathogenesis**

Canine influenza virus is spread easily by aerosolization of respiratory secretions. It remains viable in the environment for up to 24–48 hours and can be transmitted by fomites, such as food and water bowls and the hands and clothing of humans handling an infected dog. The incubation period is short for canine influenza and signs are typically seen in 1–3 days after exposure. Viral shedding can begin even before development of clinical signs and is highest in the first week of infection. Early evidence suggests that the H3N2 viral shedding period is longer than what has been seen

## Map B. H3N2 Influenza Virus RealPCR™ Test positives reported by IDEXX Reference Laboratories (March 2015–November 2016)



previously with H3N8, with some cases still testing positive by PCR for as long as 2–3 weeks following initial clinical presentation.

Regardless of the subtype or species infected, influenza causes significant respiratory disease. In our companion animals, clinically manifest influenza infection is generally characterized by high fever, cough, inappetance, weight loss, and interstitial pneumonia, and can be fatal in rare cases.

### Diagnosis

Diagnosis of canine influenza is based on recognition of classic clinical signs (fever and respiratory signs being predominant), history of potential exposure, and positive serology or molecular tests. Real-time PCR tests detect the presence of the viral RNA in respiratory secretions and are considered confirmatory for infection in a patient with consistent clinical signs. Peak viral shedding occurs early in infection (days 2–3 of clinical signs) and correlates with the best chance of obtaining a PCR-positive result. H3N8 canine influenza virus may shed for only 1 week following onset of clinical signs, while H3N2 virus has demonstrated longer shedding

periods in some patients. Ideal specimens for influenza include nasal and deep pharyngeal swabs.

On May 4, 2015, IDEXX Reference Laboratories announced the availability of an H3N2 Influenza Virus RealPCR™ Test, allowing rapid, reliable, and specific testing for the H3N2 virus. The specific H3N2 influenza virus RealPCR test was included in the Comprehensive Canine Respiratory Disease (CRD) RealPCR panels at no additional charge. In a dog with respiratory signs suspicious for influenza, the comprehensive panel is recommended to allow detection not only of H3N2 virus but also to detect other common respiratory pathogens, which may be a coinfection or an alternate cause of clinical signs. However, for situations in which testing for only influenza virus is desired, a Canine Influenza Virus RealPCR™ Panel is also available. This panel includes the three influenza virus strains (H3N8, H3N2, H1N1) currently recognized to cause disease in dogs in North America, along with the nonspecific Influenza A Virus RealPCR Test directed against the matrix protein to detect the appearance of new strains.



Serology tests detect antibodies against canine influenza; a positive result can occur with exposure, infection, or vaccination (in the case of H3N8 virus). Antibody tests typically are not positive early in infection, and paired acute and convalescent titers are recommended to confirm a rising titer consistent with infection. Serology is most useful in patients presenting with chronic clinical signs when the patient is less likely to be shedding virus for detection by PCR.

## Prevention and management

There are two vaccines for H3N8 canine influenza virus available in the U.S., but there are no commercially available vaccines directed against H3N2 canine influenza in the U.S. Cross-protection against H3N2 from the H3N8 vaccine is unlikely, but this has not yet been studied. In regions where both H3N2 and H3N8 are active, vaccination against H3N8 may reduce the risk of coinfection with the two strains and limit the opportunity for viral reassortment and emergence of a new canine influenza strain.

Because of the highly contagious nature of the influenza virus, owners of dogs in areas affected by a canine influenza outbreak should be advised to restrict their dogs from areas where dogs congregate (e.g., dog parks, shelters, boarding kennels, grooming facilities, doggy daycare). Owners should understand that they can serve as fomites and carry the virus home on their hands and clothing if they interact with infected dogs outside their household.

In the clinic or kennel setting, canine influenza virus can be killed with common disinfectants, including quaternary ammonium compounds, phenols, and bleach. In affected regions, we recommend a protocol to isolate dogs with respiratory signs and limit their contact with other dogs.

## Influenza infection in cats

Although cats can be readily infected with influenza virus, they rarely develop clinical signs.<sup>12</sup> The presence of other respiratory coinfections, common in shelter cats, appears to increase the risk of complications and more severe clinical signs. Stress and crowded conditions are also contributory factors in the spread of disease and development of clinical signs with influenza infections in cats. Sporadic isolated cases of human H1N1 pandemic influenza virus have been reported in cats. Natural infection with the H3N2 canine influenza virus in cats with clinical signs is rare but, as demonstrated in our case study (see page 6), it does occur. In April 2016, a group of cats with respiratory signs in a shelter in the Midwest were found to be infected with H3N2 virus. To date, cats do not appear to be susceptible to the H3N8 canine influenza virus.

More recently, in December 2016, an outbreak of avian H7N2 influenza virus occurred in a New York City shelter, resulting in the quarantine of over 500 cats. Infection was confirmed in over 380 cats and one veterinarian. The initial case was traced back to a kitten who had died of complications of pneumonia in November 2016. This outbreak represented the first report of a feline infection with this avian-lineage strain. Clinical signs were mild in the majority of the cats with ocular discharge and malaise as the most common manifestations. However, secondary pneumonia resulted in the death of two cats. A universal influenza type A matrix RealPCR test is included in the Feline Upper Respiratory Disease RealPCR Panel, allowing rapid recognition of the emerging H7N2 strain in cats. In January 2017, IDEXX Reference Laboratories

developed a specific H7N2 RealPCR test. This specific test has been added to the feline respiratory panels at no additional charge.

## Additional resources for management

The American Veterinary Medical Association (AVMA) offers resources for pet owners concerned about canine influenza at the following website: [avma.org/public/PetCare/Pages/CanineInfluenza.aspx](http://avma.org/public/PetCare/Pages/CanineInfluenza.aspx)

Guidelines for managing canine influenza for veterinary clinics are available on the AVMA website at [avma.org/KB/Resources/Reference/Pages/Canine-Influenza-Backgrounder.aspx](http://avma.org/KB/Resources/Reference/Pages/Canine-Influenza-Backgrounder.aspx) and for shelters via Maddie's® Shelter Medicine Program website at [sheltermedicine.vetmed.ufl.edu/shelter-services/tools-tips-fact-sheets/canine-influenza/](http://sheltermedicine.vetmed.ufl.edu/shelter-services/tools-tips-fact-sheets/canine-influenza/)

## Ordering information

### Recommended canine respiratory RealPCR testing options

test code	test name and contents
<b>2524</b>	<b>Respiratory Disease (CRD) RealPCR™ Panel (Comprehensive)—Canine</b> <i>Bordetella bronchiseptica</i> , canine adenovirus type 2, canine distemper virus (CDV) Quant, canine herpesvirus type 1 (CHV-1), canine parainfluenza virus, canine pneumovirus, canine respiratory coronavirus (CRCoV), H3N2 canine influenza virus, influenza A virus (includes H3N8, H1N1, H7N2), <i>Mycoplasma cynos</i> , and <i>Streptococcus equi</i> subsp. <i>zooepidemicus</i> RealPCR™ tests. Includes quantification of distemper viral particles if PCR positive. Includes influenza A strain identification if PCR positive.
<b>3036</b>	<b>Respiratory Disease (CRD) RealPCR™ Panel (Comprehensive) with Culture (if Indicated)—Canine</b> Respiratory Disease (CRD) RealPCR™ Panel (Comprehensive)—Canine (test code 2524). If the RealPCR™ test is positive for <i>Bordetella bronchiseptica</i> or <i>Streptococcus equi</i> subsp. <i>zooepidemicus</i> , a culture with susceptibilities on selective media will be automatically performed at no additional charge.
<b>3731</b>	<b>Influenza Virus RealPCR™ Panel—Canine</b> H1N1 pandemic influenza virus, H3N2 canine influenza virus, H3N8 canine influenza virus, influenza A virus RealPCR™ tests. Includes influenza A strain identification if PCR positive. Includes influenza A strain identification if PCR positive.

### Recommended feline respiratory RealPCR testing options

test code	test name and contents
<b>2512</b>	<b>Upper Respiratory Disease (URD) RealPCR™ Panel—Feline</b> <i>Bordetella bronchiseptica</i> , <i>Chlamydomphila felis</i> , feline calicivirus, feline herpesvirus type 1 (FHV-1) Quant, influenza A virus (includes H3N2, H1N1, H3N8), H7N2 avian influenza virus, and <i>Mycoplasma felis</i> RealPCR™ tests. Includes quantification of feline herpesvirus type 1 (FHV-1) viral particles if PCR positive. Includes influenza A strain identification if PCR positive.
<b>3037</b>	<b>Upper Respiratory Disease (URD) RealPCR™ Panel with Culture (if Indicated)—Feline</b> Upper Respiratory Disease (URD) RealPCR™ Panel—Feline (test code 2512). If the RealPCR™ test is positive for <i>Bordetella bronchiseptica</i> , a culture with susceptibilities on selective media will be automatically performed at no additional charge.

**Specimen requirements:** For RealPCR™ panel: Deep pharyngeal swab (with visible organic material on swab; please rub firmly) and a conjunctival swab (wipe eye clean; swab inside of eyelid) in the same tube. Please submit dry, plastic-stemmed swabs, without transport media, in an RTT or an empty, sterile tube; keep refrigerated. Collect specimens prior to antibiotic administration.

When ordering a panel that includes a culture, also submit one culture swab in transport media for aerobic culture if indicated.

## Turnaround time

The IDEXX nationwide network of reference laboratories provides daily courier service or IDEXX-Direct® service to pick up your specimens and forward them to our IDEXX Molecular Diagnostics Laboratory in California. IDEXX RealPCR tests are run daily, Monday–Friday. Specimens received on Saturday or Sunday are processed on Monday. You can expect results within 1–3 working days, depending on shipping time. Allow additional time for influenza A strain identification.

## Contacting IDEXX

### Laboratory Customer Support

If you have any questions regarding test codes, turnaround time, or pricing, please contact our Laboratory Customer Support Team at 1-888-433-9987.

### Expert feedback when you need it

Our medical specialty consulting service is available for expert and complimentary consultation. Call 1-888-433-9987 if you have questions.

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1. Smith W, Manch MD, Andrewes CH, Laidlaw PP. A virus obtained from influenza patients. *The Lancet*. 1933;222(5732):66–68.
2. Taubenberger JK, Reid AH, Krafft AE, Bijwaard KE, Fanning TG. Initial genetic characterization of the 1918 “Spanish” influenza virus. *Science*. 1997;275(5307):1793–1796.
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11. Data on file at IDEXX Laboratories, Inc. Westbrook, Maine USA.
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# H3N2 canine influenza virus infection confirmed in a cat



## Patient

Poly, 5-month-old, spayed female domestic shorthair

## Presenting reason

Evaluation for one-week history of mild respiratory signs progressing to general malaise, lethargy, and inappetence in the last 24 hours.

## History

Poly and her housemate, Pumpkin (an unrelated 7-month-old, neutered male domestic shorthair), were both adopted from the local New York City shelter 10 days prior to presentation. Pumpkin had developed lethargy, inappetence, and sneezing one day after the two cats were adopted. Pumpkin's respiratory illness quickly progressed to more significant respiratory signs with open mouth breathing, conjunctivitis, and fever. He was hospitalized for 3 days, receiving supportive care including intravenous fluids, appetite stimulants, and antibiotics. At the time Poly presented to the clinic, Pumpkin was still showing respiratory signs, but he was being managed at home with subcutaneous fluids and antibiotics.

Poly presented with a history of sneezing, decreased appetite, and "acting like she doesn't feel well." Poly's clinical signs were less severe than those seen in her housemate, but her owner was concerned she might progress as Pumpkin had.

## Physical examination

Poly was quiet, alert, and responsive. Her temperature was 102.2°F. She had mild conjunctivitis with dark discharge from both eyes and pale yellow discharge from both nares. Her breathing was very congested nasally. She had a slow skin tent and appeared subjectively to be approximately 5%–8% dehydrated.

## Diagnostic plan

A Feline Upper Respiratory Disease (URD) RealPCR™ Panel was submitted to IDEXX Reference Laboratories in the hope of finding the infectious cause of both Poly and Pumpkin's respiratory signs. Poly, rather than Pumpkin, was selected for testing because Pumpkin had already received both antibiotics and antiviral medications at that point, which could interfere with testing.

## Laboratory findings

### Feline Upper Respiratory Disease (URD) RealPCR Panel result

The respiratory RealPCR panel was positive for *Mycoplasma felis* and feline herpesvirus type 1. The feline herpesvirus result was quantified to differentiate between latent and active infection. In Poly's case, the viral load at 101,000 viral particles per swab was intermediate between what is typically seen in latent infection (less than 50,000) versus the much higher viral counts usually seen in active infection (greater than 150,000). This suggested that the herpesvirus may not be the initiating cause of the clinical signs, but rather that it may be either a new infection or a latent infection in the process of reactivating because of the stress of concurrent infection, which is further contributing to her disease. *Mycoplasma felis* is associated with conjunctivitis, upper respiratory disease, rhinosinusitis, and pneumonia, and could be consistent with Poly and Pumpkin's clinical signs.

## Initial therapy

While awaiting results, Poly was given subcutaneous fluids and started on the same medications that Pumpkin was receiving, including terramycin ophthalmic ointment, lysine, oral doxycycline, and famciclovir.

## Update from the IDEXX Molecular Diagnostics Laboratory

Three weeks later, following the release of the new H3N2 Influenza Virus RealPCR™ Test, retrospective reanalysis of all respiratory specimens submitted in April was performed. Poly's specimen was found to be positive for H3N2 canine influenza. The veterinarian managing the case was notified by personal telephone communication of the positive result.

## Case outcome

Poly responded well to specific antiviral therapy for herpesvirus, antibiotics for *Mycoplasma felis*, and general supportive care. By the time the H3N2 infection was recognized, she had fully recovered. Pumpkin's condition improved, but he re-presented two months later with a history of continued mild, chronic, intermittent cough. At that time, a Feline Upper Respiratory Disease (URD) RealPCR Panel was performed and was negative for all organisms, including *Mycoplasma felis* and feline herpesvirus. An H3N2 canine influenza virus RealPCR test was also negative. Pumpkin's persistent cough was suspected to be due to ongoing inflammation in the respiratory passages as a sequela to his earlier respiratory infection.



POLY



2015 APR 8



**4/08/2015** (Order Received)  
**4/17/2015 @10:36 am** (Last Updated)

**IDEXX Reference Laboratories**

[Show Details](#)

**Chlamydophila felis RealPCR** NEGATIVE

**Feline Calicivirus RealPCR** NEGATIVE

**Feline Herpesvirus 1 RealPCR** POSITIVE

**FHV-1 Quantity** <sup>a</sup> 101 THOUS/SWAB

**Fold Difference Above Cutoff** 0.67 TIMES

**FHV-1 Interpretation** <sup>b</sup> ~

**Bordetella bronchiseptica RealPCR** NEGATIVE

**Mycoplasma felis RealPCR** POSITIVE

**H1N1 Influenza Virus RealPCR** <sup>c</sup> NEGATIVE

<sup>a</sup> 3 Ranges for FHV-1 Quantity:

- 1) FHV-1 latent infection: Below 38 Thous (38,000) FHV-1 viral particles per swab
- 2) Indeterminate: Between 38 Thous (38,000) and 150 Thous (150,000) FHV-1 viral particles per swab(s)
- 3) FHV-1 active infection: Above 150 Thous (150,000) FHV-1 viral particles per swab(s)

<sup>b</sup> Indeterminate: The FHV-1 viral load dose not discriminate between latent and active infection. Retesting after 5 days is recommended to help determine if FHV-1 is likely contributing to clinical signs.

<sup>c</sup> A POSITIVE FELINE URD PANEL PCR result indicates the detected organism(s) is present in the sample. In animals with clinical signs this supports infection. For FHV-1 infection, the quantitative result helps to determine clinical significance. Additional causes of clinical signs should be assessed separately. Vaccination with a modified live vaccine may result in positive results for up to a few weeks post-vaccination.

A NEGATIVE FELINE URD PANEL PCR result indicates that the organism was not detected in this sample and suggests the absence of an infectious cause, by these organisms, for the clinical signs. However, a negative PCR result may be caused by the numbers of organisms following treatment or chronic carrier state, or the occurrence of a new strain.

## Discussion of significance of H3N2 Influenza Virus RealPCR™ Test result

Natural infection of cats with H3N2 canine influenza virus has been previously documented in Asia.<sup>1-3</sup> Ferrets and guinea pigs have been experimentally infected with H3N2 virus<sup>4</sup> and may also be at risk of natural infection. Although theoretically possible, there is no evidence of risk of transmission to humans from either H3N2 or H3N8 canine influenza.

Poly is the first confirmed case of natural H3N2 canine influenza infection in a cat in the United States. It is likely that her housemate, Pumpkin, was also infected with the virus, but he was not tested to confirm this suspicion when he initially presented. Because of concurrent infection, it is impossible to determine which clinical signs can be attributed to the influenza virus versus the *Mycoplasma felis* and feline herpesvirus infections. In general, cats appear to be more resistant to infection with H3N2 canine influenza virus than their canine counterparts, but cats can be naturally infected. Cats identified to be infected with H3N2 canine influenza can serve as a source for other neighborhood dogs and cats, and they should be kept isolated from other animals.

Infection is more likely in cats that are elderly, young, or otherwise immunocompromised. The concurrent *Mycoplasma felis* and herpesvirus infections, recent shelter experience, and relocation to a new home may have contributed to the cat(s) becoming infected with the H3N2 canine influenza virus. Identification of the coinfections allowed specific treatment directed against these organisms, while supportive care was instituted for the influenza, resulting in a better outcome.

## References

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