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Comparison of commercial influenza A virus assays in detecting avian influenza H7N9 among poultry cloacal swabs, China

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ABSTRACT

Background: Avian H7N9 virus emerged in China in February 2013 and has since spread widely among China's poultry, causing numerous human infections.
Objectives: To compare World Health Organization (WHO) and US commercial influenza assays in detecting avian H7N9 virus in poultry cloacal specimens.
Study design: Between April 6 and July 15, 2013, 261 cloacal swabs were collected from commercial poultry in Nanjing and Wuxi City, Jiangsu Province, China. Swabs were screened with the WHO's influenza A and H7N9 real-time RT-PCR (qRT-PCR) assays. A blinded panel of 97 specimens (27 H7N9-positive and 70 influenza A-negative) was then used to compare 3 antigen based commercial assays (Remel Xpect Flu A&B, Quidel Quickvue influenza, and Quidel Sofia Influenza A + B), and 2 molecular commercial assays (Quidel Molecular Influenza A + B assay and Life Technologies VetMAX™-Gold SIV Detection Kit). None of these commercial assays were approved for use with poultry specimens.
Results: Considering the WHO H7N9 qRT-PCR assay as the gold standard, all assays except the Quidel Quickvue influenza assay had high specificity (ranging from 96 to 99%). Regarding sensitivity, the Life Technologies VetMAX™-Gold SIV Detection Kit (100%; 95% CI 87–100%) and the Quidel Molecular Influenza A + B assay (85%; 95% CI 66–96%) performed the best. The sensitivities of the non-molecular antigen detection assays were either unable to detect small amounts of H7N9 viral RNA or were inhibited by specimen type.
Conclusions: The Life Technologies VetMAX™-Gold SIV Detection Kit and the Quidel Molecular Influenza A + B assay are comparable in performance to the WHO H7N9 qRT-PCR assay in detecting H7N9 from poultry cloacal specimens.

1. Background

Since the first detections in February 2013, avian H7N9 viruses have spread widely in China, causing at least 142 humans to be infected (33% mortality) [1–4]. Originating in Southeastern China, these viruses are now thought to be enzootic among birds in at least 12 of China's 34 provinces [4]. Avian H7N9 viruses are unlike previous avian H5N1 influenza A threats in humans, in that they cause little symptoms among domestic poultry, and are thus difficult to detect in the environment [5]. This low pathogenicity characteristic makes outbreak preparedness more difficult as emerging subtypes can appear in humans without warning. Having available diagnostic assays, with broadly reactive detection capabilities in humans, is critical in detecting novel viruses.

While there are multiple commercial influenza A assays available and commonly used to detect influenza in humans, they have not been well-studied as tools to specifically detect H7N9. This is primarily because the virus is newly emergent, and access to H7N9-positive human samples for testing is extremely limited. However,

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we reasoned that if certain commercial influenza assays were effective in detecting H7N9 virus among avian species fecal swabs, they may serve as an additional diagnostic option in H7N9 endemic areas.

2. Objectives

We sought to compare World Health Organization (WHO) and US commercial influenza assays against avian influenza A H7N9 virus in poultry cloacal specimens.

3. Study design

This study involved a first round of testing (or screening), with a goal to identify 100 specimens (30 H7N9-positive) to be used in a second round of testing, evaluating commercial assays. All assay work was performed by Chinese and US scientists at the Institute of Microbiology and Epidemiology, Beijing (BIME), which is part of the Academy of Military Medical Sciences.

3.1. Sites for specimen collection

Chinese investigators identified hot spots in Nanjing and Wuxi City, Jiangsu Province, China, where H7N9 virus or H7N9-infected patients had been previously identified. In these locations, markets or farms with active poultry operations were recruited for sampling.

3.2. Cloacal swab collection

In the field, technicians donned personal protective gear including gowns, gloves, boots, face shields, and N-95 masks (or Powered Air Purifying Respirators also called PAPRs) to collect specimens from poultry. Specimens were collected from live chickens, ducks, and pigeons. All poultry were humanely captured by hand and sampled as to minimize trauma, and then released. A sterile Dacron swab with plastic shaft was inserted approximately 1 cm into the cloaca, twisted 180° and withdrawn (<3 s). The swab was then inserted into a cryovial containing 3 ml of sterile universal transport media, the swab stem broken or cut off, and the cryovial capped.

3.3. Sample processing

Each sample was labeled and transported to the BIME laboratory in proper transport containers on ice packs or wet ice. Upon receipt in the BIME lab, all samples were preserved at −80 °C until testing could be performed. Each subsequent aliquot of the specimen to be used in the second round of testing was similarly labeled with the same accession number.

3.4. Nucleic acid extraction

RNA was extracted and purified from cloacal swabs using the QIAamp MinElute Virus Spin Kit (Cat. No. 57704, Qiagen) for the 3 molecular assays (WHO real-time RT-PCR (qRT-PCR), Quidel Molecular Influenza A + B assay, and Life Technologies VetMAX™-Gold SIV Detection Kit) by manual methods per kit instructions. To avoid prolonged exposure to elevated temperatures, purified RNA specimens were handled on wet ice and then promptly placed back at −80 °C after use.

3.5. Sample testing

For the first round of testing, nucleic acid extraction was performed using 0.2 ml of volume from each specimen and screened with the WHO qRT-PCR assays for any influenza A and avian H7N9 virus [6]. The remainder of each specimen was promptly returned to storage at −80 °C.

Later, WHO qRT-PCR H7N9-positive specimens and specimens negative using both the WHO qRT-PCR influenza A and H7N9 assays were selected for use in the second round of testing. This final panel of swab specimens was tested in a blinded fashion employing: Remel Xpect Flu A&B assay, Quidel Quickvue influenza assay, Quidel Sofia Influenza A + B assay, Quidel Molecular Influenza A + B assay, Life Technologies VetMAX™-Gold SIV Detection Kit, and the WHO qRT-PCR H7N9 assay (repeat testing) (Table 1). Each assay was performed according to the manufacturer's instructions. The 3 molecular assays were run on a 7500 Real-Time PCR System platform (Life Technologies, Foster City, CA, USA). C_t values ≤35 were considered as positive for the Quidel Molecular A + B assay, and C_t values <38 were considered as positive for the Life Technologies VetMAX™-Gold SIV Detection Kit, and the WHO qRT-PCR assays.

3.6. Statistical analysis

Commercial assay results were compared against the second run of the WHO qRT-PCR H7N9 assay. For the sensitivity and specificity calculations (second round of testing) we ran each antigen detection assay twice and each molecular assay three times. For the antigen detection assays, if either of the two runs were positive then we considered that specimen as positive. For the molecular assays, a specimen was considered positive if at least two of the three molecular runs had C_t values <38 (≤35 for the Quidel Molecular Influenza A + B assay). Sensitivity, specificity, and confidence intervals around each parameter, were calculated using SAS v9.3 (SAS Institute, Inc., Cary, NC, USA).

4. Results

During the period April 6–July 15, 2013, study staff collected cloacal swabs from commercial poultry in Nanjing and Wuxi City, Jiangsu Province, China. With the agreement of administrators or owners, a total of 15 agriculture fair markets and 8 medium-scale farms were selected for sampling.

A total of 261 poultry cloacal swab specimens from chickens, ducks, and pigeons were collected from live birds without signs of illness. Of the 261 cloacal swab specimens initially screened, 27 were found to be H7N9-positive, which were paired with 70 random-number selected specimens that were negative by both the WHO influenza A and H7N9 assays.

There was excellent concordance between the first and second WHO H7N9 assays. The Quidel Quickvue influenza assay failed to detect any influenza A specimens among the 97 specimens in the panel and will not be further discussed. Employing the WHO H7N9 assay as the gold standard, the other four assays had excellent specificity, ranging from 96 to 100% (Table 2). The Life Technologies VetMAX™-Gold SIV Detection Kit had the highest sensitivity at 100% (95% CI 0.87–1.00), though 3 of the 70 WHO H7N9-negative samples tested positive, reflecting a false positive probability of 4% (95% CI 1–12%). The Quidel Molecular Influenza A + B assay had a sensitivity of 85% (95% CI 66–96%), detecting 2 of the 70 WHO H7N9-negative samples as positive; a false positive probability of 3% (95% CI 0–10%).

The VetMAX™-Gold SIV Detection Kit and the Quidel Molecular Influenza A + B assays were discrepant with the WHO H7N9 assay for 7 specimens (Table 3). Examining the discrepant specimens, both the Life Technologies VetMAX™-Gold SIV Detection Kit and the Quidel Molecular Influenza A + B assay were positive when