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3 **CROSS-CANADA DISEASE REPORT/**

4 **RAPPORT DES MALADIES DIAGNOSTIQUÉES AU CANADA**

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6 **Genetic relatedness of recent Canadian equine influenza virus isolates with**  
7 **vaccine strains used in the field**

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25 Equine influenza is one of the most economically important contagious respiratory diseases of  
26 horses(1, 2). This disease is characterized by rapidly spreading signs that include pyrexia,  
27 depression, anorexia, a harsh dry cough, a nasal discharge, and secondary bacterial respiratory  
28 infections(3). Chronic sequelae, including bronchopneumonia and exercise-induced hemorrhage,  
29 have been identified(4). Outbreaks of equine influenza have been reported worldwide. Influenza  
30 is endemic in the horse populations of Europe and North America, and several outbreaks have  
31 been reported throughout Canada(4-7). In the most recent epidemiological study done in Ontario,  
32 equine influenza virus was found to be the causal agent in 56% of 23 outbreaks of acute viral  
33 respiratory disease investigated over a 2-year period(7). In this study, the vaccination rate was  
34 quite low, as only 36% of the horses had been given an equine influenza vaccine at least once  
35 during the previous year. This is far below the 70% vaccination rate that has been suggested as  
36 being necessary to prevent epidemics of influenza in horses(1).

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38 Over the years, strain variants of equine influenza virus have emerged due to antigenic shift and  
39 antigenic drift(8, 9). In an attempt to monitor the evolution of equine influenza virus in Canada,  
40 the Diagnostic Veterinary Virology Laboratory (DVVL) of the Faculté de médecine vétérinaire  
41 (FMV) of the Université de Montréal isolated 7 equine influenza virus strains from clinically  
42 affected animals between 2003 and 2006. These strains were isolated from embryonated eggs  
43 inoculated with nasal swabs. Five of these strains were recovered from horses in Quebec and 2  
44 were recovered from horses in Alberta (Table 1). The subtype (H3N8 or equine-2; and H7N7 or  
45 equine-1) of each influenza virus isolate was determined with an hemagglutination-inhibition test,  
46 using specific antibodies for each subtype. All isolated strains were subtype H3N8, which was  
47 not a surprising result since subtype H7N7 has not been isolated for over 35 years in Canada.

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49 After isolation, either the viruses or the hemagglutinin (HA) and the neuraminidase (NA) RNA  
50 virus sequences were sent for genomic characterization at the Animal Health Trust (AHT)  
51 laboratory in the UK, a World Organization for Animal Health (OIE) reference laboratory for  
52 equine influenza and equine rhinopneumonitis. A phylogenetic tree was built by the AHT to  
53 determine the relatedness of Canadian isolates to worldwide reference strains. Moreover, equine  
54 influenza virus vaccine strains currently used in Canada and the Canadian equine influenza virus  
55 isolates recovered prior to 2003 were included in the phylogenetic analysis. The HA gene was  
56 target in the phylogenetic analysis, because its mutation rate has been demonstrated to be greater  
57 than that of other viral genes and because it is one of the main targets of the humoral protective  
58 immune response(10).

59  
60 As shown in Figure 1, the 7 Canadian equine influenza viruses isolated since 2003 by the DVVL  
61 are variant American (Florida) lineage strains. The HA1 amino acids (aa) identity between the  
62 recent 7 Canadian isolates was 98.21% to 100% (data not shown). Interestingly, the HA1 aa  
63 identity between recent and older (2002 to 1997) Canadian isolates was 95.24% to 99.4% (data  
64 not shown), confirming the existence of a constant evolution of the genetic code of equine  
65 influenza, as previously reported(8). Several vaccines are currently used in Canada, or will be in  
66 a near future. Consequently, we were interested in evaluating the genetic relationship between  
67 recent Canadian equine influenza isolates and the equine influenza vaccine strains that are listed  
68 in Table 1.

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70 As illustrated in Figure 1, the Kentucky/97 vaccine strain (Genbank accession number  
71 AF197249) is the virus most genetically related to recent Canadian equine influenza field isolates

72 and the only commercially available vaccinal strain that belongs to the same lineage; the variant-  
73 American (Florida). Indeed, the HA1 aa identity between the Kentucky/97 vaccine strain and  
74 recent Canadian isolates was established to be 97.02% to 98.21% (Figure 1, data not shown).  
75 The 2<sup>nd</sup> most related vaccine strain to recent Canadian isolates was Kentucky/93, a vaccinal strain  
76 belonging to the mainstream American (Kentucky) lineage that shares an HA1 aa identity with  
77 recent Canadian isolates of 96.13% to 97.32% (Figure 1, data not shown). The HA1 aa identities  
78 of other vaccine strains with recent Canadian strains were established to be 93.45% to 96.73%  
79 (Figure 1, data not shown).

80  
81 None of the recent Canadian strains belongs to the Eurasian lineage. To our knowledge,  
82 Saskatoon/90 was the only strain isolated in North America that belongs to this lineage. This  
83 suggests that this strain did not spread out in the North American horse population and probably  
84 became extinct.

85  
86 It is interesting to pinpoint that the Kentucky/97 strain contained in the Fluvac Innovator®  
87 commercial vaccine is the most closely genetically related vaccine strain to recent Canadian  
88 influenza virus isolates, based on HA1 nucleotide and protein comparison analysis (Figure 1, data  
89 not shown). How important this antigenic closeness is in regards to field vaccine efficacy is  
90 difficult to assess. It was previously reported that the antigenic disparity between the circulating  
91 virus and the vaccine strain is a significant factor for vaccine failure(1, 11). Antigenicity and  
92 sequencing studies suggested that significant antigenic drift contributed to reduce vaccine  
93 efficacy; therefore, it was recommended that vaccines be updated with the most representative  
94 recent virus strain approximately every 4 years(12). However, what constitutes a significant

95 antigenic drift is not clearly defined. Yates and Mumford reported that the ability of a vaccine to  
96 reduce virus excretion correlates directly to the antigenic relatedness of the vaccine strain with  
97 the challenge strain(13). Lai *et al*(11) concluded that 2 phylogenetic lineages, the mainstream  
98 American (Kentucky) and variant-American (Florida), are antigenically distinct. In their  
99 experiment, horse sera reacted differently to viruses of 1 lineage, with a higher reactivity towards  
100 the homologous virus and viruses in the homologous lineage(11). In an experimental challenge  
101 study, the less efficient vaccine was the vaccine made with the most distant strain from the  
102 challenge strain(13). Field outbreaks were reported in the UK and in Italy in horses vaccinated  
103 with a mainstream American (Kentucky) strain and were subsequently found to be infected with a  
104 variant American (Florida) strain(3, 14). In the Italian outbreak, the field infecting strain  
105 displayed 9 aa changes in the HA1 subunit protein with respect to the vaccine strain.

106  
107 In the present report, the most closely related vaccine strain (Kentucky/97) displayed 6 to 10 aa  
108 changes in the HA1 subunit protein with respect to recently isolated Canadian influenza strains.  
109 On the other hand, the 2<sup>nd</sup> most related vaccine strain (Kentucky/93) and the least related vaccine  
110 strain (Newmarket/2/93) displayed 9 to 13 and 19 to 23 aa changes, respectively, in the HA1  
111 subunit protein with respect to recent Canadian strains. As previously reported(14), use of an  
112 equine influenza vaccine strain closely related to field isolates may not guarantee the efficacy of  
113 protection induced in the vaccinated animals, but, the probability of inducing a protective  
114 immune response and reducing clinical disease is higher when the vaccine strain used is closely  
115 related to the field isolate.

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## FIGURE LEGENDS

**Figure 1.** Phylogenetic analysis of the HA1 gene nucleotide sequence of recent Canadian equine influenza virus isolates. Original names of Canadian isolates and vaccine strains, and their origin, and date of isolation are given in Table 1. Canadian isolates are identified with an arrow and vaccine strains used in Canada are identified with an asterix. The numbers at the main partitions of the phylogenetic tree are ML bootstrap values. Horizontal branch lengths are drawn to scale with the bar indicating 0.004 nucleotide substitutions per site on HA1.



164 **Table 1.** Canadian equine H3N8 influenza virus isolates and H3N8 vaccine strains description  
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<b>Strain name</b>	<b>Geographical origin</b>	<b>Year of isolation</b>	<b>Related vaccine trademark name</b>
Saskatoon/90	Canada/Saskatoon	1990	n.a.
Ontario/97	Canada/Ontario	1997	n.a.
Ontario/01	Canada/Ontario	2001	n.a.
Ontario/02	Canada/Ontario	2002	n.a.
Manitoba/43433/01	Canada/Manitoba	2001	n.a.
Canada/2447/03	Canada/Quebec	2003	n.a.
Canada/3112/04	Canada/Quebec	2004	n.a.
Canada/2/04	Canada/Quebec	2004	n.a.
Quebec/05	Canada/Quebec	2005	n.a.
Quebec/06	Canada/Quebec	2006	n.a.
Alberta/1/06	Canada/Alberta	2006	n.a.
Alberta/2/06	Canada/Alberta	2006	n.a.
Kentucky/91	United States/Kentucky	1991	FluAvert®
Kentucky/93	United States/Kentucky	1993	Prestige® <sup>a</sup>
Kentucky/95	United States/Kentucky	1995	Calvenza® <sup>b</sup>
Newmarket/2/93	United Kingdom/Newmarket	1993	Calvenza® <sup>b</sup>
Kentucky/97	United States/Kentucky	1997	Fluvac Innovator® <sup>c</sup>
Kentucky/98	United States/Kentucky	1998	Flumune®

166 n.a.: None applicable.

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 168 <sup>a</sup>The Prestige® vaccine also contains one H7N7 strain; Pennsylvania/63.  
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170 <sup>b</sup>The Calvenza® vaccine also contains one H7N7 strain; Newmarket/77.  
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172 <sup>c</sup>The Fluvac Innovator® vaccine also contains one H7N7 strain; Prague/56.  
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