Brief Communication Communication brève

Seroprevalence of antibodies to canine influenza virus in dogs in Ontario

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Abstract — A cross-sectional study evaluating the seroprevalence of antibodies to canine influenza virus in dogs in Ontario was performed. The prevalence was 0.4% (1/225), and the only seropositive dog was a greyhound that originated in Florida.

Résumé — Séroprévalence aux anticorps du virus de l'influenza canine chez des chiens de l'Ontario. Une enquête transversale a été menée sur des chiens en Ontario pour évaluer la séroprévalence aux anticorps du virus de l'influenza canine. La prévalence était de 0,4 % (1/225) et le seul chien séropositif était un Lévrier anglais originaire de la Floride.

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Influenza virus is an important pathogen in a number of species, including humans, birds, and horses (1). Most influenza viruses have highly evolved relationships with their chosen host and are unlikely to infect other species. For cross-species transfer to happen, a series of events must occur. These include mutation of the virus in a manner that makes it able to survive in the new host, access to the new host at the time of viral evolution, active infection of the new host with shedding of virus, and prompt exposure to other susceptible individuals of the new host, so that the virus can be disseminated (2).

Influenza virus has, traditionally, not been considered to be a pathogen of dogs. While previous studies have demonstrated seroconversion of dogs exposed to different strains of influenza virus (3–5), the 1st evidence of influenza virus resulting in significant clinical infection was in 2004 when outbreaks of disease were identified in greyhounds at racing facilities in Florida (6). Two main clinical syndromes were observed: 1) mild illness with pyrexia and cough, and 2) sudden death with hemorrhagic tracheitis, bronchitis, bronchiolitis, and suppurative bronchopneumonia. The initial case fatality rate was 36%; however, subsequent anecdotal reports have indicated a lower mortality rate. Molecular analysis of isolates from dogs identified that the canine influenza virus was A/canine/Florida/43/2005 or canine/FL/04 and that it shared > 96% sequence identity with equine influenza A2 H3/N8 and had a lesser relationship with all other tested viruses (6). This strongly suggested that canine influenza originated from H3N8 equine influenza virus, the predominant equine influenza viral strain in horses in North America (7,8).

Outbreaks of canine influenza were then reported at racetracks in several American states in 2004 and 2005 (6). The report of a study of dogs in a shelter in Florida and veterinary clinics in Florida and New York stated a seroprevalence of 97% (6). This indicated that the influenza virus was not restricted to specific populations such as racing greyhounds, and raised concern about potential effects of canine influenza virus infection in pet dogs. Reports of canine influenza have not been limited to the United States. An outbreak of disease in quarry hounds in the UK in 2002 was subsequently identified as being caused by canine influenza virus (9). A later seroprevalence study in the UK identified antibodies to H3N8 equine influenza virus in 37.5% of foxhounds; however, the seroprevalence was 0% in dogs born after April 1, 2003, and 90% in dogs born before Nov 1, 2002 (10). Interestingly, the higher prevalence period coincided with the time that the H3N8 influenza virus was circulating in the British equine population (10). It was hypothesized that close contact between dogs and horses, as would be present in hunting animals, combined with circulating H3N8 equine influenza virus in horses, could have led to interspecies transmission. It is also interesting that canine influenza virus is not believed to be currently circulating in the British dog population, despite previous reports of infections (9,10).

The finding of evidence of similar strains of this virus in dog populations on 2 continents, whether it be from independent emergence of canine influenza virus from H3N8 equine influenza virus or trans-Atlantic transmission, suggests that exposure of the dog population in Ontario to the virus is possible. The objective of this study was to determine the prevalence of canine influenza virus in selected dog populations in Ontario.

A cross-sectional study was performed, using a convenience sample of dogs from 9 veterinary practices in Ontario. The practices were located in the regions of Aurora, Barrie, Kitchener-Waterloo, Niagara Falls, Ottawa (2 hospitals), Thunder Bay,

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Toronto, and Windsor. Each practice collected serum samples from 25 dogs. Dogs presented for any reason were eligible for inclusion, but they were excluded if their owners declined to provide consent or if blood collection would have posed undue stress on the animal, based on its clinical condition. Practices were allowed to start sample collection on any date, but they were required to collect samples from 25 consecutive eligible dogs once collection was underway. This study was approved by the University of Guelph Animal Care Committee.

Sera were tested for antibody to canine influenza virus in a hemagglutination-inhibition test. Positive and negative control canine sera (kindly provided by Dr. E. Dubovi, Diagnostic Laboratory, New York State College of Veterinary Medicine, Cornell University, Ithaca, New York, USA) and test sera were treated in duplicate in sterile 96-well V plates for 12–18 h at 37°C, using 25 μL volumes, with 100 μL of 100 units of receptor destroying enzyme (Cambrex Bio Science, Walkersville, Maryland, USA) diluted in 0.1% calcium saline, pH 7.4. Subsequently, 75 μL of a 2.5% sodium citrate solution was added to each well and sera heated at 56°C for 30 min. Sera were adsorbed with 50 μL of 0.5% turkey red blood cells (TRBC), diluted in tryptose phosphate broth (TPB) with 1% fetal bovine serum (TPB + FBS), for 30 min at room temperature until the TRBC settled. The final dilution of the treated sera was 1/10. Treated sera were transferred to another 96-well V plate and each serum was diluted 2-fold in Dulbecco's phosphate buffered saline to create a dilution series of 1/20 to 1/2560. Subsequently, 4–8 hemagglutination units of equine influenza virus H3N8 (G04-54701) in 50 μL of TPB + FBS was added to the 1st row of each diluted serum. Fifty microliters of TBP + FBS without virus was added to the 2nd row of diluted sera to serve as an individual negative control for each serum. Plates were incubated at room temperature for 30 min, followed by the addition of 50 μL of 0.5% TRBC to each well. The plates were incubated at room temperature for 30–60 min until the TRBC settled. The endpoint titer was the last well where hemagglutination inhibition was observed. Positive control canine sera had an antibody titer of 1/80, while negative control canine sera had a titer of $< 1/20$.

Samples were collected from 225 dogs between January 15 and June 20, 2006. Antibodies against canine influenza virus were detected in only 1 (0.4%; 95% confidence interval 0–1.2%) dog. The seropositive (1/40) dog was a greyhound living in Kitchener that was clinically normal, with no recent history of respiratory tract disease. Interestingly, the dog had originated from a racing facility in Florida. No information was available with respect to the influenza activity or the dog's medical history while the dog was at the facility. Risk factor analysis was not performed because of the low prevalence.

In some respects, it was surprising that the seroprevalence was so low, considering the variety of reports of canine influenza in various American states, including the bordering state of New York, and the minimal restrictions on cross border movement of dogs. Presumably, high-risk groups such as racing greyhounds and shelter animals are less likely to be transported across the border. Also, movement of sick animals is presumably relatively uncommon, although no objective data are available. While the duration of shedding in dogs has not been reported, based on information from other species, it can be presumed that shedding postinfection is short- and long-term carriers should not be a concern. It is possible, therefore, that limited cross-border movement of infectious dogs is the reason for the low seroprevalence in Ontario.

The role of canine influenza virus in respiratory disease in Ontario is unclear. This was a population prevalence study, not a study focusing on clinically ill animals. However, the very low prevalence in this study suggests that canine influenza is currently rare in the province. It is unclear whether the only seropositive dog was exposed in Ontario, but considering that this dog originated from a racetrack in Florida, a region that has experienced canine influenza outbreaks (6), it is perhaps more likely that the dog was exposed or infected in Florida prior to adoption. No historical information was available from the facility of origin and the duration of persistence of canine influenza antibodies is unknown, so no conclusions can be made of location of infection.

Potential biases of the sample population should always be considered. This study involved dogs presented to primary care veterinary clinics. While commonly used for prevalence studies, this type of population may be biased, as there is presumably a subset of animals that are rarely or never presented for veterinary care. Veterinary clinic sampling based on collection of serial samples from individual animals is also likely to introduce as age bias, as younger animals are presented to veterinarians more often than older animals because of the increased frequency of preventive health visits (vaccination) and sterilization procedures. Another limitation in interpreting these data is the paucity of peer-reviewed studies involving canine influenza. While canine influenza has been a relatively high profile disease and there are various anecdotal reports and reports on the Internet, the scientific literature is surprisingly sparse.

While the seroprevalence of antibodies to the canine influenza virus was very low and no cases of canine influenza have been identified at the University Guelph, Laboratory Services, Animal Health Laboratory (unpublished observation), continued diligence is required. If this virus is still circulating in dog populations in the United States, and if there is the potential for further transmission from horses to dogs, there is a continued risk of exposure for dogs in Canada. Active and passive surveillance for clusters of infectious respiratory tract disease and submission of appropriate specimens for the testing of potentially infected animals are important to detect the emergence of this canine pathogen of potential concern.

Authors' contributions

Dr. Carmen performed the laboratory testing. Drs. Kruth and Weese enrolled clinics and recorded the data. The manuscript was written, reviewed, and approved by all authors.

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References

1. Hansen L. Influenza. Clin Evid 2004:1095–1102.
2. Parrish CR, Kawaoka Y. The origins of new pandemic viruses: The acquisition of new host ranges by canine parvovirus and influenza A viruses. Annu Rev Microbiol 2005;59:553–586.
3. Todd JD, Cohen D. Studies of influenza in dogs. I. Susceptibility of dogs to natural and experimental infection with human A2 and B strains of influenza virus. Am J Epidemiol 1968;87:426–439.
4. Nikitin A, Cohen D, Todd JD, Lief FS. Epidemiological studies of A-Hong Kong-68 virus infection in dogs. Bull World Health Organ 1972;47:471–479.
5. Romvary J, Rozsa J, Farkas E. Infection of dogs and cats with the Hong Kong influenza A (H3N2) virus during an epidemic period in Hungary. Acta Vet Acad Sci Hung 1975;25:255–259.
6. Crawford P, Dubovi E, Castleman W, et al. Transmission of equine influenza virus to dogs. Science 2005;310:482–485.
7. Lai A, Chambers T, Holland R, et al. Diverged evolution of recent equine-2 influenza (H3N8) viruses in the Western Hemisphere. Arch Virol 2001;146:1063–1074.
8. Mumford E, Traub-Dargatz J, Carman J, et al. Occurrence of infectious upper respiratory tract disease and response to vaccination in horses on six sentinel premises in northern Colorado. Equine Vet J 2003;35:72–77.
9. Smith KC, Daly JM, Blunden AS, Laurence CJ. Canine influenza virus (letter). Vet Rec 2005;157:599.
10. Newton R, Cooke A, Elton D, et al. Canine influenza virus: Cross-species transmission from horses (letter). Vet Rec 2007;161:142–143.

Book Review
Compte rendu de livre

Veterinary Herbal Medicine

Wynn SG, Fougère BJ. Mosby Elsevier, St. Louis, Missouri, USA, 2007. 736 pp. ISBN-13: 9870-3230-2998-8. CDN$114.00.

Veterinary Herbal Medicine is a comprehensive, readable, current, and informative treatise on the use of herbs in veterinary practice. With its rational approach, background material, and references, this book discusses the credible use of herbs in veterinary science. This is an excellent addition to every open-minded veterinary practitioner's desk.

The book is divided into 5 sections preceded by an introduction. Here, the principal authors outline the advantages of herbs in chronic conditions that are inadequately served by conventional pharmacology. All the chapters have clear subheadings with numerous tables and references; useful Web sites are also included.

Unlike nearly all the other books about herbal medicine, the materia medica is only 1 chapter. It is comprehensive at over 200 pages and the authors describe the numerous plants, their actions and clinical uses, and the doses and practical dosing information completely; all are well-referenced.

The 5 sections are written by Wynn, Fougère, and 20 guest authors. The section "Historical Relationship between Plants and Animals" is fascinating and includes chapters clearly explaining traditional Chinese medicine and Ayurvedic (East Indian) medicine. The authors have included a section titled Herbal Medicine Controversies, in which research, quality control, regulations, and even counter-arguments are discussed.

The section on plants provides the background information to the use of herbs therapeutically. This includes medical botany, plant biochemistry, toxicity, and drug interactions (with useful descriptions and tables). Both the diagnoses and the herb selection process are clearly explained. Fougère and Wynn follow up with practical and essential (but often omitted) information about the various forms that herbal medicines come in, along with dosage and dosing, and drug interactions. There are also chapters about purity and concentrations, manufacturing processes, gardening and preserving our world plant biodiversity.

One of the issues with traditional native, Indian/Ayurvedic and Chinese medicine is that, often, they do not incorporate recent (within the last 200 y) information about biochemistry, physiology, pathophysiology, and disease-causing factors. Scientific medicine is integrated into this text, especially in the section "Veterinary Clinical Uses of Medicinal Plants." Fougère discusses this dilemma as she describes the various approaches to prescribing herbs, especially in chronic conditions: "herbalism is vitalistic and holistic in its approach." She places a Western medical diagnosis within the herbal medicinal frame of reference, this is within the "context of the much bigger picture of the individual patient...it's mental, emotional and physical experience." This includes the patient's diet, exercise, and environment in addition to the disease process and secondary imbalances. The aim it to not only treat the cause of the problem but to reverse the abnormal signs and to restore the health and vitality of the patient.

The chapter on a systems-based approach to veterinary herbal medicine is a model of evidence-based logic, clarity, and practical advice, even for the herbal newcomer. Wynn and Fougère incorporate physiology, the latest information on immunity, disease etiology, pathology, and pharmaceuticals. The authors recommend herbs based on their biochemical activity rather than on traditional Chinese medicine principles.

This extensive chapter, with its emphasis on dogs and cats, is followed by chapters on equine practice and dairy cows, including relevant materia medica. Wynn and Fougère have thoughtfully included a chapter on getting started, in which they make many useful and practical suggestions for veterinarians venturing into this field. These include sources of information, pricing, patient selection, dispensing advice, and how to have fun doing it all. In the Appendix titled "Suppliers," Canadian companies are listed. The index is not extensive, but it is clear.

In conclusion, this is an essential reference for veterinarians who want to help their patients by using herbal medicine: clear, comprehensive, and practical.

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