World Organisation for Animal Health (OIE) visit to the People's Republic of China to investigate influenza A (H7N9) infections in poultry

25 April – 1 May 2013

OIE Team: Keith Hamilton and David Swayne
Accompanied by **Huang Baoxu** (China Animal Health and Epidemiology Centre) and in close cooperation with **Hualan Chen** (Harbin Veterinary Research Institute – OIE Reference Centre)

Objective: to understand the steps taken to investigate the animal source of influenza A (H7N9), to summarise preliminary findings, and to inform the international perspective.

Schedule:

25 April – Beijing: Meeting with OIE Delegate, Ministry of Agriculture, Minister of Health and Family Planning, Ministry of Forestry, China Animal CDC; flight to Shanghai.

26 April – Shanghai: Meeting with Provincial Agriculture Department, China Animal CDC, Public Health (China CDC). Visit to one Wholesale live poultry market; two retail live poultry markets, and to provincial laboratory; train to Hangzhou.

27 April – Hangzhou: Meeting with Provincial Agricultural Department, China Animal CDC, and Public Health. Visit to two wholesale markets, one retail live poultry market, and provincial laboratory.

28 April – Harbin: Flight to Harbin. Visit to Harbin Veterinary Research Institute. Flight to Beijing.

29 April – Beijing: Summing up and press conference (press brief as Annex).

Assumption:

That the influenza A (H7N9) virus is of avian origin and is responsible for causing the infections detected in positive human cases.

General data and summary of main findings

Observations and conclusions are based on discussions that took place during the mission, on the data provided in the meetings, and on observations made during the field visits

China's Ministry of Agriculture had launched emergency H7N9 animal surveillance aimed at finding the animal source, the host range, route of transmission, and transmission route to facilitate elimination of H7N9 from the animal population. At national level the surveillance strategy is adapted to 3 zones: 1) Core areas (provinces

where human and/or animal cases of H7N9 have been detected); 2) Key areas (provinces bordering the core area); 3) Ordinary areas outside of zones 1 and 2. Surveillance is targeted to chickens, waterfowl, raised pigeons and quail, wild birds, and pigs. Sampling strategy focuses on live poultry markets, poultry slaughterhouses, poultry farms, wild birds, pig slaughterhouses. Samples taken from birds are blood plus cloacal and throat swabs; from pigs – nasal swabs. Environmental samples are also taken. PCR for m-gene and H7 was carried out at provincial level and referred to the National Reference Lab (Harbin) if positive for H7. If H7 positive at provincial level, movement restrictions were imposed on the poultry site and if confirmed by MoA (Harbin) all birds and contacts were destroyed – cleaning and disinfection takes place, and markets (and slaughterhouses) were closed.

At least 300, 000 (range 300,000 – 400, 000) diagnostic samples had been taken from birds (poultry, pigeons, wild birds, and ducks) or environments that housed birds. At the time of the mission there had been no positive detections of H7N9 on poultry farms. There were no known human cases on farms or amongst veterinarians. There had been at least 46 positive results for H7N9 from environmental and poultry samples taken in live poultry markets. Human cases predominantly inhabited an urban environment.

The incubation period of H7N9 in humans was estimated to be approximately 5–7 days. Positive laboratory confirmation of human H7N9 infection would likely take several days more. Therefore human cases would have been exposed to infection at least 7–10 days before an animal source tracing investigation took place.

The movement of poultry from farms through a live poultry market system includes 'wholesale' and 'retail' establishments and would be dynamic, not static. It appeared unlikely that any live birds would be kept in a live poultry market for 7–10 days, and none would have been kept isolated and segregated, for infectious disease control. Poultry that could have been the exposure source from a human case could be traced from a retail live poultry market, to a wholesale market and a farm through a license and certificate system, but the source of the infecting virus cannot be immediately attributed to the supplying farm because of high probability of virus amplification within the wholesale markets, and to a lesser degree in the retail markets. Therefore, the source of infection of poultry was most likely to be another infected bird or contaminated environment in the live poultry market system, and low probability of being the farm of origin.

At the time of the mission trace back investigations to poultry farms following detection of H7N9 in the live poultry market were negative. This could be due to one of several factors or a combination of factors:

- The poultry testing positive for H7N9 was infected in the market (not at the poultry farm source)
- All poultry at the farm source had since recovered and were not shedding virus when trace back sampling took place
- All infected birds at the farm of origin had also been sent to markets, killed, and sold. Most of the farming operations supplying the 'yellow chickens' to wholesale markets raise one age of birds, sending all the chickens to the market at one time.

More general market analyses on epidemiological data may help to identify areas where the H7N9 virus was more likely to have originated.

Conclusion: H7N9 infection had been maintained in the live poultry market.

Possibility: The prevalence in poultry farms was currently undetectable with the wholesale markets being sporadically seeded with H7N9. Some infection in wholesale markets may result from transportation of birds or equipment between different poultry market.

Live poultry markets

'Wholesale' markets

When markets are operating, farmers bring poultry from farms (including from outside the province) to individually owned booths within the wholesale market (pictures 2 and 3); e.g. the largest wholesale market in Hangzhou had 295 booths. Species include chicken, quail, and pigeons (in Shanghai markets ducks have been banned from live poultry markets following the emergence of H5N1 since 2004) are held and sold in the wholesale markets. Up to 80,000 birds per day are processed in larger markets. Birds are sold from the numerous booths and sent to individual retail poultry markets or restaurants. Shanghai had three wholesale poultry markets supplying 461 retail poultry markets. In Shanghai 80% of birds were being supplied from outside the province. The market system is complex.

Some wholesale poultry markets would be open 24 hours a day every day of the month apart from during festivals. According to informal discussions with experts, on average most birds would have been held in wholesale markets for 1 day, however some birds would be held in the market over 3 days thus creating an opportunity for virus infection and amplification in the wholesale markets. Biosecurity appeared to be poor in some poultry markets and there appeared to be no epidemiological separation between birds from different farms. One respondent said that there were also movements of birds between wholesale markets and a few farmers sent birds directly to retail poultry markets.

Conclusion: an environment existed for H7N9 virus to spread between different batches of poultry and to be maintained / amplified in the wholesale markets. Of minor importance, H7N9 virus may also spread between wholesale markets.

'Retail' live poultry markets

When markets are open, poultry from wholesale markets are sold to consumers at retail markets (pictures 4 and 5). These are small live poultry stalls within a larger market which also has stalls selling other products (vegetables and fruit, fish, crabs, eels, pork, frogs, etc.). In some retail markets hygiene appears poor. From observations made it seems likely that airflow would pass from the rear of the shop and be concentrated through the small window where the customer collects the slaughtered bird. In the stall the bird would be killed, eviscerated and de-feathered (using a centrifugal mechanical de-feather) potentially creating aerosols. This environment would put the customer at risk of exposure to aerosols created during the slaughter process.

Market stalls selling other produce and not selling poultry could be found, not far from the retail live poultry stall.

Following closure of the live poultry markets there had been a significant reduction in the number of human H7N9 cases reported. The team understood that no human cases had occurred in Shanghai more than one incubation period after closure of the live bird market.

Conclusion: The live poultry retail market may provide an opportunity for humans to be exposed to H7N9 virus through aerosols created during the slaughter process. It is possible that some humans may have been exposed in these large mixed wet markets without having an obvious link to poultry. For cases that did not report contact with live birds, further investigation may be required to establish the source of exposure.

Disparity in detection rate between humans and animals

The positive human cases had shown severe clinical signs; therefore human surveillance for H7N9 could be targeted to clinically ill humans. There was no information available concerning detection of H7N9 in humans showing no signs of disease; i.e. seropositive humans without disease.

Based on field and laboratory data poultry did not show clinical signs and there is no opportunity for clinical surveillance. Trace back investigations around human cases took place at least 7–10 days after the human was exposed, and poultry to which the human was exposed may have been killed, recovered or moved somewhere else.

Trace back of consignments to the poultry farm of origin is likely to be of no value if the birds were infected in the wholesale market. Less than 2,000 samples were analysed by Harbin Veterinary Research Institute having been taken from 22 live poultry markets, with 13 markets having one or more positive samples. If this estimate includes the larger wholesale markets, then the population size would have coverage of larger populations (at least 100,000 birds) and the detection rate in the *at risk* live poultry markets would therefore realistic, compared to a broad national poultry survey (approximately 12 billion poultry) which had been the source of most of the samples reported as negative.

Swab samples had tested positive for H7N9 by virus isolation in embryonating chicken eggs at Harbin Veterinary Research Institute and for influenza A (matrix real-time reverse transcriptase polymerase chain reaction [RRT-PCR] test) and H7 RRT-PCR test at provincial laboratory level. All H7 positive samples from provincial laboratories were confirmed at HVRI before being officially reported as H7 positive. There was no way to assess whether there had been false negative H7 laboratory results from poultry samples. However, the provincial laboratories had used RRT-PCR for H9N2 virus detection with a rate of 7.8% positive for live poultry market samples in 2011 for Shanghai. The provincial veterinary services diagnostic laboratories did not have a validated and harmonised, national RRT-PCR test to detect H7N9 viruses and the antigen in the haemagglutination inhibition antibody detection assay should be updated to a current H7N9 virus strain[1]. Lack of such unified and current testing methods raised concerns about the possibility of false negative results. Both assays should be incorporated within a national proficiency test for provincial and county veterinary diagnostic laboratories.

Conclusion: Sensitivity of surveillance in animals would inherently be lower than humans owing to the probable epidemiology. Detection rate in live poultry markets was realistic accounting for the sample population vs. sample size, and historical use of RRT-PCR technology to detect H9N2.

1 China has now updated the antigen reagent for H7N9 diagnostics.

General recommendations

- Enforce live poultry market controls until the full epidemiological situation is understood and appropriate elimination measures have been taken at the poultry farm level. If markets are to reopen, science based management measures must be developed and implemented to avoid maintenance/amplification of infection through marketing of poultry from H7N9 free farms and regular closures of markets for full cleaning and disinfection.

- Strict biosecurity measures on all poultry premises should be implemented to avoid silent spread of infection

- A national surveillance programme for LPAI H7N9 should be developed and implemented to determine the extent of infection and to eliminate infection when detected

- Retrospective epidemiological analyses, including market chain analyses, to identify poultry holdings that have been or are at greater risk of H7N9 infection

- Implement proficiency testing (led and coordinated by Harbin Veterinary Research Institute) for nationally unified RRT-PCR and serology tests, carried out in provincial and county veterinary laboratories, which should incorporate the influenza A(H7N9) strain (H7 RRT-PCR and H7 HI tests)

Considerations

- There is currently no official vaccination policy, moreover, unofficial or official vaccination for H7N9 would complicate serological surveillance in poultry and make detection of virus infections difficult and would likely encourage enzootic maintenance of the virus.

Questions

- Did the virus originate in the live poultry market system as a reassortment event and then spread between markets where it was maintained and amplified?

- Did the virus originate on a poultry farm or a number of poultry farms? Where?

- If the virus originated on a poultry farm(s) is infection still prevalent?

Questions for public health

- Do humans seroconvert to H7? If so is it possible to estimate baseline exposure/ infection level?

- Have any cases occurred outside one incubation period of the markets being closed?

Acknowledgements

The team would like to thank all staff at Central and Provincial level, staff at Harbin Veterinary Research Institute, and the translators for their considerable efforts and patience in making the team feel most welcome, and for facilitating the fruitful discussions with access to comprehensive data and field information.

Photographs

Picture 1 – Wet market – Shanghai

Picture 2 – Closed wholesale market – Shanghai

Picture 3 – Closed wholesale market – Hangzhou

Picture 4 – Closed retail market – Hangzhou

Annex – press brief

Opening statement by Dr Keith Hamilton

World Organisation for Animal Health

Experts from the World Organisation for Animal Health, the OIE, visited China following an invitation from the Ministry of Agriculture, P. R. China.

The following team were appointed by the Director General of OIE and the Chinese Ministry of Agriculture – Dr Keith Hamilton, expert from the OIE Headquarters (Paris), the Director of the OIE Collaborating Center for Research on emerging avian diseases, also a key scientist in OFFLU OIE/FAO network of expertise on animal influenza, Hualan Chen, and Huang Baoxu.

The OIE collaborates with the FAO and the WHO within the tripartite framework. This mission is linked with tripartite activities and is working in cooperation with the FAO.

Representing the Director General of OIE, I would like to thank the Government of China for inviting us to visit the country in relation to the current influenza A (H7N9) situation.

This visit has been very helpful and allowed us to visit key sites linked to infections of H7N9 in humans and poultry, as well as laboratories involved in the ongoing investigations of these outbreaks. We appreciate the availability and transparency of the Chinese authorities, the veterinary services, and other key personnel who have willingly shared important information. We acknowledge the efforts that they have made to keep the international community informed about the disease situation.

OIE has received timely notification of and follow-up reports on H7N9 infections in birds through OIE's World Animal Health Information System (WAHIS).

Over the last 5 days our team has visited MOA officials in Beijing, poultry markets in Shanghai and Hangzhou – which have been closed as an H7N9 control measure – the veterinary lab in Harbin, and spent time with key experts from the Veterinary Services.

According to the information and data collected during the mission we can confirm that some human cases of H7N9 appear to have a link with live bird markets. To date there have not been any known human cases or animal infections of H7N9 on poultry farms. Based on available data, the team have a strong suspicion that live bird markets play an important role in human and poultry H7N9 infections. Humans could be infected through exposure to infected birds or a contaminated environment. The team recommend that control measures continue to be enforced in live bird markets, whilst wider investigations aim to confirm this situation. It remains a challenge to fully understand dynamics of H7N9 infections in poultry and humans. This is why the mission recommends that more investment be made in surveillance, targeting animals in markets, and at the farm level to support these investigations.

We recognise that OIE Reference labs for avian influenza, including Harbin Veterinary Research Institute, as well as members of the FAO/OIE network OFFLU, are conducting research on H7N9 to better understand the characteristics of this virus and to ensure that diagnostic testing protocols are publicly available.

We will now provide some feedback on concerns that may be raised by the public.

1. Relating to the H7N9 Influenza A outbreaks, the public has become concerned about the consumption of poultry and poultry products. Poultry production in China has suffered great losses. We would like to share some points on this.

   There is no evidence to suggest that the consumption of poultry or eggs fit for human consumption could transmit the AI virus to humans.

   Animals which have been culled as a result of control measures implemented in response to a positive detection of H7N9 virus in accordance with relevant Chinese Law, should be disposed of with adherence to strict biosafety, and cannot enter the food and feed chain.

   It is safe to eat properly prepared and cooked meat including from poultry and game birds.

2. At present, small scale farming still forms a significant portion of poultry production in China, which makes preventing and controlling animal diseases, including H7N9, even more difficult. We have some suggestions to help farmers respond to the current H7N9 situation.

   We now know that currently virus infection does not cause visible disease in poultry meaning veterinarians must be extra vigilant in preventing its further spread.

   Biosecurity measures should be applied to prevent introduction of infection to farms. This includes controlling the entry of people into and out of poultry farms. Access to industrial poultry farms should be restricted to essential personnel and they should wear protective clothing and be disinfected before entering and leaving. Vehicles, apparatus and all outer clothing should be disinfected before entering or leaving a farm. Small scale farmers should also adapt their practices to avoid spreading the disease between backyard flocks.

   An “all-in & all-out” model should be adopted to prevent poultry that have not been sold from going back to poultry farms from live bird markets.

3. Compared with H5N1, H7N9 is not pathogenic in poultry, which makes prevention, surveillance, and control of the infection in poultry quite difficult.

   This mission confirms the importance of an extensive laboratory based surveillance programme in animals to establish the full extent and distribution of the H7N9 virus in poultry. We highlight the importance of limiting contact between infected animals and people, and other influenza-susceptible animals.

   A full understanding of the extent of the disease in animals is essential for effective control.

   The Chinese government has reacted rapidly to investigate possible animal sources of this disease. Further extensive investigations in animal populations are needed to fully understand the extent of animal infections.

   Effective surveillance will require cooperation between poultry owners and government veterinary services. Because this disease is likely to show little or no visible signs in poultry, the use of reliable and

accurate laboratory tests, complying with OIE Standards and OFFLU guidance, will be critical for surveillance and control of this disease in poultry.

It is possible that the characteristics of this virus may change with time so that it causes disease in poultry or becomes more pathogenic in poultry, therefore any signs of increased mortality or loss of production should be reported to the veterinary services so that they can further investigate.

In case of outbreaks, destruction of infected poultry by the veterinary services as well as poultry that have been in contact with the infected poultry is recommended. Culling of animals must be done following requirements for acceptable killing methods as described in the OIE *Code*.

More research is needed to know whether poultry vaccination could be considered as a control option for H7N9.

Finally, we would like to highlight the importance of veterinary services for prevention and control of zoonosis.

Veterinary services stand in the front line in reducing public health threats from zoonotic infections. Strong action and investments in a proactive response must be taken now to fully understand the disease situation and to take action to avoid H7N9 infections becoming entrenched in poultry populations. This principle should apply to all zoonotic infections.