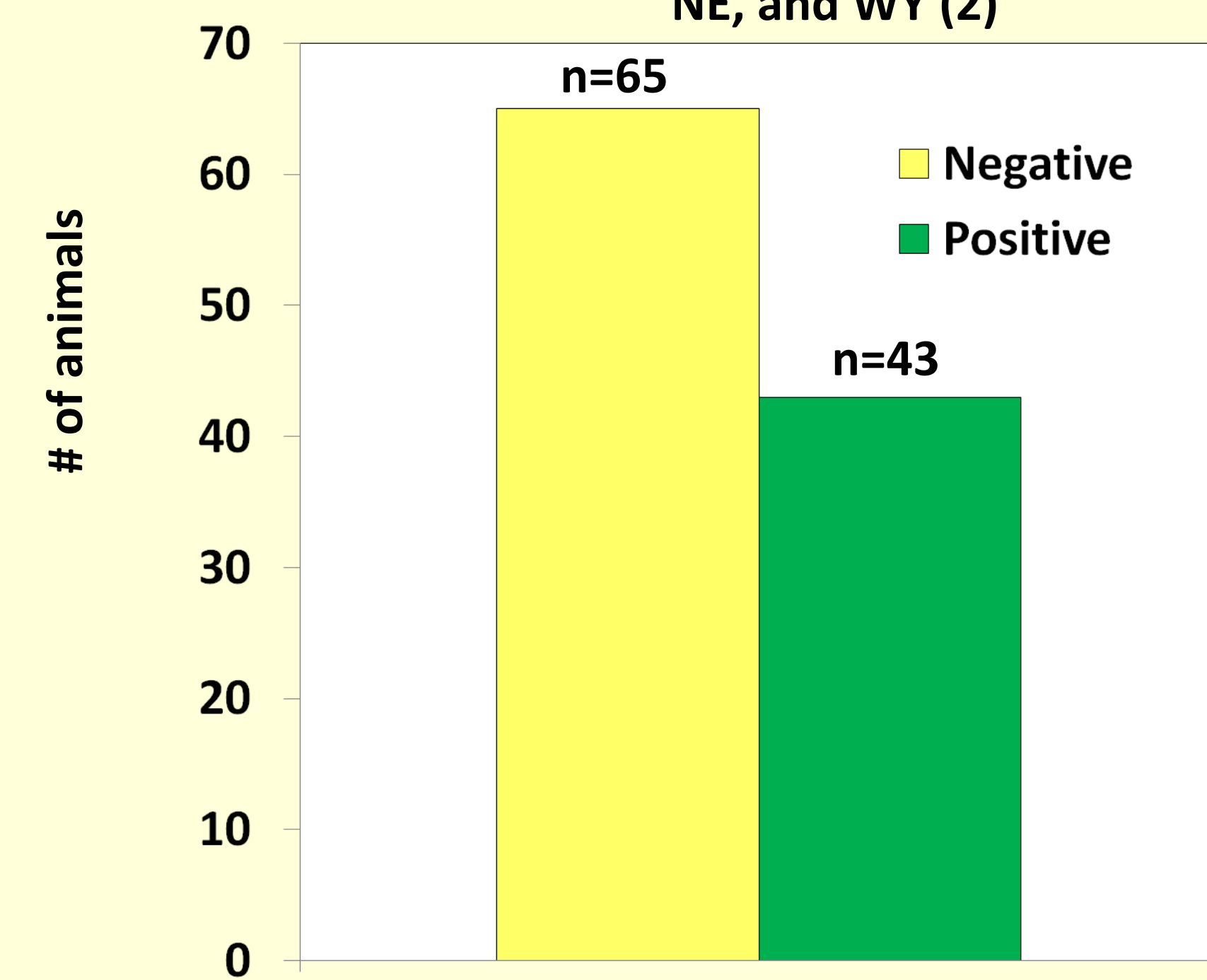


Background

Historically, dogs were not regarded as hosts for Influenza A virus. However, in 2004, an outbreak of respiratory disease in racing greyhounds in Florida provided evidence of transmission and maintenance of an influenza virus closely related to contemporary equine H3N8 influenza viruses (1). Since first isolated, canine influenza virus (CIV) has spread widely throughout dog populations in the United States. Recent studies performed in our laboratory indicate that CIV is an important pathogen in dogs housed in humane shelters in Colorado and adjacent states.

CIV positive nasal-swab samples in dogs with clinical signs of kennel cough in CO, NE, and WY (2)



As high titers of CIV are shed in respiratory secretions, dogs housed in groups such as boarding facilities, shelters, etc. appear to be more commonly affected. In contrast, little is known about the significance of CIV household animals. Our research aims to determine the seroprevalence of CIV in Colorado pet dogs and to identify risk factors that may predispose household dogs to CIV infection. Based on the demonstration that CIV is an important pathogen in dogs with clinical signs of kennel cough in Colorado, we hypothesize that there has been an increase in CIV seropositivity in household dogs since the emergence of the pathogen in 2004. Furthermore, we propose that because known risk factors for influenza exist in other species, similar predisposing factors will exist for CIV infection in pet dogs. To test these hypotheses, serum samples were collected from dogs being seen at the Veterinary Medical Center at Colorado State University. To identify risk factors for CIV infection, owners completed questionnaires at the time of sample collection. Levels of antibodies in sera were determined via hemagglutination inhibition assays. Three genetically distinct viruses were used to account for any potential antigenic drift that may have occurred since 2004: A/Ca/Florida/43/04, A/Ca/Fort Collins/224986/06, and A/Ca/Colorado Springs/234550/09. Additionally, banked serum samples from dogs seen at the Veterinary Medical Center prior to 2004 were assayed to evaluate presence of influenza in dogs before it's first isolation.

Research Aims

To test our hypothesis, we have set the following aims:

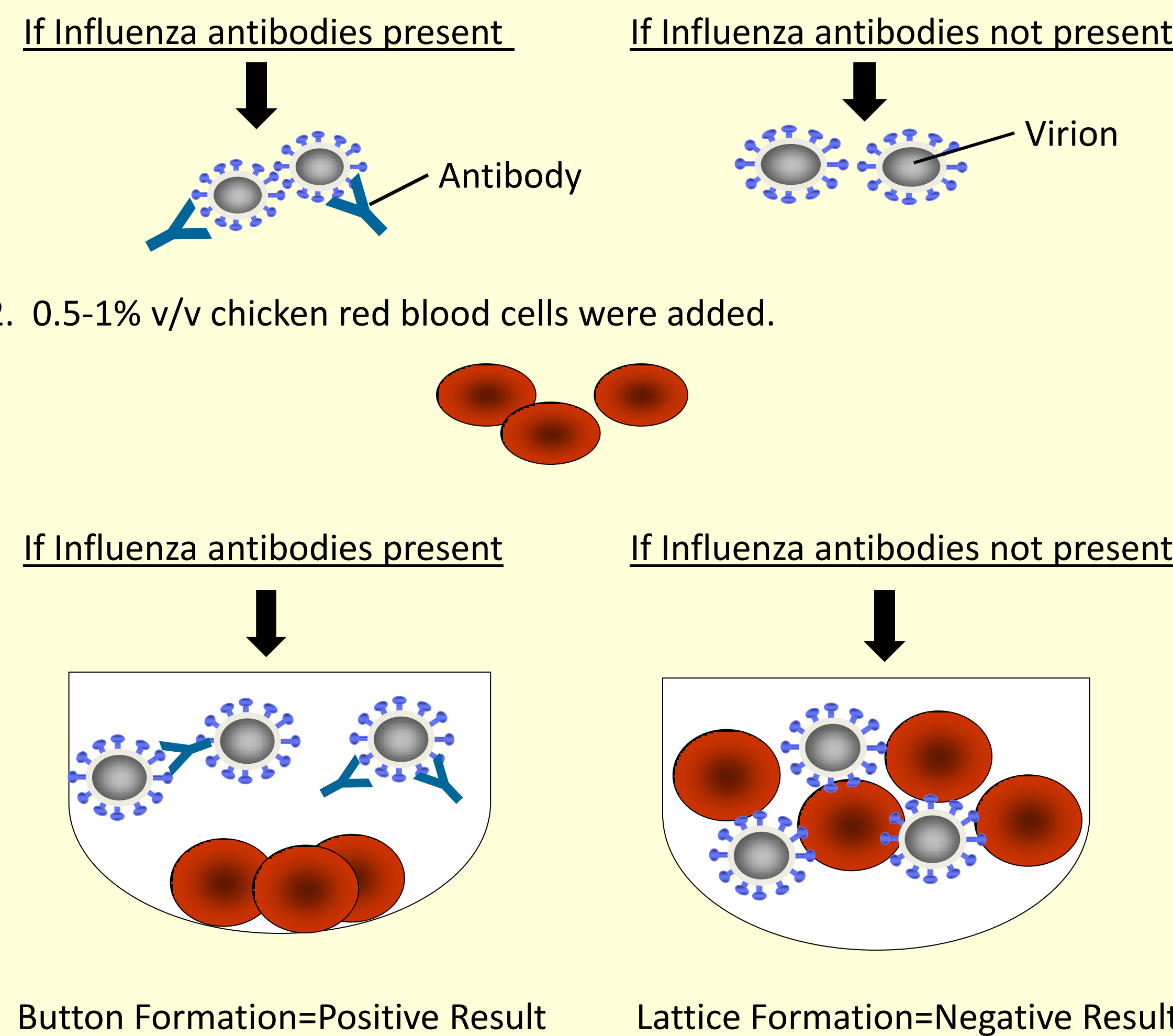
Aim 1: To determine the seroprevalence of CIV in household dogs in Colorado, both prior to and following 2004.

Aim 2: To identify risk factors for CIV seropositivity in household dogs in Colorado.

Materials and Methods

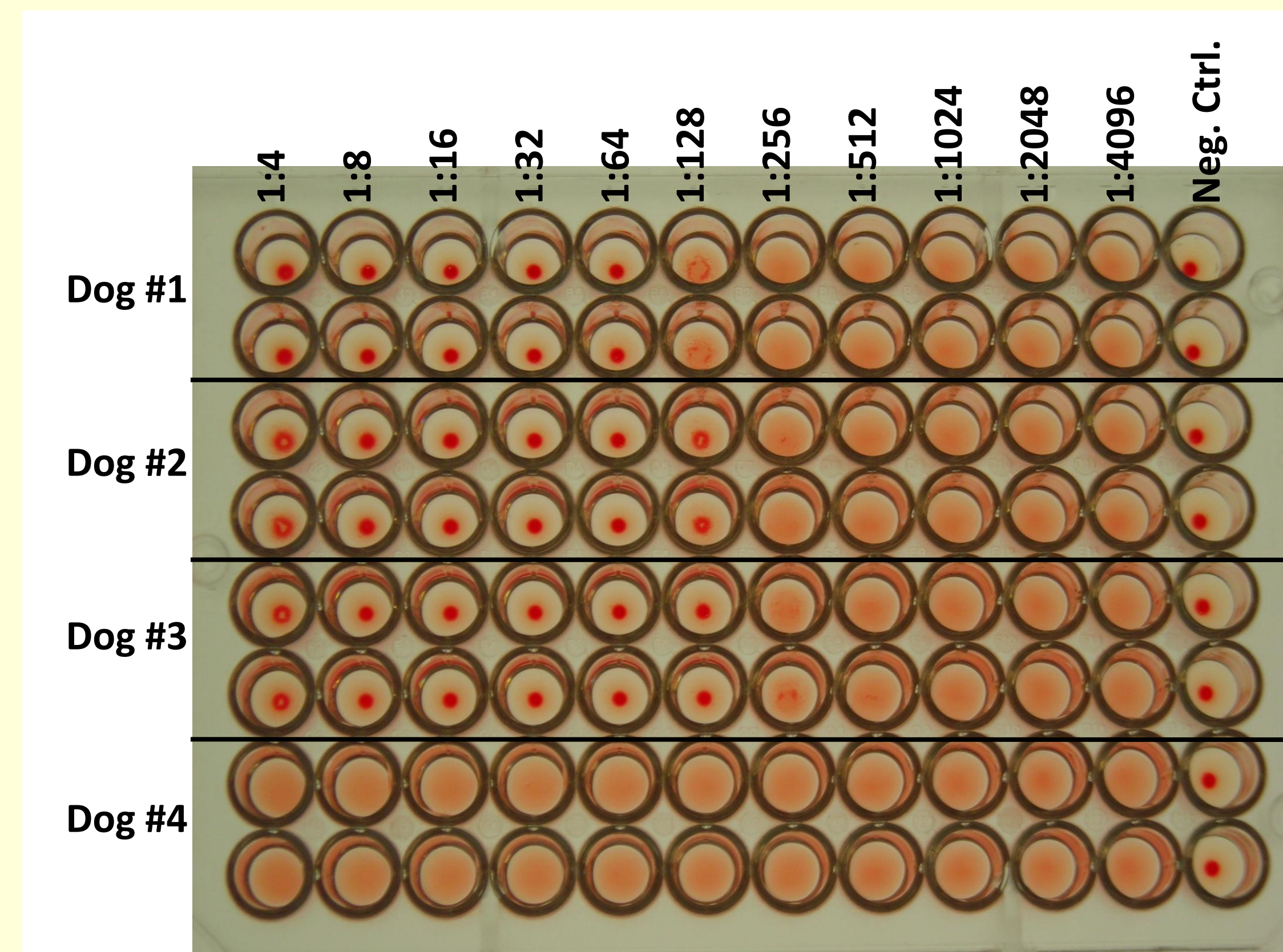
Aim 1: To determine CIV seroprevalence in household dogs in Colorado

- Serum samples were treated with Receptor Destroying Enzyme (RDE), which was later heat-inactivated. Serial dilutions of serum were then combined with one of three canine influenza virus isolates: A/Ca/Florida/43/04, A/Ca/Fort Collins/224986/06, and A/Ca/Colorado Springs/234550/09.



- Seropositivity was defined as an HI titer of $\geq 1:8$. Each serum sample was run in duplicate and the assay was repeated to ensure accuracy.

Hemagglutination Inhibition Assay Results



Dog #1 has an HI antibody titer of 1:64. Dogs #2 and 3 both have an HI antibody titer of 1:128. Dog #4 has a negative HI antibody titer. Well #12 was left as a negative control for all rows.

Materials and Methods (cont.)

Aim 2: To identify risk factors for infection of household dogs with CIV

A questionnaire was distributed to owners to address behaviors and practices that may predispose dogs to infection. These questions covered:



Background on the dog supplying the sample.



Regular veterinary and health care.



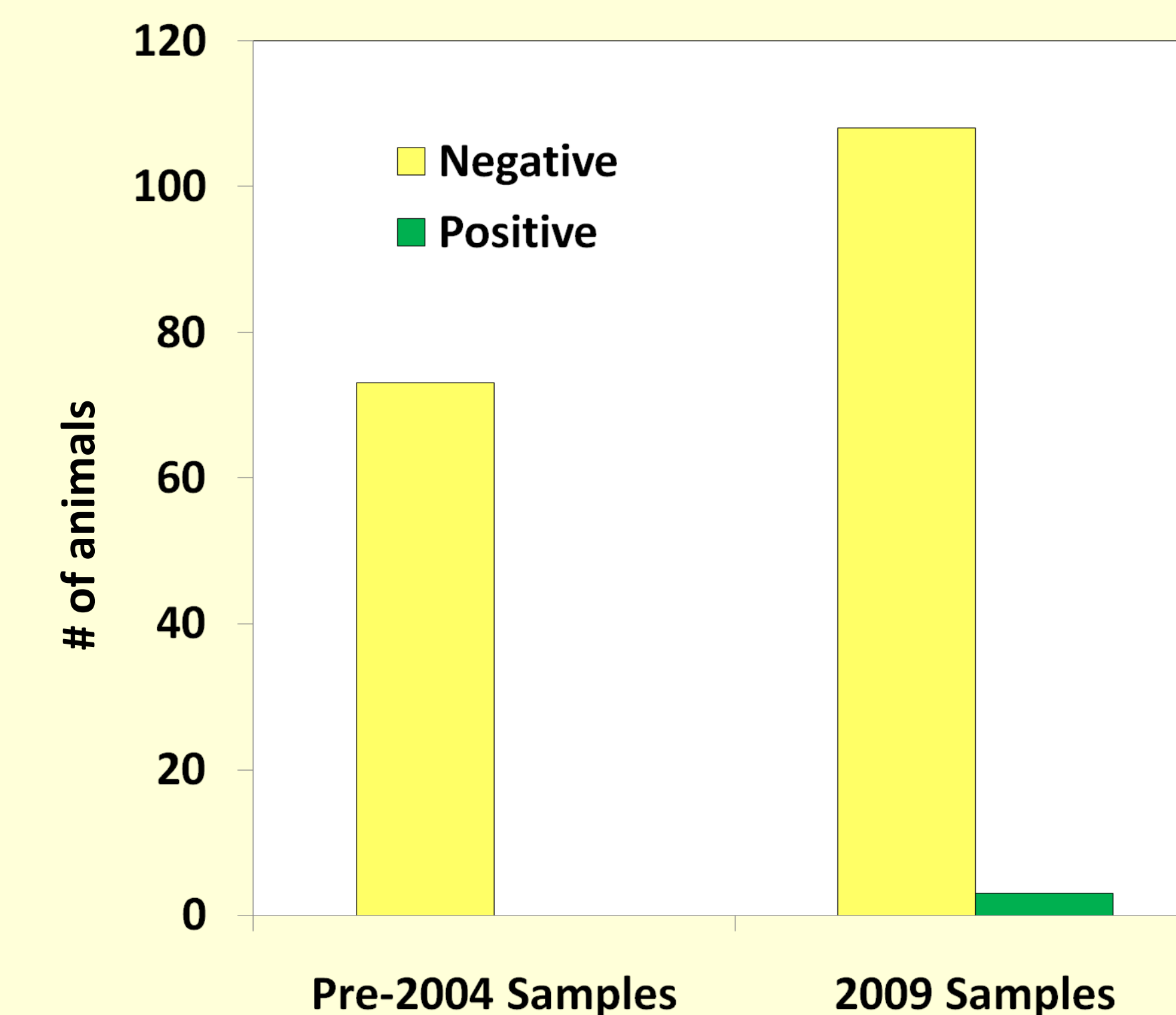
Other dogs in the household with whom that dog has contact.



Social interactions that dog has with other animals.

Results

At this time, 111 of the proposed 250 household dogs have been sampled. Of those 111 dogs, three were positive for CIV using the HI assay. This yields a seroprevalence of 2.7%. No differences were seen in titers between the three different influenza viruses. Additionally, HI has also been performed on 73 serum samples collected from dogs seen at the Veterinary Medical Center prior to 2004. Of those 73 samples, none were positive for CIV using any of the three influenza isolates.



Summary and Conclusions

Based on our results, we have come to the following conclusions:

- Seroprevalence of CIV is low (<5%) in the Colorado household dog population, although it has increased since CIV was first isolated in 2004.
- As no differences were detected in the titers of the three virus isolates, antigenic drift has likely not occurred in the canine influenza lineage.
- Due to the small number of seropositive dogs, we were unable to identify risk factors predisposing dogs to CIV at this time.

References and Acknowledgements

- Crawford, P.C., Dubovi, E.J., Castleman, W.L., Stephenson, I., Gibbs, E.P., Chen, L., Smith, C., Hill, R.C., Ferro, P., Pompey, J., Bright, R.A., Medina, M.J., Johnson, C.M., Olsen, C.W., Cox, N.J., Klimov, A.I., Katz, J.M., Donis, R.O. 2007. Transmission of equine influenza virus to dogs. *Science* 310(5747): 482-485.
- Spindel, M.E., Lunn, K.F., Dillion, S., Landolt, G.A. 2007. Detection and quantification of canine influenza virus by one-step real-time reverse transcription PCR. *Journal of Veterinary Internal Medicine* 21(3): 576.