SEPRL NC1180 Station Report

Project: Control of emerging and re-emerging poultry respiratory diseases in the united states

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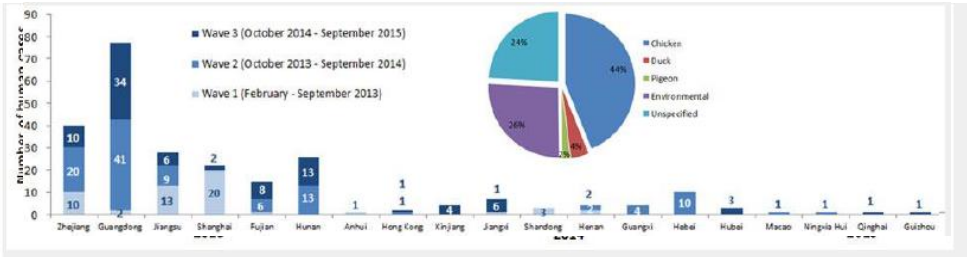
Projects addressing objective 1:

Identify reservoirs of infectious respiratory disease agents in wild birds and poultry

Studying the pathogenesis of H7N9 low pathogenicity avian influenza virus in chickens

681 human cases
271 deaths



- We evaluated the infectious dose and pathogenesis of H7N9 viruses in White Leghorns (table-egg layers) and White Plymouth Rocks (meat chickens).
- No morbidity or mortality were observed with doses of 10^6 or 10^8 EID_{50}/bird when administered intranasally, and the mean infectious dose (10^6 EID_{50}) was higher than expected, suggesting that the virus is poorly adapted to chickens.
- **IMPACT:** For chickens to serve as a primary reservoir for the H7N9 lineage we would expect for the infectious dose to be lower. Therefore the spread of the H7N9 virus by chickens, at least early on (as the virus has continued to circulate it may have changed), may not have been efficient.

Spackman, E., Pantin Jackwood, M.J., Swayne, D.E., Suarez, D.L., Kapczynski, D.R. 2015. Impact of route of exposure and challenge dose on the pathogenesis of H7N9 low pathogenicity avian influenza virus in chickens. Virology. 477:72-81.

Identification of new sub-genotypes of virulent Newcastle disease virus with potential panzootic features

- Virulent NDV isolates from new sub-genotypes within genotype VII are rapidly spreading through Asia and the Middle East causing outbreaks of Newcastle disease characterized by significant illness and mortality in poultry.
- Viruses from sub-genotype VIIh were isolated in Indonesia (2009-2010), Malaysia (2011), China (2011), and Cambodia (2011-2012) and are closely related to the Indonesian NDV isolated in 2007, APMV1/Chicken/Karangasem
- Since 2011 and during 2012, highly related NDV isolates from sub-genotype VIIi have been isolated from poultry and occasionally pet birds, throughout Indonesia, Pakistan and Israel.
- **IMPACT:** The co-evolution of at least three different sub-genotypes reported here and the apparent close relationship of some of those genotypes from ND viruses isolated from wild birds, suggests that identifying wild life reservoirs may help predict new panzootics.

Miller, P.J., Haddas, R., Simanov, L., Lublin, A., Rehmani, S.F., Wajid, A., Bibi, T., Khan, T.A., Yaqub, T., Setiyaningsih, S., Afonso, C.L. 2015. Identification of new sub-genotypes of virulent Newcastle disease virus with potential panzootic features. Infection, Genetics and Evolution. 29:216-229.

Projects addressing objective 3:

Investigate the pathogenesis and polymicrobial interactions of specific infectious agents associated with poultry respiratory diseases

Previous infection with virulent strains of NDV reduces HPAI virus replication, disease, and mortality in chickens

- Objective: Determine if co-infection with NDV affects HPAIV replication in chickens.
- Only infections with virulent NDV strains (mesogenic Pigeon/1984 or velogenic CA/2002), and not a lentogenic NDV strain (LaSota), interfered with the replication of HPAIV A/ck/Queretaro//95 (H5N2) when given at a high dose ($10^{6.9}$ EID_{50}) two days after the NDV inoculation, but mortality was still observed.
- Chickens infected with the mNDV strain three days prior to being infected with a lower dose ($10^{5.3\text{-}5.5}$ EID_{50}) of the same or a different HPAIV, A/ck/Jalisco/2/2012 (H7N3), had reduced HPAIV replication and increased survival rates.
- **Conclusion:** previous infection of chickens with virulent NDV strains can reduce HPAIV replication, and consequently affect disease and mortality. This interference depends on the titer of the viruses used, the virulence of the NDV, and the timing of the infections.
- **IMPACT:** The information obtained from these studies helps to understand the possible interactions and outcomes of infection (disease and virus shedding) when HPAIV and NDV co-infect chickens in the field.

Costa-Hurtado M, Afonso C, Miller P, Shepherd E, Cha R, Smith D, Spackman E, Kapczynski D, Suarez D, Swayne D, Pantin-Jackwood M. Previous infection with virulent strains of NDVreduces highly pathogenic avian influenza virus replication, disease, and mortality in chickens. Vet Res. 2015 Sep 23;46(1):97.

Experimental coinfection of domestic ducks with a virulent Newcastle disease virus and low or highly pathogenic avian influenza viruses

- Infections with AIV of low and high pathogenicity and NDV are commonly reported in domestic ducks, but it is not clear if co-infections with these viruses affect the severity of the diseases they produce, virus shedding and transmission.
- Pekin ducks were inoculated with a virulent NDV virus (vNDV) and either a LPAIV or a HPAIV by giving the viruses individually, simultaneously, or sequentially two days apart.
- No clinical signs were observed in ducks infected or co-infected with vNDV and LPAIV, but co-infection decreased the number of ducks shedding vNDV and the amount of virus shed ($P<0.01$) at 4 days post inoculation (dpi).
- Co-infection did not affect the number of birds shedding LPAIV, but more LPAIV was shed at 2 dpi ($P<0.0001$) from ducks inoculated with only LPAIV compared to ducks co-infected with vNDV.
- Ducks that received the HPAIV with the vNDV died earlier than ducks that received the vNDV two days before the HPAIV.
- In conclusion, domestic ducks can become co-infected with vNDV and AIV with no effect on clinical signs but with reduction of virus shedding and/or transmission.
- **IMPACT:** These findings indicate that infection with one virus can interfere with replication of another, modifying the pathogenesis and transmission of the viruses.

Pantin Jackwood, M.J., Costa-Hurtado, M., Miller, P.J., Afonso, C.L., Spackman, E., Kapczynski, D.R., Shepherd, E.M., Smith, D.M., Swayne, D.E. 2015. Experimental coinfections of domestic ducks with a virulent Newcastle disease virus and low or highly pathogenic avian influenza viruses. Vet.Microbiology. 177:7-17.

Projects addressing objective 4:

Develop new prevention and control strategies for poultry respiratory diseases

Expression of H5 hemagglutinin vaccine antigen in common duckweed (Lemna minor) protects against H5N1 high pathogenicity avian influenza virus challenge in immunized chickens

- A synthetic hemagglutinin (HA) gene from A/ck/Indonesia/7/2003 (HPAI H5N1) was expressed in aquatic plant Lemna minor (rLemna-HA)
- Efficacy of rLemna-HA was tested on birds immunized with 0.2 or 2.3 µg HA and challenged the homologous virus strain. Both dosages conferred clinical protection and dramatically reduced viral shedding. Almost all the birds had HA antibody titers against Indo/03 antigen
- Efficacy of rLemna-HA was also tested on birds immunized with 0.9 µg or 2.2 µg HA and challenged heterologous H5N1 virus strains A/ck/Vietnam/NCVD-421/2010 or A/ck/West Java/PWT-WIJ/2006. Birds challenged with VN/10 exhibited 100% survival regardless of immunization dosage, while birds challenged with PWT/06 had 50% and 30% mortality at 0.9 µg HA and 2.2 µg HA, respectively. Viral shedding titers from 2.2 µg HA vaccinated birds were significantly lower than those from 0.9µg HA vaccinated birds.
- In conclusion, Lemna-expressed HA demonstrated complete protective immunity against homologous challenge and suboptimal protection against heterologous challenge, the latter being similar to results from inactivated whole virus vaccines.

Bertran, K., Thomas, C., Guo, X., Bublot, M., Pritchard, N., Regan, J.T., Cox, K.M., Gasdaska, J.R., Dickey, L.F., Kapczynski, D.R., Swayne, D.E. 2015. Expression of H5 hemagglutinin vaccine antigen in common duckweed (Lemna minor) protects against H5N1 high pathogenicity avian influenza virus challenge in immunized chickens. Vaccine. 33(30):3456-3462.

Vaccine protection of chickens against antigenically diverse H5 HPAI isolates with a live HVT vector vaccine expressing the influenza hemagglutinin gene derived from a clade 2.2 avian influenza virus

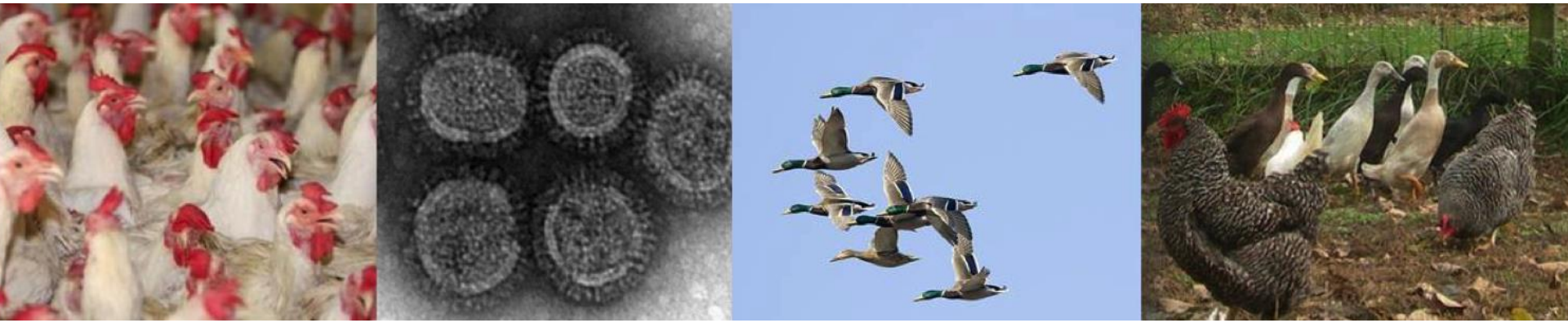
- Protection of chickens provided by a turkey herpesvirus (HVT) vector vaccine expressing the HA gene from a clade 2.2 H5N1 strain (A/swan/Hungary/4999/2006) against homologous H5N1 as well as heterologous H5N1 and H5N2 HPAI challenge was evaluated.
- All vaccinated birds were protected from clinical signs and mortality following homologous challenge and vaccinated birds had lower titers of viral shedding compared to sham-vaccinated birds. Following heterologous H5N1 or H5N2 HPAI challenge, 80-95% of birds receiving the HVT vector AI vaccine at day of age survived challenge with fewer birds shedding virus after challenge than sham vaccinated birds.
- In vitro cytotoxicity analysis demonstrated that splenic T lymphocytes from HVT-vector-AI vaccinated chickens recognized MHC-matched target cells infected with H5, as well as H6, H7, or H9 AI virus.
- **IMPACT:** These studies provide support for the use of HVT vector vaccines expressing HA to protect poultry against multiple lineages of HPAI, and that both humoral and cellular immunity induced by live vaccines likely contributes to protection.

Kapczynski D.R., Esaki, M., Dorsey, K.M., Jiang, H., Jackwood, M, Moraes, M., Gardin, Y. 2015. Vaccine protection of chickens against antigenically diverse H5 highly pathogenic avian influenza isolates with a live HVT vector vaccine expressing the influenza hemagglutinin gene derived from a clade 2.2 avian influenza virus. Vaccine. 2015 Feb 25;33(9):1197-205

Development of an improved vaccine evaluation protocol to compare the efficacy of Newcastle disease vaccines

- While there is typically 100% survivability in birds challenged with vNDV under experimental conditions, either with vaccines formulated with a strain homologous or heterologous (different genotype) to the challenge virus, vaccine deficiencies are often noted in the field.
- An improved and more stringent protocol to experimentally evaluate live NDV vaccines was developed showing that a statistically significant reduction in mortality can be detected with genotype matched vaccines.
- Using traditional and improved vaccine evaluation protocols, birds were challenged with vNDV and the efficacy of live heterologous and homologous NDV vaccines was compared.
- Under traditional vaccination conditions there were no differences in survival, but the homologous vaccine induced significantly higher levels of antibodies specific to the challenge virus. With the more stringent challenge system (multiple vaccine doses and early challenge with high titers of vNDV), the birds given the homologous vaccine had superior humoral responses, reduced clinical signs, and reduced mortality levels than those vaccinated with the heterologous vaccine.
- IMPACT: These results provide basis for the implementation of more sensitive methods to evaluate vaccine efficacy.

Cardenas-Garcia, S., Diel, D., Susta, L., Lucio-Decanini, E., Yu, Q., Brown, C.C., Miller, P.J., Afonso, C.L. 2015. Development of an improved vaccine evaluation protocol to compare the efficacy of Newcastle disease vaccines. Biologicals. 46:136-145.



Pathogenicity and transmission of Eurasian HPAI H5 clade 2.3.4.4 viruses in avian species

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USDA Agricultural Research Service

Studying the North American H5 HPAI viruses

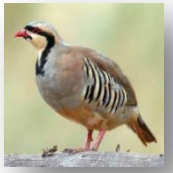
- Initial experiments used the earliest USA isolates:
  - A/Gyrfalcon/Washington/41088/2014 (H5N8)
  - A/Northern Pintail/Washington/40964/2014 (H5N2)

More recent isolates tested to evaluate how the viruses are changing

- Pathogenesis studies
- Susceptibility studies - Infectious dose and transmission
  - SPF White Leghorn chickens
  - Commercial broad-breasted white turkeys
  - Commercial Japanese quail, pheasants, partridges, guinea fowl
  - Commercial Pekin ducks and geese
  - Captive reared Mallards









Infectious dose and transmission - H5N2 and H5N8 index viruses

Species	% Mortality	MDT (days)	BID_{50} (log10)	Transmission to contacts
Chickens *(Gallus Gallus)*	60-100	3 - 4	4.3-5.7	No or only in 10^6 groups
Turkeys (*Meleagris gallopavo*)	100	5.3 - 9	5	Only in 10^6 groups
Japanese Quail (*Coturnix japonica)*	80	2.5 - 3	3.0 - 3.6	Only H5N2 in 10^6 group
Pheasants (*Phasianus colchicus*)	100	4.7 - 4.8	3.0 - 3.4	Yes, in 10^4 and 10^6 groups
Partridge (*Alectoris chukar)*	100	4.1 - 5.2	3.6	Yes, in 10^4 and 10^6 groups
Guineafowl (*Numida meleagris*)	100	2.0	Pending	Pending
Pekin ducks *(Anas platyrrhynchos var. dom.)*	0	-	3	Yes, in 10^4 and 10^6 groups
White Chinese Geese (*Anser cygnoides*)	25	7-7.5	≤ 2 -3.0	Yes, in 10^4 and 10^6 groups
Mallards (*Anas platyrrhynchos*)	0	-	≤ 2	Yes, in all groups

Infectious dose - 2015 H5N2 HPAI poultry viruses

Virus	Species	% Mortality	MDT (days)	BID50 (log10)
A/Tk/Minnesota/12582/2015	Chickens	100	2.0	3.6
A/Ck/Iowa/13388/2015	Chickens	100	2.6	3.5
A/Tk/South Dakota/12511/2015	Chickens	100	2.2	3..2
A/Tk/Arkansas/7791/2015	Chickens	88	2.3	5.1

Virus	Species	% Mortality	MDT (days)	BID50 (log10)
A/Tk/Minnesota/12582/2015	Mallards	12	9	≤ 2
A/Ck/Iowa/13388/2015	Mallards	0	-	≤ 2

Summary

- The two early H5 HPAIV strains were not well adapted to gallinaceous poultry
  - BID_{50}:100-1,000 times higher than previous H5N1 HPAIVs
  - Inefficient transmission to contacts except when placed with high challenge dose group
- Longer time to death than historic HPAIV
- Early viruses well adapted to mallards
- Susceptibility to infection:

Mallards > Pheasants, Partridges, GF, Pekin, geese > Quail > Turkeys > Chickens

- More recent H5N2 viruses are more adapted to chickens