Original Article

Recognizing true H5N1 infections in humans during confirmed outbreaks

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Abstract

Introduction: The goal of this study was to evaluate whether any characteristics that are evident at presentation for urgent medical attention could be used to differentiate cases of H5N1 in the absence of viral testing.

Methodology: Information about exposure to poultry, clinical signs and symptoms, treatments, and outcomes was abstracted from existing data in the global avian influenza registry (www.avianfluregistry.org) using standardized data collection tools for documented and possible cases of H5N1 infection who presented for medical attention between 2005-2011 during known H5N1 outbreaks in Azerbaijan, Indonesia, Pakistan and Turkey.

Results: Demography, exposure to poultry, and presenting symptoms were compared, with only the common symptoms of fever and headache presenting significantly more frequently in confirmed H5N1 cases than in possible cases. Reported exposure to infected humans was also more common in confirmed cases. In contrast, unexplained respiratory illness, sore throat, excess sputum production, and rhinorrhea were more frequent in possible cases. Overall, oseltamivir treatment showed a survival benefit, with the greatest benefit shown in H5N1 cases who were treated within two days of symptom onset (51% reduction in case fatality).

Conclusion: Since prompt treatment with antivirals conferred a strong survival benefit for H5N1 cases, presumptive antiviral treatment should be considered for all possible cases presenting during an outbreak of H5N1 as a potentially life-saving measure.

Key words: influenza, human; avian influenza; oseltamivir; antiviral agents

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Introduction

The highly pathogenic avian influenza virus of subtype H5N1 remains prevalent in many countries, causing infections and deaths. Case fatality rates have been over 80% in Indonesia [1,2] and 59% overall, with outbreaks continuing to be reported largely in Egypt and Indonesia [3]. The H5N1 virus continues to mutate, with on-going concern about its potential to become more highly pathogenic and more easily transmissible [4,5]. Although the World Health Organization (WHO) provides counts of laboratory-confirmed cases of influenza A/H5N1, cases that are tested and identified represent only a fraction of the

total cases, and little is known about them collectively, aside from survival [6].

Once an outbreak has started, medical care providers are confronted with a range of patients including true cases and possible cases who present for medical attention. Test results are rarely immediately available after presentation for medical care, and when available, may be positive, negative or have indeterminate results. Clinicians are often obliged to formulate treatment plans based upon presumptive diagnoses. The goal of this study was to describe the characteristics of confirmed cases, to compare them with possible cases who presented for medical care around the same time and in the same country, and to evaluate antiviral treatment effectiveness in both groups. Possible cases included those who presented for medical attention during a confirmed local outbreak through the same medical systems as did true cases, but who tested negative for H5N1, had indeterminate results, were never tested, or died before testing.

Methodology

The Avian Flu Registry (www.avianfluregistry.org) is an international patient registry for human cases of influenza A (H5N1). All cases were identified through record systems in regions with known outbreaks of H5N1 between 1997 and 2011. Information about exposure to poultry, clinical signs and symptoms, treatments, and outcomes were collected in a web-enabled database. Data were abstracted from clinical records, published case series and governmental agency reports using a standardized data collection form. Avian exposure was classified as "in the vicinity of live poultry" and where available, direct or indirect contact with sick or dead poultry or wild birds was noted. The registry methods for case acquisition, data collection, and institutional review and approval are fully described elsewhere in detail [7,8].

The registry classifies cases according to certainty of diagnosis, with confirmed and possible cases used for these analyses. These categories were adapted from the WHO classification scheme [9] based on practical considerations from field experience and differ from the roughly equivalent WHO categories in a few important ways. The WHO classification scheme begins with suspected cases who had exposure to poultry or wild birds or infected persons. Possible cases have had exposure and also have infiltrates or evidence of an acute pneumonia or who were linked in time and place and exposure but died before testing could be performed. Confirmed cases met all the criteria for a suspected or probable case and had a positive laboratory confirmation of H5N1 from an influenza laboratory whose avian influenza or human pandemic influenza test results are accepted by WHO as confirmatory. The registry's confirmed cases are based on *any* laboratory report confirming infection with influenza A/H5N1, and is not restricted to reports from WHO reference laboratories. The definitions of influenza-related symptoms were broadened for the registry to accommodate practical application to these observational data. For example, the registry records a case as having had a fever if the patient record indicated "fever" but the actual body temperature was not available. In addition to symptoms used by the WHO (acute pneumonia on chest radiograph plus evidence of respiratory failure), gastrointestinal, and both upper and lower respiratory symptoms are recognized here as symptomatic, since these characteristics were noted in outbreaks of confirmed cases. Further, "close contact" was defined broadly based on available data. For example, presence of an infected family member in the household was an acceptable proxy for close contact, in contrast to the WHO definition of being within one meter of an infected person. Possible cases include people who presented for medical attention during known outbreaks of H5N1 and were thought to be true cases, but for whom confirmation of infection with H5N1 was not documented (including negative and interdeterminate laboratory reports).

As of April 30, 2012, the registry contained 647 cases from 12 countries that were reported as H5N1 ("all cases"), including 407 cases confirmed as influenza A (H5N1). To minimize differences in data availability by country, this analysis is restricted to cases from countries that reported both confirmed and possible cases of H5N1. Nineteen possible cases were excluded because data were missing for age, gender or outcome. Four countries met the criteria for inclusion: Azerbaijan (9 confirmed, 8 possible), Indonesia (127 confirmed, 132 possible), Pakistan (4 confirmed, 7 possible) and Turkey (13 confirmed, 53 possible), yielding 353 cases: 153 confirmed and 200 possible cases, all occurring between 2005 and 2011.

The characteristics of confirmed and possible cases were compared by examining differences in demography, clinical presentation and treatment effects. Differences in the prevalence of symptoms at presentation for medical care were compared using Fisher's Exact test. Differences in treatment with

oseltamivir and survival were compared using the Chi-square test for statistical significance.

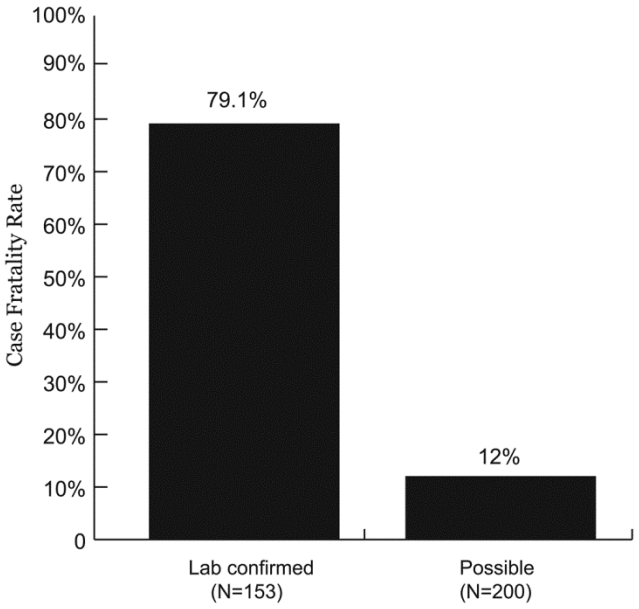
Survival by timing of treatment initiation was analyzed using 2x2 tables, with relative risks used to describe the risk of death in the treated patients compared with the same risk in untreated patients, with probability determined by use of a Fisher's exact test. A delayed cohort entry strategy was used, with cases eligible to be included in the risk calculation for each treatment interval only if they had presented for medical care by the first day of the interval. Statistical significance was determined with α set at .05. All analyses were conducted using SAS v. 9.2 (SAS Institute, Cary, USA).

Results

Of 153 confirmed cases, 44.4% were male, with a median age of 17.0 years (range 1.5-67.0). In contrast, 57.5% of 200 possible cases were male, with a median age of 13.0 years (range 0.4, 80.0). True cases were more likely than possible cases to have had direct contact with another confirmed case. Exposure to sick or dead poultry or wild birds was not a reliable aid for recognizing true cases (Table 1).

Possible cases were more likely to present for medical attention at an emergency room (45.0% v. 28.1%, respectively) and presented for medical care later than true cases (median of 2 v 0 days after symptom onset). Possible cases were hospitalized more quickly after symptom onset than confirmed cases (3 v 6 days), had viral testing sooner (3 v 7 days), and had antiviral treatments initiated much more quickly (3 v 7 days) than confirmed cases. Confirmed cases had a substantially higher case fatality rate (79.1% v. 12.0%, p<.01) (Figure 1).

Figure 1: Case fatality rate in confirmed and possible cases



The prevalence of symptoms at presentation for confirmed and possible cases are displayed in Table 2. Confirmed cases were more likely to present with fever (87.8% v 76.8%, p = 0.01) and headache (43.5% v 29.5%, p = 0.05). Tachypnea, a potential warning sign for clinicians, was also frequent in both confirmed and possible cases (32.5% v 25.6%, p = 0.22.) In contrast, possible cases were more likely to have unexplained respiratory illnesses (82.8% v. 65.7%, p < 0.01); sore throat or pharyngitis (62.4% v 44.1%, p < 0.01), excessive sputum production (37.8% v 4.4%, p < 0.01) and rhinorrhea (29.1% v. 11.2%, p < 0.01).

Table 1: Exposure to poultry, wild birds and infected humans

Exposure	Cases		
	Confirmed (N =153) n (%)[a]	Possible (N = 200) n (%)[a]	p-value
Direct contact with sick or dead poultry	61/153 (39.9%)	98/200 (49.0%)	0.09
Indirect contact with sick or dead poultry	33/153 (21.6%)	89/200 (44.5%)	< 0.01
Direct contact with sick or dead wild birds	3/153 (2.0%)	5/200 (2.5%)	1.00
Indirect contact with sick or dead wild birds	6/153 (3.9%)	3/200 (1.5%)	0.18[b]
In the vicinity of live poultry	55/153 (36.0%)	37/200 (18.5%)	< 0.01
Contact with a confirmed human case	29/153 (19.0%)	19/200 (9.5%)	0.01

[a]Multiple exposures could be reported for a single case; categories are not mutually exclusive: [b]Fisher's exact test.

Table 2: Prevalence of symptoms at presentation for confirmed and possible cases

Symptom at presentation	Cases		
	Confirmed (N=153) n (%)[a]	Possible (N=200) n (%)[a]	p-value
Fever	122/139 (87.8%)	152/198 (76.8%)	0.01
Unexplained respiratory illness with cough, shortness of breath or difficulty breathing	92/140 (65.7%)	164/198 (82.8%)	< 0.01
Tachypnea	38/117 (32.5%)	40/156 (25.6%)	0.22
Abnormal breath sounds	10/61 (16.4%)	33/129 (25.6%)	0.16
Sore throat/pharyngitis	41/93 (44.1%)	108/173 (62.4%)	< 0.01
Cyanosis	4/74 (5.4%)	3/137 (2.2%)	0.24[a]
Excessive sputum production	3/68 (4.4%)	48/127 (37.8%)	< 0.01
Rhinorrhea	10/89 (11.2%)	48/165 (29.1%)	< 0.01
Diarrhea	15/97 (15.5%)	19/180 (10.6%)	0.24
Abdominal pain	20/89 (22.5%)	26/163 (16.0%)	0.20
Vomiting	19/99 (19.2%)	22/177 (12.4%)	0.13
Headache	40/92 (43.5%)	28/95 (29.5%)	0.05
Fatigue or malaise	23/92 (25.0%)	24/108 (22.2%)	0.64
Myalgia	11/67 (16.4%)	21/92 (22.8%)	0.32
Neurologic involvement	2/70 (2.9%)	3/129 (2.3%)	1.00[a]
Psychiatric	2/59 (3.4%)	1/121 (0.8%)	0.25[a]
Bleeding gums and/or nose	4/97 (4.1%)	9/185 (4.9%)	1.00[a]
Enlarged liver	1/60 (1.7%)	2/138 (1.5%)	1.00[a]
Conjunctivitis	2/74 (2.7%)	5/169 (3.0%)	1.00[a]

[a]% =(no. patients with symptom/no. patients for whom presence or absence of symptom was recorded)*100.
[b]Fisher's exact Test.

Table 3: Relative risk of survival for treated vs. untreated patients by time from symptom onset to oseltamivir treatment

From symptom onset to treatment initiation	Oseltamivir treated survived/total (%)	No antiviral survived/total (%)	Difference in % Survived	Relative Risk	95% CI	P value
Confirmed cases (N = 153)						
0-2 days	5/8(63)	11/97 (11)	0.51	5.51	2.54, 11.94	0.002
3-5 days	6/18 (33)	13/97 (13)	0.20	2.49	1.09, 5.68	0.076
6-8 days	2/22 (09)	13/76 (17)	-0.08	0.53	0.13, 2.18	0.509
9-11 days	1/14 (07)	12/42 (29)	-0.21	0.25	0.04, 1.75	0.149
≥12 days	0/1 (0)	12/21 (57)	-0.57	0.00	NA	0.455
Possible cases (N=200)						
0-2 days	43/46 (93)	70/79 (89)	0.05	1.05	0.95, 1.18	0.533
3-5 days	50/50 (100)	69/79 (87)	0.13	1.14	1.05, 1.25	0.007
6-8 days	7/10 (70)	69/76 (91)	-0.21	0.77	0.51, 1.16	0.088
9-11 days	3/5 (60)	67/72 (93)	-0.33	0.64	0.31, 1.32	0.063
≥12 days	2/4 (50)	66/69 (96)	-0.46	0.52	0.20, 1.39	0.022

A larger proportion of possible cases were treated with oseltamivir (60.5% v. 47.1% respectively). Overall, oseltamivir treatment showed a benefit, with a tripling in the survival benefit in confirmed cases compared with possible cases (a 10.4% reduction in case fatality for confirmed cases versus a 3.2% reduction in possible cases, $p < 0.01$). The CFR in untreated cases was significantly higher ($p < 0.01$) in confirmed cases compared to possible cases (84.0% v 13.9%). The greatest benefit is seen for confirmed cases treated with oseltamivir within two days of symptom onset (RR of survival = 5.51, 95% CI 2.54-11.94, $p = 0.002$) with a benefit of treatment still evident when treatment was initiated up to five days after symptom onset (RR=2.46, 95% CI 1.09-5.68, $p = 0.076$) (Table 3). In possible cases, however, the benefit of early treatment is not evident (RR of survival when treated 0-2 days after symptom onset =1.05, CI 0.95, 1.18).

Discussion

We undertook this study to examine whether any symptoms at presentation for medical care or avian exposure would differentiate true cases from possible cases occurring during the time of a confirmed H5N1 outbreak, and to understand treatment effectiveness for these two groups. Recognizing the limitations of retrospective analyses which depend on the widespread availability of reliable clinical and outcome data from many countries, it is also worth nothing that the WHO definition of confirmed H5N1 may be overly restrictive since many cases with documented H5N1 infection in this registry had symptoms that were different from the WHO definition. Nonetheless, not surprisingly, the mortality among true H5N1 cases was much higher than possible cases (79.1% v. 12.0%). This marked difference in mortality appears unrelated to medical care, since it appears that once a H5N1 outbreak has been confirmed, possible cases present directly to emergency care facilities and are hospitalized and treated quickly, most likely due to increased awareness.

Direct or indirect contact with sick or dead poultry or wild birds did not serve to differentiate true and possible cases. Indirect exposure to sick or dead poultry was more common in possible cases, most likely reflecting the fact that these suspected cases occurred in regions where outbreaks were occurring. True cases were more likely to have reported contact with another confirmed case.

The available data on symptoms at presentation indicate few differences between true and possible cases. The largest differentiator for true cases was a 47.4% increase in the rate of headache. Even classic warning signs such as tachypnea and cyanosis gave no guide to etiology. In contrast, possible cases were 8.6 times as likely to present with excessive sputum production (37.8% v. 4.4%) and 2.6 times as likely to present with rhinorrhea (29.1% v. 11.2%) as true cases. It is worth noting that rhinorrhea at presentation should not be excluded in the differential diagnosis of H5N1, since we have shown that rhinorrhea at presentation for true H5N1 infection in children aged 5 years or younger is associated with improved survival [10].

Oseltamivir showed substantial effectiveness for H5N1, but not for ILI. Further, cases who survived at least 6 days from symptom onset without having been treated with an antiviral did not benefit from delayed initiation of oseltamivir. The low survival rates shown when oseltamivir is initiated more than a week or so after symptom onset for both confirmed and possible cases may reflect a last-ditch treatment in situations where death is imminent, but also may simply be an artifact caused by small numbers of cases. Thus these findings provide empirical evidence for the benefits of even delayed initiation of antiviral treatment for confirmed cases, a notion that was posed in 2005 [11] but not confirmed until 2010 [4].

Examination of the times from symptom onset to various interventions reveals that in an outbreak, cases that present early in the outbreak's course are unlikely to be immediately recognized, and are consequently disadvantaged by delays in diagnosis and initiation of appropriate antiviral therapy, with the lack of clinical differentiators to signal true H5N1 infection further compounding the difficulties clinicians face [12]. It is evident that oseltamivir confers a survival benefit for true cases of H5N1, and when confronted with cases with influenza-like symptoms during an outbreak and in the absence of immediate virological testing, clinicians should seriously consider initiating antiviral treatment as soon as possible. Such an approach may be life-saving, especially in settings devoid of rapid virological diagnostic services.

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References

1. Adisasmito W (2010) Epidemiology of avian influenza in Indonesia-2005-2009: a descriptive analysis. Med J Indones 19: 64-70.
2. Kandun IN, Tresnaningsih E, Purba WH, Lee, V, Samaan G, Harun S, Soni E, Septiawati C, Setiawati T, Sariwati E, Wandra T (2008) Factors associated with case fatality of human H5N1 virus infections in Indonesia: a case series. The Lancet 372: 744-749
3. http://www.who.int/influenza/human_animal_interface/EN_GIP_20120810CumulativeNumberH5N1cases.pdf
4. World Health Organization. Evolution of H5N1 avian influenza virus does not increase risk to public health. 30 August 2011. Available at: www.who.int/influenza/human_animal_interface/avian_influenza/h5n1-2011_08_30/en/index.html. Accessed 10 October 2011.
5. Fouchier RAM, Herfst S, Osterhaus ADME. (2012 Public health and biosecurity. Restricted data on influenza H5N1 virus transmission. Science 336: 662-663.
6. Zaman M, Toovey S, Dreyer NA (2011) An extended review of human infection with influenza H5N1 virus in North West Frontier Province (NWFP), Pakistan in 2007. Emerg Infect Dis 17: 1056-1059
7. Adisasmito W, Chan PK, Lee N, Oner AF, Gasimov V, Aghayev F, Zaman M, Bamgboye E, Dogan N, Coker R, Starzyk K, Dreyer NA, Toovey S (2010) Effectiveness of antiviral treatment in human influenza H5N1 infections: analysis from a global patient registry. J Infect Dis 202: 1154–1160.
8. Dreyer NA, Toovey S, Oner AF, Bamgboye E, Dogan N, Zaman M, Gasimov V, Adisasmito W, Coker R, Chan PKS, Lee N, Tsang O, Hanshaoworakul W, Phommasack B, Touch S, Swenson A, Reddy D (2013) Investigating outbreaks of novel infectious disease: an international case study. Journal of Clinical Studies 5: 52-53.
9. http://www.who.int/influenza/resources/documents/case_definition2006_08_29/en/. Last accessed 17 May 2013
10. Oner AF, Dogan N, Gasimov V, Adisasmito W, Coker R, Chan PK, Lee N, Tsang O, Hanshaoworakul W, Zaman M, Bamgboye E, Swenson A, Toovey S, Dreyer NA (2012) H5N1 Avian Influenza in Children. Clin Infect Dis 55: 26-32.
11. The Writing Committee of the World Health Organization (WHO) Consultation on Human Influenza A/H5 (2008) Update on Avian Influenza A (H5N1) Infection in Humans. New Engl J Med 353: 1374-1385.
12. Adisasmito W, Nur Aisyah D, Yoga Aditama T, Kusriastuti R, Trihono, Suwandono A, Dwi Sampurno O, Prasenohadi, Sapada NA, Mamahit MJN, Swenson A, Dreyer NA, Coker RJ (2013) Human influenza A H5N1 in Indonesia health care service-associated delays in treatment initiation. BMC Public Health 13:571-577.

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