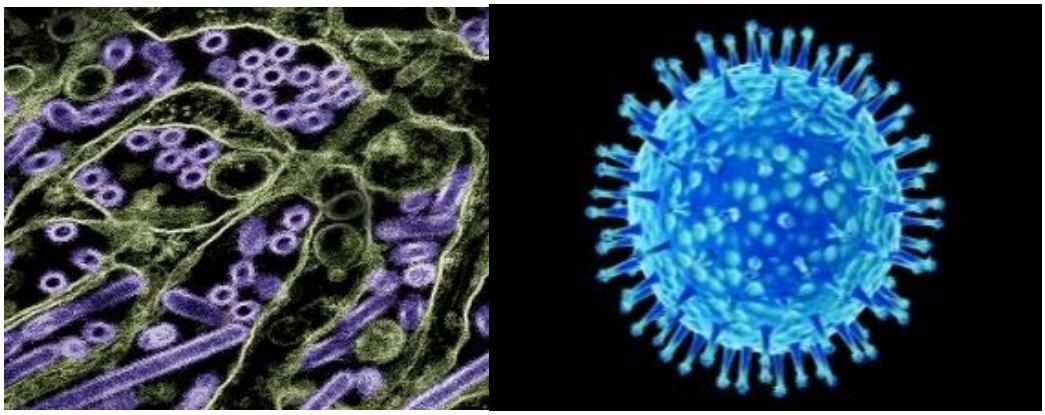


H5N1 Commissioned Testing Summary

Testing for **avian influenza (H5N1)** presents multiple scientific, logistical, and regulatory challenges. These challenges affect both **animal health monitoring** and **human public health preparedness**. Here's a breakdown of key difficulties:

1. Viral Mutation and Evolution

- **Rapid mutation:** Influenza viruses, including H5N1, mutate quickly (antigenic drift). This can make existing diagnostic tests less accurate over time.
- **Reassortment:** H5N1 can mix genetic material with other influenza strains, creating new variants that escape detection by older assays.
- **Need for constant updates:** PCR primers and probes used in molecular diagnostics must be frequently revised to match circulating strains.

2. Sampling and Biosafety Constraints

- **High biosafety level:** Confirmatory testing requires **BSL-3** laboratory conditions (due to H5N1's pathogenicity), limiting where testing can occur.
- **Risk to lab personnel:** Handling live or highly pathogenic samples requires strict containment and specialized training.
- **Degradation risk:** Viral RNA can degrade quickly if samples (like bird swabs or tissues) aren't handled and transported under cold, sterile conditions.

3. Field and Farm-Level Testing Limitations

- **Subclinical infections:** Some birds (especially wild waterfowl) can carry H5N1 without showing symptoms, making it hard to know where to test.
- **Wide host range:** The virus infects many bird species and some mammals (including humans), requiring broad testing strategies.
- **Logistics:** Collecting samples from large commercial flocks or migratory birds is time-consuming, expensive, and labor-intensive.

4. Diagnostic Method Challenges

Testing Method	Advantages	Limitations
RT-PCR (gold standard)	Sensitive and specific	Requires lab infrastructure, trained personnel, and sequence updates for new strains
Rapid antigen tests	Useful for screening	Less sensitive; often miss low viral loads or new variants
Serological (antibody) tests	Detect past exposure	Can't distinguish between vaccination and natural infection without special markers
Next-Gen Sequencing (NGS)	Provides full viral genome	Expensive, slow, and data-heavy for routine use

5. Surveillance and Data Coordination

- **Underreporting:** Many outbreaks (especially in wild bird populations or remote farms) go undetected or unreported.
- **Cross-border transmission:** Migratory birds spread H5N1 across regions, but international coordination of surveillance can be inconsistent.
- **Limited global lab capacity:** Many regions lack molecular diagnostic tools, trained personnel, or cold-chain infrastructure.

6. Emerging Human Testing Complications

- **Non-specific early symptoms:** H5N1 infections in humans resemble seasonal flu, complicating early diagnosis.
- **Low case numbers:** Because human infections are rare, it’s hard to validate and standardize human diagnostic assays.

In Summary

Testing for **H5N1 avian influenza** is difficult because:

- The virus evolves fast, requiring constant diagnostic updates.
- Testing demands high biosafety infrastructure.
- Field conditions and sample logistics are challenging.
- Global surveillance coordination is inconsistent.

Summary of Work Performed at BSL-3 Lab:

In May of 2025, Midwest Ag Solutions LLC along with Heartland Energy Group, Ltd commissioned high pathogen testing on H5N1 at a University BSL-3 lab. The following is a summary of testing performed:

Report of Xtreme Bio against HPAIV H5N1

Tested reagents:

Xtreme Bio at final concentration of 0.5 oz/gallon or 2.0 oz/gallon in water; 1 × Phosphate Buffered Saline (PBS).

Protocol:

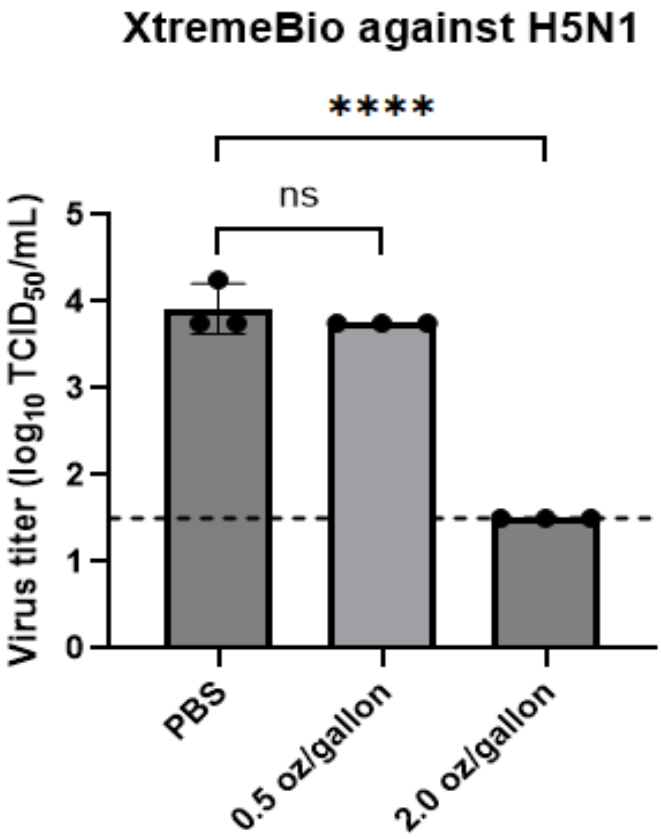
1. 10^4 $TCID_{50}$ of highly pathogenic avian influenza (HPAI) H5N1 virus (A/turkey/USA/1181-1/2024) in 0.5 mL of PBS was added to 6-well plates (3 wells for each reagent tested).
2. Virus was treated with PBS, or different concentrations of Xtreme Bio (0.5 oz/gallon and 2.0 oz/gallon) for 10 min at RT via unintentional spray.
3. Virus was collected and titrated on MDCK cells. After 3-4 days incubation, cells were fixed with cold methanol. The viral titers were determined by antibody staining.

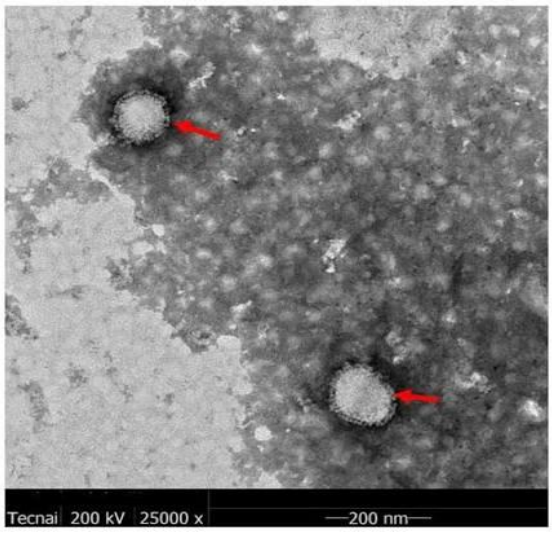
Result:

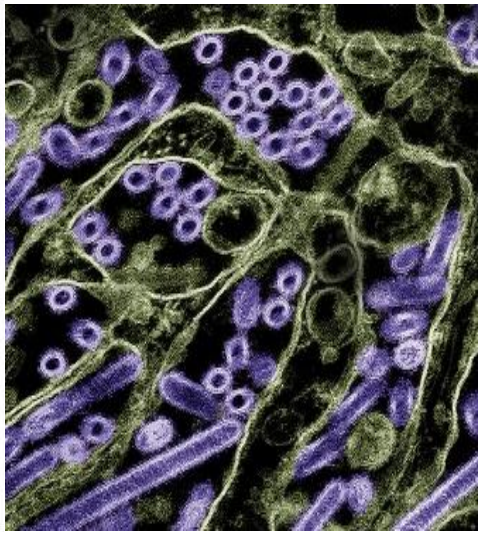
0.5 oz/gallon of Xtreme Bio cannot significantly inactivate HPAI H5N1 virus, compared to PBS treatment. However, 2.0 oz/gallon of Xtreme Bio can totally inactivate HPAI H5N1 virus (dashed line indicates the limit of detection − $10^{1.5}$ $TCID_{50}$/mL).

Note:

The test was conducted in the BSL-3 facility at the University of Missouri Laboratory for Infectious Disease Research (LIDR).







**Electron microscope / colorized microscope images of *highly pathogenic* avian influenza (H5N1) virus particles.

WENJUN MA, PHD - Professor

RESEARCH INTERESTS

Dr. Ma's research interests are viral diseases, with an emphasis on emerging zoonotic viral infections. His current research focuses on understanding mechanisms of pathogenesis and transmissibility, virus-host interaction and developing vaccines and antivirals for different zoonotic viral pathogens, such as Influenza virus, Rift Valley fever virus, SARS-CoV-2, Hantavirus and other viral infections. He is also interested in development of rapid detection systems for viral diseases. His long-term goals are to apply molecular biology and virology approaches to generate countermeasures and scientific knowledge that can be used to address current challenge from influenza and other important diseases for industry and public health needs.

TEACHING

MICROB 9001 Advanced Virology
MICROB 8303 Foundations of Virology
VPB 5558/8458 Veterinary Public Health

SELECTED PUBLICATIONS

A full list of publications can be found in the **W Ma publications**

1. Zhang J, Yang W, Roy S, Liu H, Roberts, M. R, Wang L, Lei Shi L, **Ma W**. Tight junction protein Occludin is an internalization factor for SARS-CoV-2 infection and mediates virus cell-to-cell transmission. Proc Natl Acad Sci U S A. 2023 Apr 25;120(17):e2218623120
2. Wang L, Zheng B, Shen Z, Deb Nath N, Li Y, Walsh T, Mitchell W, Li Y, He D, Lee J, Moore S, Tong S, Zhang S, **Ma W**. Isolation and identification of mammalian orthoreovirus from bats in the United States. J Med Virol. 2023 Feb;95(2):e28492.
3. Ganti K, Bagga A, Ferreri L, Geiger G, Carnaccini S, Caceres C, Seibert B, Li Y, Wang L, Kwon T, Li Y, Morozov I, **Ma W**, Richt J, Perez D, Koelle K, Lowen AC. Influenza A virus reassortment in mammals gives rise to spatially distinct sub-populations. Nature Communications. 2022. 13: 6846.
4. Artiaga BL, Morozov I, Ransburgh R, Kwon T, Balaraman V, Indran S, Madrid DM, Gu W, Henningson J, **Ma W**, Driver JP, Richt JA. Evaluating α-galactosylceramide as an adjuvant for live attenuated influenza vaccines in pigs. Animal diseases. 2022, 2:19.

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9. Gaudreault NN, Trujillo JD, Carossino M, Meekins DA, Morozov I, Madden DW, Indran SV, Bold D, Balaraman V, Kwon T, Artiaga BL, Cool K, García-Sastre A, **Ma W**, Wilson WC, Henningson J, Balasuriya UBR, Richt JA. SARS-CoV-2 infection, disease and transmission in domestic cats . Emerg Microbes Infect. 2020; 9(1): 2322–2332.
10. Meekins DA, Morozov I, Trujillo JD, Gaudreault NN, Bold D, Carossino M, Artiaga BL, Indran SV, Kwon T, Velmurugan Balaraman V, Madden DW, Feldmann H, Henningson J, **Ma W**, Balasuriya UBR, Richt JA. Susceptibility of swine cells and domestic pigs to SARS-CoV-2 . Emerg Microbes Infect. 2020; 9(1): 2278–2288.
11. Ciminski K, Ran W, Gorka M, Lee J, Malmlov A, Schinköthe J, Eckley M, Murrieta RA, Aboellail TA, Campbell CL, Ebel GD, Ma J, Pohlmann A, Franzke K, Ulrich R, Hoffmann D, García-Sastre A, **Ma W**, Schountz T, Beer M, Schwemmle M. Bat influenza viruses transmit among bats but are poorly adapted to non-bat species. Nat Microbiol. 2019 Dec;4(12):2298-2309.
12. Wang R, Zhu Y, Lin X, Ren C, Zhao J, Wang F, Gao X, Xiao R, Zhao L, Chen H, Jin M, **Ma W**, Zhou H. Influenza M2 protein regulates MAVS-mediated signaling pathway through interacting with MAVS and increasing ROS production. Autophagy . 2019 Jul; 15 (7) :1163-1181.
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14. Lang Y, Li Y, Jasperson D, Henningson J, Lee J, Ma J, Li Y, Duff M, Liu H, Bai D, McVey S, Richt JA, Ikegami T, Wilson WC, **Ma W**. Identification and evaluation of antivirals for Rift Valley fever virus. Vet Microbiol. 2019 Mar;230:110-116.
15. Sunwoo SY, Pérez-Núñez D, Morozov I, Sánchez EG, Gaudreault NN, Trujillo JD, Mur L, Nogal M, Madden D, Urbaniak K, Kim IJ, **Ma W**, Revilla Y, Richt JA. DNA-protein vaccination strategy does not protect from challenge with African swine fever virus Armenia 2007 strain. Vaccines (Basel) . 2019 Jan 28; 7 (1).