THE UNIVERSITY OF BRITISH COLUMBIA

Dr. Steven Pelech
[redacted]
Vancouver, B.C., Canada [redacted]

Date: 1 April 2025

Re: Expert Report – The Process Involved and Merits of Testing newly laid eggs from the ostriches to determine infection, transmissibility and immunity and merits of culling order

For the case involving Universal Ostrich Farms Inc. represented by Mr. Michael Carter of Cleveland & Doan Barristers & Solicitors, and Mr. Lee Turner of Doak Shirreff Lawyers LLP.

1. My full name is Steven Daniel Pelech, [redacted] British Columbia, Canada. My Ph.D. and post-doctoral training is in the area of biochemistry, and I have been on the faculty of the University of British Columbia as a professor in the Department of Medicine for over 36 years.

2. In particular, in the letter that was transmitted by e-mail to me by Mr. Turner on March 28, 2025 (Exhibit A), I was requested to provide the following information:

   i. My name, address, area of expertise and a copy of my *curriculum vitae* (provided as Exhibit A);
   ii. My qualifications and employment and educational experience in my area of expertise that pertain to the issues I have been asked to opine on;
   iii. The instructions that was provided to me in relation to the proceedings of the Universal Ostrich Farms Inc. (UOF) verses The Canada Food Inspection Agency (CFIA) (as described Mr. Turner's March 28, 2025 letter, which is provided as Exhibit A);
   iv. The nature of the opinion being sought and the issues in the proceeding to which the opinion relates;
   v. My opinion respecting those issues; and

vi. My reasons for my opinion, including

(a) a description of the factual assumptions on which my opinion is based,

(b) a description of any research relied upon that led me to form my opinion, and

(c) a list of every document relied upon by me in forming my opinion.

4. In addition, I have been asked to certify that:
   i. I am aware of my duty to assist the Federal Court of Canada;
   ii. I am not an advocate for any party in these proceedings;
   iii. I have prepared my report in conformity with my duty; and
   iv. I will, if called upon to give oral or written testimony, give that testimony in conformity with my duty.

5. I do certify that I will fulfill my responsibilities to the Federal Court of Canada in full compliance to these obligations and requirements. Note that I have already certified this as documented in Exhibit B of my January 29, 2025 expert report, which has already been filed with the Court.

PART 1: SCOPE OF THE EXPERT REPORT AND QUALIFICATIONS

6. The specific questions that I was asked to address are the following:
   i. Is there a test that could be performed either on the ostriches themselves or their eggs that can be performed safely by the farmers themselves on the farm, that would pose no risk of infection or transmission to other wildlife or humans, such that these test samples could then be provided to me so that I could analyze in my laboratory to determine whether or not these birds have been exposed H5N1, have developed antibodies to H5N1, whether they are immune to H5N1, and whether they pose any risk of spreading infection of H5N1 to other wildlife or humans?
   ii. If the answer to this question is yes, can I describe test that could be done, how that sample can be safely transported to me, and what that test would tell the Court?
   iii. Explain why the test is safe for the farmers to perform, why it is safe to transport to me, so that the reader of my report understands clearly why the test and transporting its samples to me for testing would present no risk to the public or other wildlife.

iv. If the test results demonstrate that the surviving ostriches have full antibodies to the strain of H5N1 that was detected by CFIA through their own testing either at the Abbotsford Lab or the Winnipeg lab or otherwise, please explain what the significance of that is with respect to the risk of transmission of the virus to other wildlife or humans and what the merits of culling the ostriches would be as a result.

7. To address above questions posed by Mr. Turner of the firm of Doak Shirreff, this required a broad and comprehensive assessment of the accessible scientific literature with respect to available knowledge about the testing of immunity, and the effectiveness of natural and vaccine-induced immunity in preventing or reducing illness and spread of respiratory RNA viruses. Previously, I was asked by Mr. Carter of the firm of Cleveland & Doan to provide my expert opinion related to the flock of ostriches located at the UOF and the risks of transmission of the H5N1 strain of influenza, which is responsible for the current waves of highly pathogenic avian influenza (HPAI). In addition, I was also requested to comment upon the value and applications of these birds with respect to the advancement of biomedical knowledge and production of diagnostics, vaccines and therapeutics. I have previously commented upon these topics in two reports, dated January 29, 2025, and February 12, 2025, which have already been filed with the Court as expert witness documents.

8. With my training and experience, I am confident that I am fully able to provide a qualified expert opinion in the previous reports and for this report. In my January 29, 2025, expert report, a full copy of my *curriculum vitae*, dated January 25, 2025, was already provided to the Court as Exhibit C. Furthermore, in "Part 5: Qualifications and Acknowledgements as an Expert on Immunology" (para. 74 to 85) of the same report, I have already summarized my relevant training and experience.

9. The simplest definitions of "Immunology" encompass the study of immune systems in all organisms that provide protection against infectious diseases and cancer, whilst avoiding autoimmune responses in the body and other common and benign factors in the environment. Viruses are major causes of infectious diseases, and I have been actively involved in experimental

research related to the immune response to RNA virus infections, including caused by the SARS-CoV-2 virus, and more recently influenza. My research, as does most immunology-based research, involves using experimental animal models of human disease, because what is applicable to animals is usually translatable into improved understanding of human diseases. My own research has previously encompassed the use of a wide range of species ranging from starfish, sea urchins, frogs, chickens, mice, and rats as well as human cells as the main experimental model systems in which I have published. Epidemiology involves not only the study of rates of transmission of infectious diseases, but also understanding the underlying molecular mechanisms of transmission as well as techniques for specifically tracking the spread of the pathogen to permit preventative and mitigating measures. While I have received formal training in immunology and virology in my undergraduate and graduate courses, for over 40 years since then, I have continued to update and expand my knowledge in these areas from hands-on experimentation, teaching graduate and medical students on these subjects, reading thousands of scientific articles on these subjects, and attending weekly grand-rounds and seminars that often included these topics. Finally, the fact that the H5N1 influenza virus is capable, to varying degrees, of infecting such a wide range of bird and mammal species in addition to humans clearly demonstrate that the virus is exploiting the same molecular mechanisms to infect and replicate in an extensive host range, and has targeted common host proteins to achieve this. The influenza genome only features 10 genes that contained in 8 chromosomal segments, which encode 10 viral proteins that are essential for successful entry, reproduction, and exit of the virus particles. These 10 viral proteins are optimized to the host to hijack its reproductive proteins and to try to evade the host's immune system. Thus, while a trained medical doctor or veterinarian can provide established treatments to fight an infectious disease, it is those with research training like myself that develop and test such treatments.

10. I have previously provided two expert reports dated January 29, 2025, and February 12, 2025, in response to questions that were put to me by the firm of Cleveland & Doan related to the flock of ostriches (Herd) located at the Universal Ostrich Farms Inc. (UOF) near Edgewood, B.C. and the risks of transmission of the H5N1 strain of influenza, which is responsible for the current

waves of highly pathogenic avian influenza (HPAI). Counsel for the Respondents declined the opportunity to cross examine me on my qualifications or the contents of my reports.

PART 2: COMMENTS RELATED TO THE AFFIDAVIT OF DR. SUMINDER SAWHNEY

11. As of March 5, 2025, the CFIA lists on their website that 20 premises in Canada have active HPAI virus infections and another 511 HPAI infections were previously documented, which has resulting in the culling of 14,566,000 birds in Canada.[1] With at least 531 separate outbreaks of HPAI in domestic bird flocks, and a policy of originally 28 days without avian influenza infections for "free status", it would seems that Canada has not held such status for at least 3 years, although a stamping out policy has been in effect since 2004 starting in British Columbia. Moreover, since the HPAI virus remains rampant and unchecked in the wild fowl population, it is hard to envision how Canada would achieve such status, which is now even harder with the new 3-month requirement for no cases of HPAI outbreaks. In any event, without the HPAI "free status" it is evident that Canada has still been able to continue its trade in products from commercial poultry operations (see Exhibit "F" of Dr. Sawhney's affidavit for exported bird products).

12. Dr. Sawhney discussed in para. 50 of his affidavit about how Canada would not accept poultry-related products from France, because this country adopted a preventative program that involved vaccination of poultry against HPAI. South Africa ultimately also permitted the vaccination of poultry in their commercial industry, but adopted strict guidelines for those farms that adopted this practice. One issue is that it is difficult to distinguish the production of antibodies in vaccinated birds from the vaccines and from natural infection. Influenza vaccines also have a significant breakthrough infections; by moderating the signs of infections, infected birds may be more able to transmit the disease, although with much reduced viral loads.

PART 3: COMMENTS RELATED TO THE AFFIDAVIT 2 OF DR. CATHY FURNESS

[1] https://inspection.canada.ca/en/animal-health/terrestrial-animals/diseases/reportable/avian-influenza/latest-bird-flu-situation/investigations-and-orders#dataset-filter

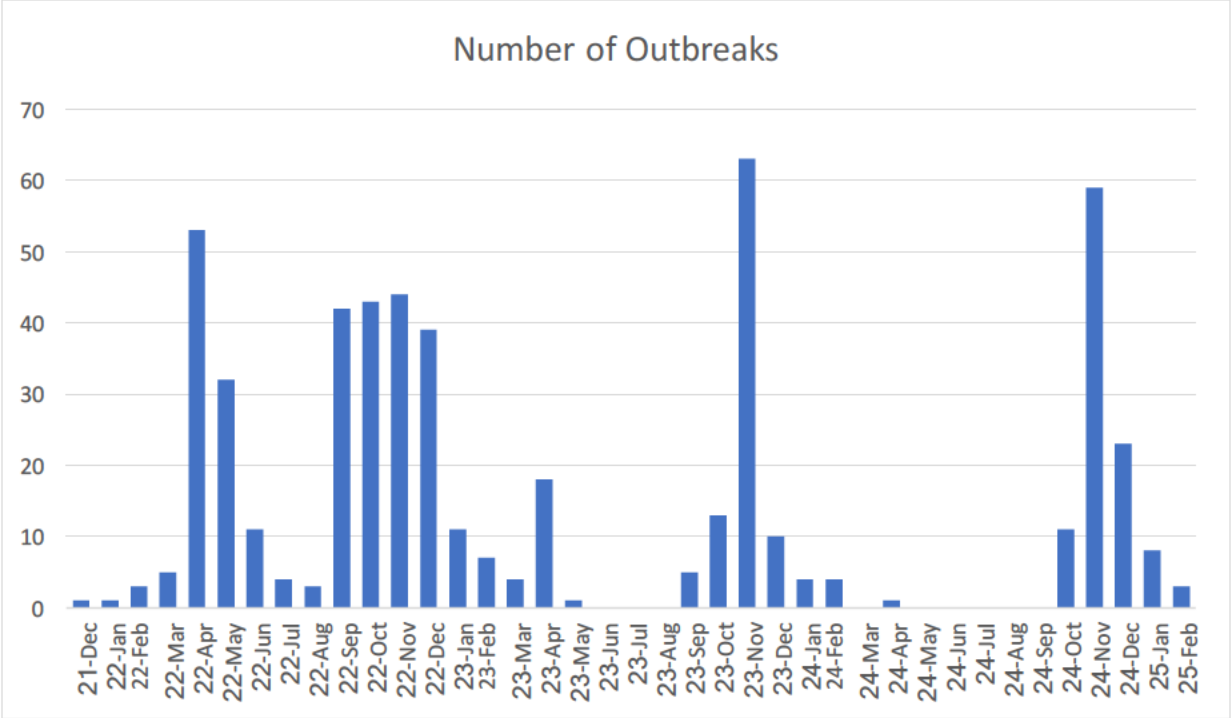
13. In para. 6 of Dr. Furness's affidavit, she noted that she is involved in Canada's planning and preparedness for HPAI in dairy cattle. Although there are no reported cases of HPAI in cattle in Canada, there have been outbreaks in 17 states in the US that has resulted in around 966 infected herds of dairy cows since March 25, 2024.[2] In the US, the policy of stamping out is not implemented for HPAI-infected dairy cattle, but rather they are isolated, treated for their illness and allowed to recover. It begs the question of whether the CFIA will follow the US lead with respect to not stamping out HPAI-infected dairy cattle in Canada. However, like cattle, ostriches are large and expensive animals, and one should expect consistency. HPAI infected cattle pose a greater risk to human health than ostriches, since they are mammals like humans.

14. The best protection against future infections appears to be from recovery from a natural infection, which is not at all considered with the current CFAI policy or the WOAH guidelines in Chapter 10.4 of the Terrestrial Code. Presumably, if a bird can be demonstrated to already possess antibodies that immunoreact with H5N1 viral proteins, and they are not sick or shown to be PCR- or rapid antigen test-positive for the virus, even if others in the flock have evidence of a HPAI infection, it is illogical to cull them too since they have already successfully recovered from an H5N1 virus infection.

Figure 1. Monthly frequency of HPIA outbreaks in Canada (includes non-commercial, and no-poultry as well) from December 2021 to February 2025. Data was retrieved from the CFAI website.[3] Note that although the wild bird migrate southward in the spring and northward in the autumn in British Columbia, the HPIA outbreaks are primarily in the late fall in the last three years, and much less so during the spring migration. Consequently, the risk of wild birds getting infected with H5N1 in the spring and summer is very low.

[2] https://www.avma.org/resources-tools/animal-health-and-welfare/animal-health/avian-influenza/avian-influenza-virus-type-h5n1-us-dairy-cattle

[3] https://inspection.canada.ca/en/animal-health/terrestrial-animals/diseases/reportable/avian-influenza/latest-bird-flu-situation/investigations-and-orders#dataset-filter



15. In para. 67, Dr. Furness reiterates that "*quarantine and the use of additional testing are not "treatment" within the meaning of subsection 48(2) of the HAA, and are also not considered by CFIA to be an appropriate mitigative measure for HPAI.*" Again, this disregards acquired natural immunity, which should be potentially higher in long-lived birds such as ostriches. I suspect that this is not such a hard and fast policy when it comes to larger animals such as dairy cows should they become infected with HPAI virus. The CFAI has adopted the stamping out policy for foot and mouth disease in cattle, which is highly contagious, and can result in 100% morbidity in a herd, be fatal in young cattle, and result in permanent reduction in milk production. By contrast, HPAI infection of cattle are rarely fatal with typically 10-20% morbidity, and less than 5% of these sick cattle die. In the US, they are not routinely using a stamping out policy for entire cattle herds with evidence of H5N1 influenza infection.

16. In para. 78, Dr. Furness disclosed that the specimens collected from the deceased ostriches from the UOF site were subjected to full genome sequencing of the 8 chromosomes of the HPAI virus (a chromosome is a separate fragment of the genome of an organism). This is why the CFAI had suggested without any clarification of the methodology used that "*the HPAI strain detected in the ostriches at UOF represents a unique reassortment (genotype) of H5N1 not previously*

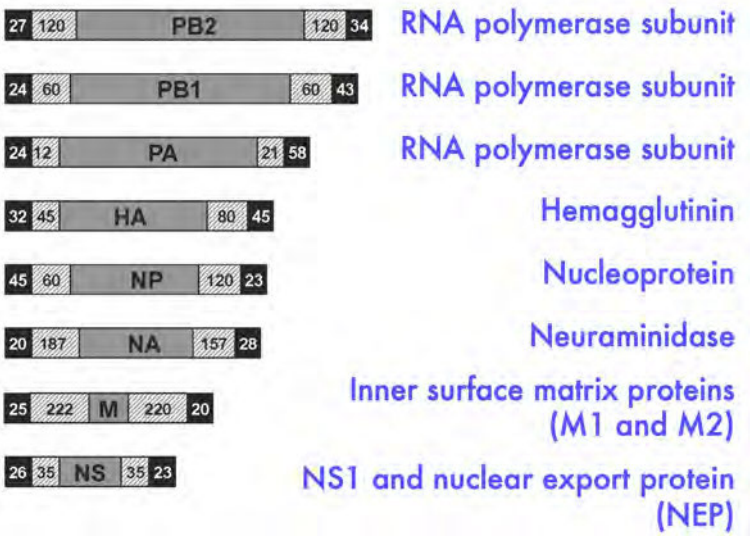
identified in Canada." This analysis was apparently undertaken in early January, 2025, and the data was available to the CFAI on January 8, 2025, based on the time stamp of the report (Exhibit "T"). It is unclear whether this viral genome sequencing was performed on the samples from both ostriches separately or mixed together, although it seems that the samples were pooled. If both birds had the same reassortment, this increases the prospects that the wild duck that originally infected the flock may have already had this reassortment. Such a reassortment is more likely in a flock of hundreds of different freely travelling ducks than in a relatively small and contained herd of ostriches. According to this report, from the result of the 2 vials/submitter samples, it is indicated that "*genes segments PB2, PA and NP belonging to North American lineage and gene segments PB1, HA, NA, M and NS belonging to Eurasian lineage*" were identified. This means that five of the viral chromosomes arose from a high pathogenicity avian influenza virus (*i.e.,* H5N1 2.3.4.4b clade) and three of the viral chromosomes arose from a low pathogenicity avian influenza virus (not specified in the report). Figure 2 provides a diagram of the genetic organization of the influenza virus genome. More information about the reassortment of influenza A virus chromosomes is available in Dadonaite *et al.* (2019).[4]

Figure 2. **Influenza A virus (IAV) genome organization and virus particle (virion) structure.** (**A**) Genome organization: The eight single-stranded, negative-sense, viral (v)RNA segments PB2, PB1, PA, HA, NP, NA, M and NS of IAV are shown. M encodes the inner surface envelop matrix 1 (M1) protein and the ion channel matrix 2 (M2) protein. NS encodes the NS1 protein and nuclear export protein (NEP). The Black boxes at the end of each of the vRNAs indicate the 3′ and 5′ non-coding regions (NCR). Hatched boxes indicate the packaging signals present at the 3′ and 5′ ends of each of the vRNAs that are responsible for efficient encapsidation into nascent virions. Numbers represent nucleotide lengths for each of the NCR and packaging signals; (**B**) Virion structure: IAV is surrounded by a lipid membrane bilayer containing the two viral glycoproteins hemagglutinin (HA), responsible for binding to sialic acid-containing receptors; and neuraminidase (NA), responsible for viral release from infected cells. Also in the virion membrane is M2 protein. Under the viral lipid bilayer is a protein layer composed of M1 protein, which plays a role in virion assembly and budding; and NEP involved in the nuclear export of the viral ribonucleoprotein (vRNP) complexes. Underneath is the core of the virus made of the eight vRNA segments that are encapsidated by the viral nucleoprotein (NP). Associated with each vRNP a

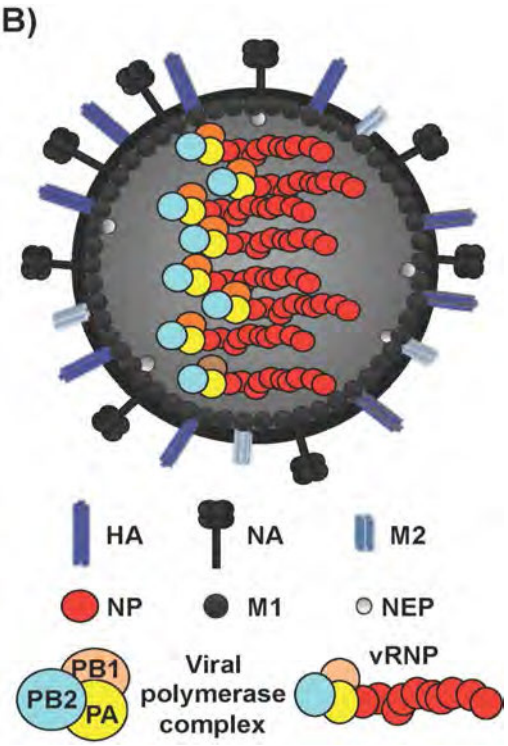
[4] Dadonaite, B., Gilbertson, B., Knight, M.L., Trifkovic, S., Rockman, S., *et al.* (2019) The structure of the influenza A virus genome. Nat Microbiol. 4(11):1781-9. doi: 10.1038/s41564-019-0513-7. https://pmc.ncbi.nlm.nih.gov/articles/PMC7191640/

complex is the viral RNA-dependent RNA polymerase (RdRp) complex made of the three polymerase subunits PB2, PB1 and PA that, together with the viral NP are the minimal components required for viral replication and transcription. This figure and information are reproduced from Breen *et al.* (2016).[5]

A)



B)



17. More insight into the results of the genome sequencing of the H5N1 influenza virus recovered from the two UOF ostriches that died and were tested by the CFAI is revealed in an internal January 4, 2025 e-mail memo between CFIA AI Lab Liaison to CFIA AI Commander (see Exhibit B for full memo). In particular, it is stated:

> *"The entire genome of the virus was amplified from two original swab samples and submitted to our Genomics group for NANOPORE sequencing. Eight gene segments of the virus were sequenced. The HA of the virus from the samples belong to Eurasian Gs/GD lineage HPAI H5N1 (2.3.4.4B) with cleavage site motif of "PLREKRRKR/GLF" compatible with HPAI viruses that came to Canada via the Pacific flyway. The H5N1 virus is a reassortant*

[5] Breen, M., Nogales, A., Baker, S.F., Martinez-Sobrido, L. (2016) Replication-competent influenza A viruses expressing reporter genes. Viruses. *8*(7):179. https://doi.org/10.3390/v8070179

*virus with PB2, PA, and NP originated from North American lineage **low pathogenic avian influenza virus**, and PB1, HA, NA, M and NS gene segments from Eurasian viruses. The virus is similar to the D1.1 viruses circulating in North America, but has the neuraminidase segment identical to WIN-AH-2022-OTH-0033 virus. PB2 627E (avian)."*

18. This correspondence confirms that three of the chromosomes (specifying the PB2, PA and NP genes) found in the retrieved samples of the influenza strain were derived from a low pathogenicity strain of avian influenza virus. It also indicates that the PB2 gene featured the E627 allele of this RNA polymerase subunit. This is important, because it is the E627K variant that has been linked with more pathogenic strains of H5N1 that can infect mammals.

19. The PB2 protein is a subunit of the viral RNA polymerase (the enzyme complex that transcribes the viral genes to make mRNA's to produce the viral proteins and allows for replication of the genome of the virus to make new virus particles), and it has also been implicated in inhibition of interferon expression by associating with the mitochondrial antiviral signalling (MAVS) protein.[6] The avian version of PB2 does not normally support mitochondrial import of this protein, and its ability to suppress immune responses via inhibition of PB2. The normal PB2-E627 form of the avian H5N1 is highly impaired in its ability to infect mammals.[7] However, this is compromised with PB2 E627K or D701N mutations.[8] However, it appears that the PB2 gene in the virus isolate from the UOF ostriches does not contain the E627K mutant, which the CFAI has been concerned might increase infectivity of humans with H5N1 influenza.

20. The bottom line is that the variant of H5N1 detected in the two ostriches was likely evolved from a mutated form of the H5N1 influenza virus that contains the surface proteins of the high

[6] Long, J.C., Fodor, E. (2016) The PB2 subunit of the Influenza A virus RNA polymerase is imported into the mitochondrial matrix. J Virol. 90(19):8729-38. doi: 10.1128/JVI.01384-16. https://pmc.ncbi.nlm.nih.gov/articles/PMC5021425/

[7] Bogs, J., Kalthoff, D., Veits, J., Pavlova, S., Schwemmle, M., *et al.* (2011) Reversion of PB2-627E to -627K during replication of an H5N1 Clade 2.2 virus in mammalian hosts depends on the origin of the nucleoprotein. J Virol. 85(20):10691-8. doi: 10.1128/JVI.00786-11. https://pmc.ncbi.nlm.nih.gov/articles/PMC3187502/

[8] Steel, J., Lowen, A.C., Mubareka, S., Palese, P. (2009) Transmission of influenza virus in a mammalian host is increased by PB2 amino acids 627K or 627E/701N. PLoS Pathog. 5(1):e1000252. doi: 10.1371/journal.ppat.1000252. https://pmc.ncbi.nlm.nih.gov/articles/PMC2603332/

pathogenicity, 2.3.4.4b clade of the avian influenza virus, which was already present in the infected ducks that transmitted the virus to the ostriches. However, due to the substitution of the three of the viral genes for internal proteins in the influenza particles that are critical for its replication of the virus with variants from a less pathogenic strain, it has lower virulence than found with the HPAI 2.3.4.4b clade. It is also less likely to cause illness in humans and other mammals that are infected, because it does not feature the PB2 – E627K mutation.

21. The emergence of a less virulent but more infectious variants of a virus, as may be the case with the medium pathogenicity strain of H5N1 influenza virus in the UOF ostriches, is actually typical. With reduced pathogenicity, the infected host is less sick and more likely to successfully transmit the virus to other hosts, since the normal behaviour of the animal is less affected. However, because it features nearly identical viral surface proteins, the immunity that it induces in the host protects it well from future infections with HPAI strains. This phenomenon is exemplified by how the more infectious, but more benign Omicron variants were able to rapidly out compete the earlier SARS-CoV-2 variants during the SARS-CoV-2 pandemic. Thus, the H5N1 variant discovered in the UOF ostriches may actually perform as a natural attenuated vaccine against the more pathogenic versions of the H5N1 influenza virus.

22. In para. 96, Dr. Furness argued that "*testing cannot wholly evaluate the current and future risk of disease spread posed by an entire flock of confirmed infected birds continuing to exist on a known contaminated premises*." Basically, the point of the culling is to prevent the spread of the H5N1 virus. Yet, the CFAI chooses to ignore any evidence that the infected flock no longer is shedding the virus and that natural immunity has been established by way of production of neutralizing antibodies, all of which can be easily tested for. Moreover, it has forbidden any treatment or testing of the ostriches following the culling order that it issued to the UOF owners. It should also be appreciated that it is not necessary to individually test birds for shed virus with PCR- or rapid antigen tests, and pooled samples are sufficient for detection.

PART 4: COMMENTS RELATED TO THE AFFIDAVIT 2 OF DR. SHANNON FRENCH

23. In para. 21, Dr. French noted that "*unlike chickens and turkeys, many ducks (for example a flock only showing decreased egg laying) will recover from the infection*." This probably reflects the establishment of a level of natural herd immunity possibly from recovery from prior infection with HPIV or a highly related avian influenza strain. With the culling that occurs with domestic chickens and turkeys, such herd immunity is unable to develop.

24. In para. 22, Dr. French cited studies by Abolnik *et al.* (2007)[9] and Van Helden *et al.* (2016)[10] to support the contention that adult ostriches can shed virus without any clinical signs. However, these studies were not based on the HPAI H5N1 strains that are more virulent and likely to cause illness. The Abolnik *et al.* (2007) publication is actually based on the H6N8 and H9N2 avian influenza viruses and the Van Helden *et al.* (2016) publication is likely based on H5N2. This latter article stated that "*the presence of clinical signs on the farms was included in the case definition but did not play a large role in identifying infected properties, as the majority of positive farms did not subjectively show an increase in morbidity or mortalities of ostriches*." In view of the high rate of false-positives with the PCR test at the thermal cycle thresholds that were likely used, it is not clear that all of the birds were even necessarily infected.

25. In para. 24, Dr. French cited the publication by Abolnik *et al.* (2021)[11] to support the contention that ostriches without clinical signs of illness can shed influenza virus into the environment. This is based on H7N1 low pathogenic avian influenza (LPAI) and H5N8 HPAI viruses, and **not** the H5N1 HPAI virus, and the infection of 7-week-old ostriches, which are likely to have little immunity initially. Antibodies against H5N8 HPAI virus and H7N1 LPAI virus only appeared after day 7 post

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[9] Abolnik, C., Bisschop, S., Gerdes, T., Olivier, A., Horner, R. (2007) Outbreaks of avian influenza H6N2 viruses in chickens arose by a reassortment of H6N8 and H9N2 ostrich viruses. Virus Genes. 34:37-45. doi: 10.1007/s11262-006-0007-6. https://pubmed.ncbi.nlm.nih.gov/16927114/

[10] Van Helden LS, Sinclair M, Koen P, Grewar JD. (2016) Description of an outbreak of highly pathogenic avian influenza in domestic ostriches (Struthio camelus) in South Africa in 2011. Preventive Veterinary Medicine. 128:6-11. https://doi.org/10.1016/j.prevetmed.2016.03.019

[11] Abolnik, C., Ostmann, E., Woods, M., Wandrag, D.B., Grewar, J., *et al.* (2021) Experimental infection of ostriches with H7N1 low pathogenic and H5N8 clade 2.3. 4.4 B highly pathogenic influenza A viruses. Veterinary Microbiology. 263:109251. https://pubmed.ncbi.nlm.nih.gov/34656859/

exposure, with higher antibody titres induced by the HPAI virus compared to the LPAI virus. By 14 days post-infection, there was little if any detectable shedding by PCR testing even with 40 thermal cycles. Live virus cultivatable in eggs was not detected from the H7N1 LPAI virus if more than 30 thermal cycles was required for amplification of the viral RNA by PCR. The authors concluded that their findings "***show that LPAI and HPAI viruses are unlikely to circulate at low levels or for short periods within ostriches***."

26. In para. 24, Dr. French claimed that "*AIV have been demonstrated to survive for months or **even years** in fresh water at low temperatures, so allowing the virus to spread through a herd has the potential to create a large source of infective virus that will remain in the area even after the individual ostriches have recovered and are no longer shedding the virus themselves*." This unlikely scenario is based on a single study with a duration of up to a year by Ramey *et al.* (2022)[12] using PCR detection with up to 44 thermal cycles as corresponding to a positive result. While "positive samples" were subsequently tested via inoculation of embryonating chicken eggs, it seems highly improbable that the virus would be stable in a non-sterile environment, and it would likely become highly diluted in a pond or stream so the viral load would be very low. Moreover, this study was performed with the H5N2 influenza virus and not an H5N1 strain.

27. In para. 25, Dr. French argued that mutations can occur in avian influenza viruses that might foster cross-species transmission, especially since ostriches are allowed run more freely and potentially interact with wild animals. Firstly, the rate of mutation of the influenza viruses is dependent on the genome and its encoded proteins, such as the error rate in the viral RNA polymerase complex that facilitates its replication and not so much from mutations of host proteins. The rate of mutation in a host species is orders of magnitude much slower than a virus or bacteria. Efficient replication of a virus requires an optimization of the virus in its evolution for successfully entering, hijacking and exiting from the host's cells. The PB-E627K mutation referred to by Dr. French, which increases infectivity in mammals, was shown by the CFAI **not** to be present in the H5N1 virus RNA isolated from the UOF ostriches as discussed in para. 17 to 20

[12] Ramey, A.M., Reeves, A.B., Lagassé, B.J., Patil, V., Hubbard, L.E., *et al.* (2022) Evidence for interannual persistence of infectious influenza A viruses in Alaska wetlands. Science of the Total Environment. 210;803:150078. https://royalsocietypublishing.org/doi/full/10.1098/rspb.2020.1680

above. Secondly, historically farms were in more open environments where livestock tended to be more mobile, and grazed in wide spaces on diverse vegetation and exposed to more microbes. In a natural environment, ecosystems are extremely complex with a high degree of inter-species interactions. In the large factory farms that now dominate in the livestock industry, the animals are often confined and kept in high density, often under stressful conditions. This increases the prospects for infection by a pathogenic virus or bacteria, since stress reduces the effectiveness of immune systems and cramped conditions foster infectious disease spread. Furthermore, if we accept Dr. French's argument, then open farms should really be banned outright in general, not just ostrich farms. Moreover, we should also be discouraging the establishment of zoos, game farms, animal shelters and even wild bird refuges, since these allow the mixing of diverse species in crowded surroundings. People should also not have pets such as dogs, cats or rodents, since this also increases the chances of the spread of infectious diseases by zoonosis (*i.e*., the spread from animals to humans).

28. In para. 28, Dr. French argued that enforcement of a stamping out policy is the best way for "*minimizing viral transmission and contamination of the environment, with the final intention of viral eradication*." However, with such a wide range of wild animals that can be infected and transmit the H5N1 influenza virus, there is too large a reservoir of the virus to ever hope to eradicate it from the domestic animal and human populations. The biosecurity measures would have to be so draconian that the domestic animals on the farms would be even more inhumanely treated. Curtailment of the spread of a pathogenic virus or bacteria is more likely achieved by acquisition of natural immunity, breeding between survivors with genetics that confer more resistance to the pathogen, and the natural evolution of the pathogen to more infectious and benign forms.

29. In para. 30, Dr. French suggested that the stamping out policy has successfully stop previous HPAI outbreaks since 1957. However, as illustrated in Figure 1, influenza outbreaks tend to be seasonal, and largely wane over a few months on their own accord even without a stamping out strategy. This is clearly evidence, for example, in the rise of the flu in human populations during the winter months in the Northern hemisphere, and during the summer months in the Southern

hemisphere. It is hard to rule out that the displacement of more virulent strains by more benign and infectious strains, and acquisition of herd immunity are not also contributing to a decline in an epidemic or pandemic of a pathogen. Ultimately, it is a matter of debate whether the mass culling of all of the birds in a flock or herd results in better overall animal welfare, as compared to having a few die and most recovering from an infection with resulting herd immunity and protection from future infections.

30. In para. 39, Dr. French noted that while HPAI illness may start in the respiratory tract in birds, it can progress to the brain and other organs resulting in multiple organ failure. She distinguished this with the actions of HPAI in humans and other mammals, which is primarily respiratory. However, influenza viruses can exert effects in humans and mammals that also results in neurological damage.[13] For example, recently a house cat was infected with HPAI H5N1 virus and it developed neurological damage before it was euthanized.[14]

31. In para. 40, Dr. French largely dismissed the relevance of data transmission, treatment and prevention with vaccines learned with human influenza pandemics with respect to their applicability to avian influenza outbreaks. However, a stated major concern of the CFIA was the possibility with HPAI H5N1 virus for infection, mutation to a more pathogenic form, and the generation of a pandemic in the human population.

32. In para. 42, Dr. French suggested that difficulties associated with the PCR testing protocols identified for the SARS-CoV-2 may not be applicable to a different virus, such as the influenza virus, even though both are respiratory RNA viruses with relatively few genes and similar sized genomes. The fundamental problems associated with PCR tests at high thermal cycle (CT) cut-offs is not virus-dependent. For example, Abolnik *et al.* (2021) showed that with PCR requiring 30

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[13] Jang, H., Boltz, D., Sturm-Ramirez, K., Shepherd, K.R., Jiao, Y., *et al.* (2009) Highly pathogenic H5N1 influenza virus can enter the central nervous system and induce neuroinflammation and neurodegeneration. Proc Natl Acad Sci U S A. 106(33):14063-8. doi:10.1073/pnas.0900096106. https://pmc.ncbi.nlm.nih.gov/articles/PMC2729020/

[14] Naraharisetti, R., Weinberg, M., Stoddard, B., Stobierski, M.G., Dodd, K.A., *et al.* (2025) Highly pathogenic avian influenza A (H5N1) virus infection of indoor domestic cats within dairy industry worker households — Michigan, May 2024. MMWR Morb Mortal Wkly Rep 74:61–5. DOI: http://dx.doi.org/10.15585/mmwr.mm7405a2

or more cycles, there are no replication competent avian influenza virus particles in an ostrich-derived sample.[11]

33. To my knowledge, the PCR testing for H5N1 performed by the CFIA is not externally certified. At the time that I prepared my earlier expert reports, the CFIA had not disclosed that it had performed full sequence analysis of the viral genome samples retrieved from the two dead UOF ostriches, and the PCR tests that were described were limited to the H5 gene and did not appear to test for the N1 gene. I agree now that it is highly likely that these ostriches were exposed to an H5N1 virus, although the sequencing information revealed that the strain that infected the UOF ostriches was a likely hybrid virus from reassortment mixing with low pathogenic avian virus, and as a consequence would likely be less pathogenic than an HPAI virus.

34. In para. 43, Dr. French dismissed the utility of rapid antigen tests for the presence of active shed virus from birds of an infected flock, because the CFAI does not use such tests, even though they are more rapid, cheaper and convenient to use than PCR tests. Such H5N1 influenza rapid antigen tests are already commercially available, and they should not be ignored as a complementary strategy to monitor viral shedding on-site.

35. In para. 44, Dr. French questioned whether it was possible to develop antigens for antibodies that could distinguish different H5N1 avian virus variants. However, the epitopes for antibody recognition can be as little as a single amino acid in a peptide sequence from an antigen protein. [Peptides are polymers of two to 50 or more amino acids like beads on a chain. Proteins are longer and can feature hundreds to thousands of amino acids.] In my own lab, we have routinely developed hundreds of polyclonal antibody preparations from the serum of immunized rabbits that can distinguish a difference of the phosphorylation state of a single amino acid in an otherwise identical peptide sequence.

36. It is completely feasible to develop assays that can distinguish between different mutant forms of the various influenza proteins and even neutralizing antibodies against the hemagglutinin protein, which allow the virus particle to attach and enter host cells. For example, neutralizing monoclonal antibodies were developed against the Wuhan SARS-CoV-2 spike protein that did not

work on the Omicron variants. A single amino acid change in an epitope recognized by an antibody in an antigen can result in a profound loss of immunoreactivity.

37. In para. 45, without offering any evidence to the contrary, Dr. French disagreed with my suggestion that the study of the antibody reactivity of the naturally infected UOF ostrich herd would be a good starting point to develop a diagnostic test. This is certainly an approach that we took at my company Kinexus Bioinformatics in development of a 40-marker antibody test (against 8 of the SARS-CoV-2 proteins) that we used in a 4500-person clinical study. This blood test first involved the careful screening over 6000 possible peptide fragments predicted from the amino acids sequences of the 28 proteins encoded by the SARS-CoV-2 genome and testing with the serum from hundreds of patients that were naturally infected with SARS-CoV-2 (usually confirmed by PCR) and had symptoms of COVID-19. As shown in my February 12, 2025 expert report, we were able to identify several portions of the hemagglutinin and neuraminidase proteins of H5N1 that were highly immunoreactive in antibodies tested in the egg yolks from UOF ostriches collected in the summer of 2024.

38. In para. 46 of her report, Dr. French correctly pointed out that the sensitivity and specificity of a serological test is dependent on having samples from infected and non-infected birds or their eggs, and performing proper controls. It would be ideal to have samples from animals that were confirmed to be PCR or rapid antigen positive to follow their antibody levels, especially for specific epitopes. However, the policy of the CFIA to not permit the testing of the ostriches during and after the infection has made this difficult. Nonetheless, the most commonly recognized basis of immunity is that the human or animal possess antibodies that can clearly recognize a peptide sequence from an infectious pathogen. The more different epitopes unique to the pathogen that are detected in a specimen from the person or animal, the greater can be the confidence that they have recovered from an infection and have future protection from that or highly related pathogens.

39. In para. 49, Dr. French concurred that in her opinion, "*it is reasonably possible that the birds may have no longer been shedding virus by January 29, but it is impossible to say with any degree of certainty. The reality is that we simply do not know what the disease status of the birds was at*

that time." Since the birds have continued to show no signs of an ongoing influenza infection at the UOF up to March 7th, when Dr. French signed her affidavit, and now true up to March 31st[h], the likelihood that the UOF ostriches present a threat for spread of the HPIV only continues to decline.

40. In para. 50, Dr. French noted that in a study of 929 ostriches on South African farms,[15] that "*roughly 90% of the flock had antibodies to influenza (had seroconverted)*." While 90% of the birds in the herd had evidence of antibodies against the HPAI virus, PCR testing with a positive result was done on only one combined sample. It is likely that most of the birds were infected with the H5N2 or a related virus (not H5N1), and had only low levels of infection that did not cause serious signs in the birds. In this study, the detection of 17% of sera from a farm (identified as Farm AI18) that contained H5-specific antibodies, had no NP-specific antibodies (NP is one of the influenza virus proteins – see Figure 2). It also appears that the presence of anti-N2 antibodies was not assessed in this study. Consequently, it is feasible that many of the ostriches tracked and culled may have been infected previously with low pathogenicity H5 influenza strains. It was probably unnecessary to cull this herd.

41. In para. 53, Dr. French acknowledged that "*given all the variables, we simply cannot say with any certainty whether any individual ostriches were transmitting virus on January 29" at the UOF site.*" In view of this lack of knowledge, it would be prudent to perform PCR tests and serological tests on the ostriches before any of these ostriches are culled. This information would be valuable in understanding the effectiveness of natural immunity in this species against the H5N1 influenza virus.

42. In para. 59, Dr. French disagreed that the lack of deaths from the December 2024 – January 2025 infection of the ostriches at the UOF site that were established before 2021 could be due to natural immunity against a previous avian influenza infection. She noted that younger birds are more susceptible to death from infection. However, in my previous expert reports, I did not refer

[15] Abolnik, C., Fehrsen, J., Olivier, A., van Wyngaardt, W., Fosgate, G., Ellis, C. (2013) Serological investigation of highly pathogenic avian influenza H5N2 in ostriches (Struthio camelus). Avian Pathology. 42(3):206-14.

specifically to younger birds, and many of the birds that were added to the herd after 2020 were in fact older birds. Within the entire group of UOF 450 ostriches, there appears to be a 16% mortality rate, assuming that all of the birds were exposed to the virus, which is likely since 69 birds died. However, since none of the birds on the UOF site fromm prior to 2021 died, this is a 0% mortality rate, and strongly supports natural immunity. However, immune status can only be achieved by actually testing for antibodies, and under control conditions, looking at morbidity and mortality rates when birds are actually infected in a pre-clinical study.

43. In para. 60, Dr. French disagreed that the UOF ostrich herd could actually provide protection to wild birds, at least in a significant way. Surely, if the ostriches have herd immunity, they will not be passing on the virus to virus-naive wild ducks and geese that may land on the farm. I do agree that the ostriches will have little impact on the overall transmission of H5N1 to other commercial livestock whether they were infected or not, or be transmissible or not for the virus in view of the relatively small size of the ostrich herd and the hundreds of thousands of ducks and geese that migrate through BC annually, and the actual portion of these wild birds that specifically frequent the UOF site. The main value of preserving the UOF ostrich herd is for the valuable information that they can provide about the effectiveness and duration of natural immunity, and the important products that can be developed from the production of antibodies in their eggs for a wide range of applications, including the detection and treatment of avian bird flu in animals and humans.

44. In para. 61, Dr. French suggested that even if the UOF ostriches have natural immunity against the particular avian influenza strain that infected them in the December 2024-January 2025 period, they could still become infected with new variants of the influenza virus the following spring. She further proposed that the birds may still harbor the original influenza strain that infected them, and exposure to new variants could trigger further reassortments of their chromosomes to produce additional variants. If the ostrich herd was culled and replaced with new and likely younger ostriches that are naïve to the H5N1 virus, the chances of infection leading to sickness and death is actually much higher than with the existing herd that has already survived the infection outbreak. The sequences of the various influenza virus proteins actually have a high

degree of amino acid identity and similarity amongst the various influenza strains. Since infection of the ostriches induces a polyclonal antibody response, it would seem most likely that a high degree of immune protection would be afforded to the birds. Repeated exposures of these long-lived birds would likely naturally booster and further expand their immunity to future influenza variants. It seems highly improbable that the influenza virus would be maintained at even low levels for 6 months to a year in the ostriches to allow for potential recombination with other variants of avian influenza virus during the following migratory seasons.

45. In para. 62, Dr. French expressed the concern that HPAI virus-naïve birds could come to the UOF site and become infected by shed virus from the ostriches. However, any avian influenza virus that infected the ostriches would likely have already been placed earlier into the environment by infected migratory wild fowl. It seems much more likely that the HPAI virus-naïve birds will get infected from other wild birds than from the UOF ostriches and certainly more likely at one of the thousands of other ponds and streams throughout British Columbia than from the two at the UOF site.

46. In para. 63, Dr. French again mentioned her concern that the immune protection afforded to the UOF herd following exposure to the H5N1 strain they encountered during the recent outbreak on the farm may be insufficient against a future variant of the influenza virus. The amino acid sequences of the 10 influenza virus proteins actually exhibit a high degree of amino acid identity and similarity amongst the various influenza strains. Any mutations that might be further generated in the H5N1 strains is usually restriction to less than 1% of the overall primary amino acid structures of the virus's proteins. Most mutations compromise the infectivity of the virus, which is highly optimized for its normal host species. Consequently, the vast majority of the polyclonal antibodies that recognize avian influenza virus in these birds, along with their T-cell immunity, should still provide strong protection against newer variants.

47. In para. 69, Dr. French questioned whether UOF ostrich herd has acquired “resistance” to the H5N1 virus, despite the evidence of no deaths in the ostriches on the farm in the group on site prior to 2021 and the presence of detectable anti-H5- and anti-N1-specific antibodies in the yolks of the eggs from these ostriches collected in the summer of 2024. No arguments are offered by

her to counter the claim that having ostriches that are more resistant to future influenza virus infections would not be useful for preventing further infection and spread. This is the basis for the concept of "herd immunity." While the repertoire of antibody producing B-cells that an animal is born with is partly generated de novo by mutation *in utero* in the B-cells, it is also partly hereditary. Consequently, it is reasonable that careful breeding with ostriches that survived their infection with H5N1 virus might be able to allow increased resistance to the virus in their off-spring.

48. In para. 70, Dr. French pointed out the antibody reactivity in the egg yolks from the ostriches that were tested by Kinexus may not have arisen from an HPAI H5N1 variant. That is correct. However, the fact remains that all of the birds that were tested for the presence of the H5-specific and N1-specific peptide sequences demonstrated immune reactivity against multiple epitopes, and they did not succumb to the influenza strain that infected the flock in December 2024. If these ostriches were now tested, I fully expect that their antibody levels that can target the HPAI H5N1 would be boosted and much stronger signals would be evident on the immunoblot tests from Kinexus shown in Figure 3 of my February 12, 2025 expert report.

49. In para. 71, Dr. French noted that animals are commonly used for production of antibodies for research purposes, but there could be batch to batch issues related to reproducibility of results. Polyclonal antibodies for commercial purposes are routinely produced in larger animal species such as horses, goats, and donkeys. The yield from an ostrich egg is sufficiently high, and following affinity purification, would have the desired purity and specificity for industrial applications such as diagnostic kits and therapeutic antibodies. The UOF has already been involved in partnerships to develop antibody-based products for a wide range of applications such as treatment of acne, digestive enzyme neutralization in the gut for weight loss and reduction of blood triglycerides and sugar in diabetics, reduction of bacterial contamination of food, cosmetics and tooth paste, and improvement of masks and filters for protection against SARS-CoV-2 and other viruses and allergens.

50. In para. 72, Dr. French was unsure if selective breeding of ostriches that fully recover from infection with HPAI can permit development of more AIV-resistant birds. The fact that these birds

can breed within a couple of years and produce so many eggs in a season for over 55 years makes them highly suitable for genetic improvement in infectious disease resistance. The survival from infection would be an important criterion in a careful breeding program. Another criterion would be how marked is the antibody response in the birds when immunized with portions of the HPAI proteins such as hemagglutinin (H) and neuramidase (N).

51. In para. 75, Dr. French concurred that it is not unreasonable that the UOF ostriches are no longer shedding active avian influenza virus, but to be certain, she suggested that each ostrich should be individually tested. Rather than the costly testing of individual birds by PCR, combined specimens from multiple birds can be tested on an ongoing basis. Rapid antigen tests could also be performed. Such antigen tests could use antibodies that are generated and available in the yolks of recently produced ostrich eggs following affinity purification with peptides with amino acid sequences from H5N1 virus proteins that were found to be high immunogenic (i.*e*., antibody reactive) in the Kinexus-based tests.

52. In para. 76, Dr. French referred to PCR-based studies that indicate that viral shedding of H5N8, and other low and high pathogenic H5 influenza strains may be beyond 14 days even though the birds may present no clinical signs. The problem with all of these studies is that the PCR tests used do not necessarily detect replication competent virus particles, but rather degraded fragments of the virus that may arise from their destruction by immune cells. At 40 thermal cycles of amplification of the viral RNA in the test samples, the false-positive rate for replication-competent virus likely exceeds 95%. Note that with each thermal cycle the amount of genetic material is doubled with the polymerase chain reaction. Thus, with 40 cycles, the original starting amount of RNA is increased by 2^{40} (or 1.1×10^{12}). With each thermal cycle, the chances of false amplifications and mutations in the generated nucleotides induced by the polymerase also increases.

53. In any event, by 14 days after infection, the titers of the detected viral RNA fragments were extremely low and mostly undetectable within a week following initial infection. Since the UOF ostriches have continued to show no signs of sickness or infection since January 15th, over 9

weeks ago, the chances that any ostriches in the herd are shedding active virus in levels that support transmission is exceedingly remote.

54. In para. 77, Dr. French questioned the relevance of studies of human transmission of respiratory viruses like influenza to how the viruses are transmitted in birds, which are deemed to be less hygienic. Nevertheless, the basic principles of the chain of infection apply to animals as well as humans, and often humans may be found in less hygienic conditions too.

55. In para. 78, Dr. French suggested that it is the drinking water that is the main source of transmission of influenza for ostriches. While the study by Abolnik *et al.* (2021)[11] that she cited indicates that the H7N1 virus could be detected in drinking water from infected ostriches by PCR tests, it was not tested whether this was replication competent virus. Seven-week-old chicks were used in this study, with initial infection by direct inoculation. It was not evaluated whether the birds that acquired subsequent infection was via the shared water.

56. It remains controversial whether influenza virus can really be spread through drinking water.[16] Influenza virus is classified as a respiratory virus because that is the main means of its transmission. Although the virus might contaminate drinking water, it would also become highly diluted with the large body of water in a pond or even in a water bucket. This would significantly reduce the viral load.

57. In para. 79, Dr. French asserted that "*the traditional presentation of avian influenza in ostriches is usually mild with more severe clinical illness and mortalities generally limited to younger birds unless the birds are under significant external stress. For this reason, the fact that on UOF the mortalities were observed in younger birds is what would be expected as a result of infection and should not implicitly be interpreted as a sign of preexisting exposure to this viral subtype*." As pointed out earlier, the UOF ostriches that were the most susceptible to getting sick and dying were not necessarily younger birds, but those that were not in the herd prior to 2021. The fact

[16] WHO (2022) Guidelines for drinking-water quality: fourth edition incorporating the first and second addenda. Geneva, Switzerland: WHO Press.
https://www.who.int/publications/i/item/9789240045064
https://iris.who.int/bitstream/handle/10665/352532/9789240045064-eng.pdf

that many of the ostriches tested positive for anti-H5 and ant-N1 antibodies in the yolk of their eggs collected in the summer of 2024 further supports the contention that there was prior exposure to an H5N1-like influenza virus before this time.

58. In para. 81, Dr. French strongly expressed her disagreement with my previous statement that "*the possibility of mutations occurring in ostriches that would increase influenza infectivity in mammals is "extremely remote*" and stated that this was "*well documented in the literature*" without actually providing any references. I am unaware of any publications that show that mutations in avian influenza viruses that resulted in increased ability to infect and cause disease in mammals, which <u>were generated and identified first in ostriches</u>. Dr. French does cite the study by Shinya *et al.* (2009),[17] which documented that ostriches with H5N1 influenza variants PB2-E627K and D701N are more able to propagate the virus. There is certainly no compelling reason why the risk of generation of these mutants would be higher in ostriches than any other bird. The influenza virus identified in the UOF ostriches did not have any of these types of mutations. The risk of successful transmission to a human would be much higher from an H5N1 species that has already successfully infected a mammalian host such as a dairy cow. For example, 41 of the 70 human cases of HPAI H5N1 virus identified in the US were in those working on dairy farms and 24 were in those that worked with poultry.[2]

59. In para. 83, Dr. French described how the PB2 E627K mutant of HPAI H5N1 increases its ability to propagate in mammals, and she mentioned that the RNA polymerase is more active at lower temperatures based on citation of a review by Olivier (2006).[18] While wetter and colder months are associated with increased influenza virus detection (hence the seasonal incidence of influenza in late fall in the Northern hemisphere), this is more likely due to decreased host resistance than a more active viral RNA polymerase at lower temperatures. Generally, enzymes are less active at

[17] Shinya, K., Makino, A., Ozawa, M., Kim, J.H., Sakai-Tagawa, Y., *et al.* (2009) Ostrich involvement in the selection of H5N1 influenza virus possessing mammalian-type amino acids in the PB2 protein. Journal of Virology. 83(24):13015-8. https://europepmc.org/article/PMC/2786862

[18] Olivier, A.J. (2006) Ecology and epidemiology of avian influenza in ostriches. Developments in biologicals. 1;124:51-7. https://www.researchgate.net/publication/7327440_Ecology_and_epidemiology_of_avian_influenza_in_ostriches

lower temperatures than the normal body temperature. The immune system of the host is commonly more active at slightly higher body temperatures to more efficiently fight viral and bacterial infections. Identification of temperature-sensitive mutants is a common strategy to explore the role of a protein. However, in these rare cases, higher temperatures than physiological leads to a complete loss of function.

60. The body temperature of a bird is typically 40 to 42 °C, whereas in humans it is around 37°C. Ostriches have a normal body temperature of between 36 to 39°C depending on different reports, and this would not be ideal for a virus that has been evolved to propagated primarily in ducks and geese. A virus that normally infects a host will have its viral enzyme activities optimized for the environment in the host. Infection with a virus or bacteria typically induces increased temperatures in humans and animals.

61. In para. 85, Dr. French noted that mutations in HPAI viruses that increase infectivity do not necessarily increase virulence and the presentation of more severe clinical disease. The basis for use of attenuated viruses for vaccines is that they are able to infect a host, but they do not replicate as fast and provide time for the immune systems to respond before it is necessary to turn on further host responses that produce sickness. Thus, if a mutation increases infectivity without causing illness, this is actually desirable provided that it does not increase virulence. This exposure may induce immunity against more pathogenic variants of the virus in the future.

62. In para. 87, and based on a study by Abolink *et al.* (2007),[9] Dr. French suggested that "*there is documented molecular evidence that an endemic strain of avian influenza that circulates in chickens in South Africa most likely originated from the recombination of two strains (H9N2 and H6N8) that occurred in ostriches."* Since South Africa has the largest commercial population of ostriches, with numbers around 350,000 birds with 350 registered ostrich farms, this speculation is not unreasonable,[19] but the number of ostriches on the UOF site is a thousand-times lower, so the chances of such a recombination with H5N1 strains is also lower by this magnitude.

[19] https://stickymangorice.com/2021/04/09/ostrich-farming/

63. In para. 89, Dr. French described some of the events that would be necessary for an HPAI virus to cause a serious pandemic in humans. The transmission of H5N1 influenza virus from one human to another has yet to be documented. In the one instance of a recent human infection with H5N1 HPAI in Canada, this was a 13-year-old girl that was obese and had asthma.[20] She also infected with a viral strain that had the PB2-E627K mutation. One of the other necessary changes would be mutations that would permit the hemagglutinin protein to better binds to the alpha-2,6 sialic acids found in mammals rather than the terminal alpha-2,3 sialic acid residues found in receptors in birds, reptiles and amphibians. She also mentioned that the virus has to be more transmissible as an airborne pathogen. There are many other factors that would also be necessary too. It would have to optimize for human cell physiology, better evade the immune system of humans, and also be less virulent to more widely increase transmission.

64. In para. 93, Dr. French suggested that co-infection of the same host and the same cells with two different influenza strains could transpire such that "*a virus that may cause relatively mild disease in one host could in theory cause significant disease in an abnormal host*." This seems highly unlikely as successful viruses tend to be optimized to bind to a host cell, enter and successfully replicate, and exit the host cell. Cross-species transmission of viruses is generally a rare event, considering that hundreds of thousands of different strains of viruses are believed to infect mammals, and only around a hundred are truly pathogenic in humans. If the infections from both virus strains are mild, then it is most likely that the immune system should be able to easily handle both viruses before coinfection can occur in the same host cells. The theoretical concerns raised by Dr. French that have yet to be fully manifested with the H5N1 influenza virus in mammals, and so far, after many decades, this virus strain generally leads to mild disease in mammals with very rare instances of severe illness.

65. In para. 98, Dr. French repeated her earlier concerns that having commercial birds in an open environment with access to wild birds and other animals increases the prospects of viruses crossing over to other species. By this logic, the CFAI would seem to prefer the permanent closure

[20] Jassem, A.N., Roberts, A., Tyson, J., Zlosnik, J.E.A., Russell, S.L., *et al.* (2024) Critical illness in an adolescent with influenza A (H5N1) virus infection. N Engl J Med. 392(9):927-929. doi: 10.1056/NEJMc2415890. https://pubmed.ncbi.nlm.nih.gov/39740022/

of livestock operations that permit their animals to roam more freely outdoors in the interests of maintaining biosecurity. This all seems fruitless if these viruses are already commonly found within the wild animal communities.

66. In para. 101, Dr. French argued that selective breeding for resistance to influenza infections is not an immediate practical response to an outbreak. While this is true, it should be part of a longer-term strategy to protect commercial livestock. Farmers have had thousands of years of selective breeding practices to create the diversity of desired characteristics found in domesticated animals today.

67. In para. 105, Dr. French suggested that allowing the development of natural immunity in a herd of animals by not intervening is not an acceptable strategy on its own to deal with an outbreak of a potentially highly pathogenic virus or bacteria. I agree. It should be combined with preventative measures such a vaccination and other means of boosting the immune systems of animals, testing, isolation and treatment of infected animals, and should the animals fully recover, reintegration into the herd or flock. Culling of whole herds of animals on the basis of a few infected animals in the unit is not a viable, long term strategy as is now clearly evident. The concept of recognizing natural immunity, which was largely ignored during the COVID-19 pandemic, is in fact endorsed by a wide range of scientists and medical practitioners. In the case of the COVID-19, which is another respiratory disease with similar morbidity and mortality to influenza, it is the opinion of tens of thousands of doctors and scientists world-wide as documented in the Great Barrington Declaration,[21] the Public Health Agency of Sweden,[22] the conclusions of the US Congressional Select Subcommittee on the Coronavirus Pandemic,[23] the National Citizens Inquiry into Canada's response to COVID-19,[24] and the recent Alberta's COVID-19 Pandemic Response[25] report that natural immunity should be adopted as a viable approach

[21] https://gbdeclaration.org/
[22] https://en.wikipedia.org/wiki/Swedish_government_response_to_the_COVID-19_pandemic
[23] https://oversight.house.gov/release/final-report-covid-select-concludes-2-year-investigation-issues-500-page-final-report-on-lessons-learned-and-the-path-forward/
[24] https://nationalcitizensinquiry.ca/national-citizens-inquiry-issues-commissioners-final-report/
[25] https://open.alberta.ca/publications/albertas-covid-19-pandemic-response

to curtail pandemics with special attention to isolated and protect those that are of higher risk than the general population.

PART 5: COMMENTS RELATED TO THE ANTIBODY TESTING OF OSTRICH YOLK AND HUMAN BLOOD SAMPLES

68. In the previous parts of this report, I have described how the UOF ostriches are highly unlikely to be a threat for future transmission of the H5N1 influenza virus to other birds, animals and humans. Moreover, I have documented that transmission of HPAI H5N1 virus is unlikely to be a significant threat to the human population, and certainly highly unlikely to cause a highly lethal human pandemic. Nevertheless, it has had a significant impact on commercial poultry farms, and more effective strategies need to be developed than the current stamping out policy, which has its own devastating effects on commercial livestock operations, and increased food prices from egg and meat shortages. Such strategies require earlier monitoring of potent outbreaks, isolation of infected animals, better influenza disease treatment and preventative measures such as vaccination and others (e.g., vitamin D supplementation) that increase in the robustness of the immune systems of animals. Development of targeted immunity against the influenza virus strains, such as the production of specific antibodies against each strain, should also be tracked for evidence of the establishment of herd immunity against future infections.

69. In my January 29, 2025 expert report in para. 29 to 39, I outlined the uses and limitations of the polymerase chain reaction (PCR) tests for the detection of the genetic material generated following a viral infection (in Section 3.5 “Detection of Viral Infections by PCR”). This is the test that was used by the CFIA to determine whether the two recently deceased UOF ostriches at the end of December 2024 were infected with an H5-containing strain of avian influenza. When undertaken properly (not at high thermal cycle numbers), this test can be highly accurate. However, the test must be performed in a laboratory setting that is properly equipped to undertake the analysis, and it takes at least a day to complete.

70. In my January 29, 2025 expert report in para. 40 to 42, I also outlined the use and limitations of the rapid antigen tests for the detection of viral proteins generated following a viral infection (in Section 3.6 “Rapid Antigen Tests for SARS-CoV-2”). I provided information for detection of the

virus that caused COVID-19 as a comparison, and noted that there several commercial rapid antigen test kits for detection of the H5N1 strain influenza viral proteins, including the CTK Biotech OnSite. Influenza A/B Ag Rapid Test,[26] the Ringbio Avian Influenza Antigen Test Kit, AIV Ag Test,[27] and GlobalDx Herdscreen GDX84-2 AIV H5 Ag Test.[28] Such tests are based on the availability of specific H5- or N1-specific antibodies on a test strip that can capture viral proteins that are present within a specimen sample. These tests can be highly accurate, but they are much less sensitive, and it is not possible to amplify the levels of a protein as it is feasible for RNA or DNA using the PCR method. However, the test is rapid and can be performed on site without special equipment, with results that are evident within a few minutes. A subclinical, asymptomatic detection of influenza would be harder to detect with this method, but it can be very useful for diagnosis of flu-like illnesses. I would recommend testing some of the UOF ostriches with one of the H5N1 rapid antigen tests should any of them show signs of illness in the future to help rule out an HPAI infection. It can also be used to help prove that asymptomatic ostriches are not shedding virus.

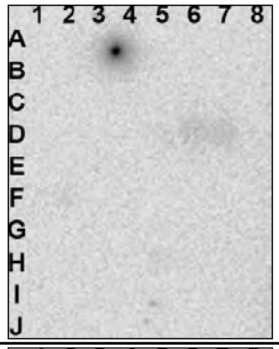
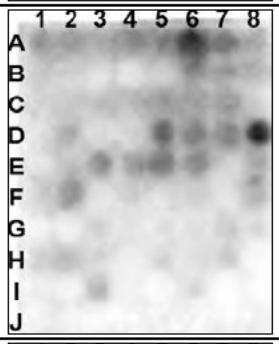
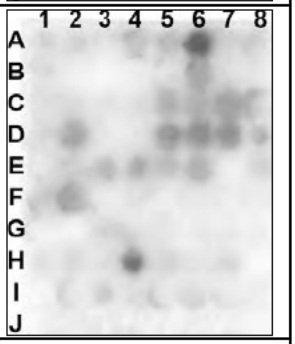
71. In para. 56 to 61 of my February 12, 2025 expert report, I provided the specifics of a serological test that was designed to test antibodies against the portions of the H5 and N1 viral proteins that were unique to the H5N1 influenza virus and not to other strain of the virus. As shown in Figure 3 of that earlier expert report, all of the egg yolk samples from 18 of the UOF ostriches collected in the summer of 2024 were found to yield multiple antibodies against the H5- and N1-derived unique peptides. This demonstrated proof of concept that such a highly sensitive test for evidence of specific immunity against this virus can be successfully developed. More recently, we obtained samples of a few drops of blood from four of the staff at the UOF. As shown in Figure 3, each of these individuals were shown to have developed antibodies that recognize the H5N1 strain of the avian influenza flu virus, although none of them had any signs of illness from the recent influenza infection on the farm. It is not surprising that these individuals have developed immune responses following their exposure to the H5N1 virus that infected the UOF ostrich herd.

[26] https://ctkbiotech.com/onsite-influenza-a-b-ag-rapid-test/
[27] https://www.ringbio.com/solutions/poultry/avian-influenza-antigen-test-kit
[28] https://globaldx.com/avian-flu/

It also demonstrates that the H5N1 virus in the ostriches was not pathogenic in humans. However, it remains unclear when it was that the UOF staff was first infected with an H5N1 virus, and this could have been even in 2020, when the UOF had an outbreak of what was diagnosed as pseudomonas bacteria, which is usually what birds actually die from after an H5N1 influenza virus infection.

72. The Kinexus H5N1 peptide SPOT array test is much more sensitive and accurate than typical serological tests that use whole recombinant hemagglutinin or neuraminidase proteins as target antigens. Such alternative tests will feature portions of the H5 or N1 proteins that are not unique to the H5N1 strains of influenza virus and allow for false positives. However, additional research is needed in order to optimize the Kinexus SPOT array test for this virus. I suspect that the yolk samples from ostrich eggs that are collected in 2025 will have very high titres of anti-H5 and anti-N1 antibodies that are much stronger than shown in Figure 3 of my February 12, 2025 expert report. Having strong positive, controls is important to establish specificity and sensitivity of such antibody tests. However, it is difficult to obtain such specimens, which is why I have been willing to test egg yolk samples from the UOF ostriches and staff member to determine whether the test does indeed work with other species besides birds as expected. Further work with negative controls, such as eggs from uninfected chickens and blood samples obtained in 2020 from human participants in our serological SARS-CoV-2 antibody testing clinical study are now underway.

Figure 3. H5N1 peptide SPOT arrays probed for IgA, IgM and IgG antibodies in serum from blood samples originally obtained from four human staff at the UOF in March 2025. The appearance of a dark spot indicates positive immunoreactivity with different 14-amino acid long peptides derived from the H5 hemagglutinin protein (Spots A1 to E7) and N1 neuraminidase protein (Spots E8 to J2).

Array #	Image	Array #	Image
KPL01CDY-03c T-TBS Neg. Control		KPL01CDY-06c K.E.	
KPL01CDY-07c S.		KPL01CDY-08c K.P.	
KPL01CDY-10c D.B.			

73. If the UOF ostrich eggs collected in 2025 do indeed have very high titres of antibodies against the H5, N1 and the other proteins found in the HPAI virus, these would be highly valuable for research purposes, to track infection with the virus in rapid antigen tests, and could potentially be used as therapeutic antibodies to treat animals and humans that are sick following H5N1 infection.

74. The CFIA has expressed concerns that ostrich samples from the UOF may contain replication-competent HPAI virus, and these should not leave the quarantine area. At this time, such concerns can be easily allayed for the following reasons:

    a) There is no evidence that there is an active infection of the H5N1 virus on the UOF site after 9 weeks since the last signs of illness in the ostriches;

b) My previous H5N1 influenza antibody tests of yolk samples from all eggs individually collected from 18 different UOF ostriches during the summer of 2024, all tested positive for multiple epitopes for both H5 and N1, indicating the birds already had a degree of natural immunity;

c) Eggs are naturally sterile as long as the egg shell has not been cracked or broken. The eggs acts as a very strong barrier to infection by viruses and other microbes. Consequently, egg yolks are extremely unlikely to be contaminated with the H5N1 virus. Moreover, the presence of the antibodies against the virus in the egg yolk would neutralize the virus even if it was possible to somehow infect the egg during early stages of egg formation;

d) The H5N1 strain that infected the UOF ostriches and staff appears to be of lower pathogenicity in the ostriches and not pathogenic to humans; and

e) No animals are housed at the Kinexus facility and none of the staff associate with any farms or commercial operations that have chickens or turkeys.

75. In view of the low risk associated with the testing of frozen yolks from eggs that are recently collected from the UOF ostriches, I am confident that they can be safely shipped to the Kinexus research facility in Vancouver for testing. I recommend that the yolk samples are:

a) in volumes of no more than 500 microlitres for each sample;

b) the yolk samples are transferred into 1.5-ml plastic, capped plastic Eppendorf tubes (or their equivalent);

c) the labelled individual 1.5-ml tubes with yolk samples are further placed up to 6 in a 50-ml plastic Falcon tube with a screw cap lid (or their equivalent); and

d) the filled Falcon tubes are further placed into a sealable plastic bag that is placed in a cardboard box with styrofoam or paper packing.

76. The samples should be sent to the Kinexus site in Vancouver, B.C. by courier or hand delivered by a member of the UOF staff.

77. These are the kind of procedures that are routinely taken by the over 2000 international and domestic clients of Kinexus over the past 26 years as we have analyzed their biological specimens for the presence of diverse proteins, including antibodies. Due to the high stability of ostrich IgY antibodies, it may also be acceptable to sterile the samples, as has been done with milk from cows, by standard Pasteurization at 63°C.[29]

Respectfully submitted by,

Steven Pelech, Ph.D.

Professor,
Department of Medicine,
University of British Columbia

President and Chief Scientific Officer,
Kinexus Bioinformatics Corporation

Vice-President, and Co-Chair,
Scientific and Medical Advisory Committee,
Canadian Citizens Care Alliance

[29] https://milkyday.com/blog/2020/02/11/methods-time-and-temperature-for-milk-pasteurization/

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Our File: 140642-1
March 28, 2025

By E-mail

E-mail -spelech@mail.ubc.ca; spelech@shaw.ca

Dr. Steven Pelech
8755 Ash Street, Suite 1
Vancouver, BC V6P 6T3

Attention: Dr. Steven Pelech

Dear Sirs/Mesdames:

Re: Universal Ostrich Farms Inc. and Canadian Food Inspection Agency
Federal Court of Canada – Docket T-294-25 (the "Action")

Further to our conversation earlier today, we confirm that we have been retained by Universal Ostrich Farms Inc.(the "**Applicant**") to obtain an expert report from you concerning options available to them to have antibody testing done of the surviving ostriches, or their eggs, in order to determine whether or not they have been exposed to H5N1, whether they are shedding the virus, and whether they have developed natural immunity, and whether they pose any risk to wildlife or humans as a result of their H5N1 exposure.

We understand you may have done some testing of eggs from these ostriches that were lead prior to the December 30, 2024 culling order and we would ask that you provide the background and results of that testing in your report. We also understand that you have recently tested the blood of the farmers who have access to these ostriches and have obtained test results concerning antibodies they may have to the H5N1 virus. **In your report can you please provide** the details of what was undertaken, including the dates that testing was done, explain the process that was undertaken to obtain this testing and the meaning of the results that were obtained with respect to risk posed by the ostriches, or the farmers, to other wildlife or human beings of spreading infection of H5N1.

Michael Carter of Cleveland Doan is still the Applicant's legal counsel at present, and he has consented to the writer contacting you for the purpose of obtaining an expert report from you as described in our letter. It is unclear at the present time whether he will continue to be the Applicant's counsel moving forward but this is expected to be known shortly. The parties attended a Case Management Conference today before Mdm. Justice Ring of the Federal Court of Canada. The hearing for final argument is set for April 15, 2025 in Federal Court in Vancouver to determine whether or not CFIA will be permitted to kill all of the surviving ostriches. We are hoping to obtain an affidavit from you in the next few days that we can attach to a motion to the court, seeking an order from the court permitting the Applicant to perform

whatever testing you recommend so that you can provide an expert opinion before April 15, 2025 concerning the results of that testing, that we could provide to the Attorney General and CFIA and the court for their consideration.

Accordingly, in addition to the opinion requested above, could you kindly provide us with your opinion concerning the following:

1. is there a test that could be performed either on the ostriches themselves or their eggs that can be performed safely by the farmers themselves on the farm, that would pose no risk of infection or transmission to other wildlife or humans, such that these test samples could then be provided to you so that you could analyse in your laboratory to determine whether or not these birds have been exposed H5N1, have developed antibodies to H5N1, whether they are immune to H5N1, and whether they pose any risk of spreading infection of H5N1 to other wildlife or humans?

2. If the answer to question 1 is yes, can you please describe test that could be done, how that sample can be safely transported to you, and what that test would tell us?

3. Please explain why the test is safe for the farmers to perform, why it is safe to transport to you, so that the reader of your report understands clearly why the test and transporting its results to you for testing would present no risk to the public or other wildlife;

4. if the test results demonstrate that the surviving ostriches have full antibodies to the strain of H5N1 that was detected by CFIA through their own testing either at the Abbotsford Lab or the Winnipeg lab or otherwise, please explain what the significance of that is with respect to the risk of transmission of the virus to other wildlife or humans and what the merits of culling the ostriches would be as a result.

Please carefully read the instructions below regarding the preparation of your report.

When you write your report, please remember that you are essentially taking on the role of a teacher in the area of your expertise. While the details of your analysis and opinion are important, what is equally if not more important is what those details mean. The reader of your report will include members of the Counsel for the Attorney General and the CFIA, the Federal Court, members of the public, and other experts who may be informed by your opinion and analysis. They need to understand your opinion and the basis for it. If you must use technical terms from your area of specialty, please define them as simply as possible in words that everyone can understand. If you are able to use diagrams, photographs, video, models or other demonstrative aids to help the reader understand your report, we encourage you to do so.

We ask that you set out in your report the following information:

1. your name, address and area of expertise (you may attach a CV as an appendix to your report). It should be clear within your CV or your description of your area of expertise, that you are qualified to offer the opinion in the areas set out above;

2. your qualifications and employment and educational experience within your area of expertise that is relevant to the opinions requested;

3. instructions that we have provided to you in relation to the proceeding, for which you can reference and attach a copy of this letter to your report if you wish;

4. the nature of the opinion being sought and the issues in the proceeding to which the opinion relates;

5. your opinion respecting those issues; your reasons for your opinion, including:

   (a) a description of the factual assumptions on which your opinion is based,

   (b) a description of any research conducted by you that led you to form your opinion, and a list of every document, if any, relied on by you in forming your opinion.

In your report, please also certify that you:

- are aware of your duty to assist the Court;
- are not an advocate for any party to me;
- have prepared your report in conformity with your duty, and
- will, if called upon to give oral or written testimony, give that testimony in conformity with your duty.

Please be sure to retain all notes and file contents pertaining to the provision of your opinion, whether digital or written, and these should be made available for production to opposing counsel at or before the hearing.

Your opinion, and the reasons for your opinion, should be expressed in the simplest of terms, bearing in mind that the challenge an expert witness faces is to make their evidence easily understood.

Please ensure that your report contains the appropriate headings if necessary, page numbers and paragraph numbers for ease of reference to specific portions of your report. While it is preferred that your report be concise, if a lengthy report is required, please include an index for the report.

Thank you for your willingness to assist on such short notice. If you need anything further please advise.

Yours truly,

DOAK SHIRREFF LAWYERS LLP

Per: 

Lee C. Turner
(Professional Law Corporation)

LCT/lct

Exhibit B

Page 213 of the filed affidavit for Court File No. T-432-25 in Federal Court between Universal Ostrich Farms Inc. (Applicant) and Canadian Food Inspection Agency (Respondent) entitled:

"Rule 318 Certified Tribunal Record of the Canadian Food Inspection Agency" filed by Cortnie Fotheringham, Incident Commander, Western HPAI Response. Dated February 22, 2025. This corresponds to internal communications listed under Tab 6D as a January 4, 2024, e-mail from CFIA AI Lab Liaison to CFIA AI Commander re Ostrich sample results.

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From:	West AI Lab Liaison / Ouest IA Liaison de laboratoire (CFIA/ACIA)
To:	West AI Commander / Ouest IA Commandant (CFIA/ACIA)
Subject:	Ostrich sample results
Sent:	1/4/2025 11:16:06 AM

Ostrich sample results from NCFAD, just in case you need this info over the weekend
Thanks
Nicki
WIN-AH-2025-FAV-0003
System ID#: 2025DCS-0000000033-4
Reason for submission: lab referral for confirmatory testing for HPAI, ref# 24-10316 (but being sent directly from the premise)
Owner: Universal Ostrich
Location: Edgewood
Submitter: Nicole Conner
Dist office: (2840) Vernon
Species: ostrich
Samples: 2 swabs
Test: AIV-PCRMX
History: Ostrich farm. Tested positive at BCMAL, duplicate samples being sent from the Vernon District office Michelle Li will be sending out the other set of samples from AHL on Monday with other positives as she is worried about the issues we have been having with shipping.
NCFAD Results 2025-01-03:
AVIN-PCRMX: Nucleic acid was extracted from the 2 swab samples and tested for the presence of influenza A genomic material using the matrix, H5 and H7 gene-based real-time RT-PCR assays. 2/2 swab samples tested **positive** on the matrix and on the **H5 gene**-based real-time RT-PCR assays, 2/2 samples were **negative on the H7** real-time RT-PCR assay. 2/2 swab samples tested **positive on the clade-specific** H5 2.3.4.4 real-time RT-PCR assay.
NCFAD Results 2025-01-04
Molecular pathotyping: The entire genome of the virus was amplified from two original swab samples and submitted to our Genomics group for NANOPORE sequencing. Eight gene segments of the virus were sequenced. The HA of the virus from the samples belong to **Eurasian Gs/GD lineage HPAI H5N1 (2.3.4.4B)** with cleavage site motif of **"PLREKRRKR/GLF"** compatible with HPAI viruses that came to Canada via the **Pacific flyway**. The H5N1 virus is a reassortant virus with **PB2, PA, and NP** originated from North American lineage low pathogenic avian influenza virus, and **PB1, HA, NA, M and NS gene segments from Eurasian viruses.** The virus is similar to the D1.1 viruses circulating in North America, but has the neuraminidase segment identical to WIN-AH-2022-OTH-0033 virus. PB2 – 627E (avian).