West Nile Virus and Influenza (H1N1) are known to cause deaths and have the potential to evolve into more pathogenic strains, like the deadly Spanish Flu, posing a severe threat to military and civilians. This project is developing a unique approach using avian therapeutic antibodies to treat emerging viral diseases such as West Nile Virus and H1N1 flu. This approach has the potential of a faster development time, rapid scalability, broader protection, and higher survival rates.

When a vaccine is not available or exposure has occurred, polyepitope therapeutic antbodies are the only possible source of reducing mortality in humans. With these emerging viruses, it is imperative to develop alternative strategy that provides a novel approach for the detection and treatment using therapeutic antibodies of those infected to prevent widespread deaths. A key element for protection is that avian therapeutic polyclonal antibodies offer the potential of broad spectrum strain efficacy. Goose therapeutic antibodies are targeted for faster development because, unlike mammalian based antibodies, avian antibodies do not require humanization for use in humans. Early studies have shown the protective potential of the goose therapeutic antibodies against West Nile Virus in large population studies.

During the current period the following key objectives were achieved:

1. Demonstration of high titer antibody production by geese following immunization with inactived H1N1 virus.

All work until this point in this project has utilized antibodies from geese naturally infected with West Nile Virus. Geese surviving West Nile Viruses infection were logically expected to produce high titer West Nile Virus specific antibodies, as the goose is a natural reservoir of West Nile Virus. There was not, however, evidence thus far as to the kinetics of goose antibody production in response to artificial immunization with a target killed virus.

Geese were immunized with an initial challenge, and 2 boosts of formalin-fixed H1N1 virus I.M. in the appropriate adjuvant. Sera were tested at 2 week intervals following the primary immunization via microtiter neutralization assay and hemaggultination assay for the presence of H1N1 neutralizing antibodies. An IgY response was detectable within 4 weeks of the initial immunization with very high titers by 6 weeks, > 1:128,000. These results were replicated.

These findings were particular interesting as it indicates that geese are capable of producing very high titer of virus-specific antibodies in a very short period of time. Theoretically, goose could be used to produce therapeutic or diagnostic antibodies specific for an emerging virus within 2 months of the identification of a newly emerged viral pathogen.

2. Completion of the epitope mapping of West Nile Virus-specific goose antibodies and initiation of epitope mapping of H1N1 flu-specific goose antibodies.

Other laboratories have demonstrated a protective West Nile Virus epitope located on the viral envelope (Diamond *et al.*) Monoclonal therapeutic antibody preparation a dependent on the conservation of that epitope through time, and it is necessary that binding of the single epitope is capable of neutralizing the virus. Therefore, polyclonal antibody preparations, such as those utilized in this project have the potential of being reactivity to numerous epitopes on the target virus. To determine the specificity of the West Nile Virus-specific goose antibodies, epitope recognition was screened utilizing PepSpot assays. The protective envelop epitope was recognized by the goose antibody preparation, as were several epitopes on the structural outer viral proteins. Interestingly, the strongest response was directed at epitopes present on non-structural proteins. (This work was presented at the annual meeting of the American Association of Immunologists, Baltimore, MD.)

To determine the protective ability of the major epitopes identified, corresponding peptides were generated, and epitope specific antibodies were purified and are currently being assayed via microtiter neutralization and hemaggultination assays.

3. Advancement in scalable purification of goose antibodies.

The efficacy and specificity studies continue to strongly support the potential of goose antibodies as both therapeutic and diagnostic tools. Antibodies could potentially be harvest from both goose sera and goose eggs, with technical advantages for both sources. The greatest potential problem in utilizing antibodies purified from eggs is the very high lipid content in goose eggs, compared to other avian sources. Significant effort was put into developing a protocol that was scalable for purifying antibodies from the high lipid content. At the same time protocols were being developed to purify the same antibodies from goose sera. At was determined that the sera were the desirable source as high titers and sufficient quantities could be purified from this source for either use.

Interestingly, avian antibodies were also demonstrated to have significant diversity in reactivity to Protein A purification techniques, i.e. subpopulations of WNV-specific and H1N1-specific antibodies could be identified that bound to, or did not bind to, Protein A columns. Each population has been analyzed via HPLC and gel electrophoresis for other potential biochemical differences. This characterization is still ongoing.

These advancements are critical in the progress of this project to be able to develop and purify high titer goose antibodies to be used for both therapeutic and diagnostics applications. They were necessary to be completed for the 2010 period objectives to be undertaken, including:

1. Test the diagnostic capabilities of the goose antibodies against different viral WNV strains.
2. Determine by epitope mapping, the target epitope binding sites of WNV antibodies.
3. Evaluate the efficacy of goose therapeutic epitope specific antibodies and polyclonal goose antibodies on the survival rate of animals prior to infection or infected with the WNV.

4. Develop and quantify assays to ensure production quality and conformance of goose antibodies.
5. Scaled production of purified goose anti-WNV antibodies produced under SPF conditions.