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TESTING OF HUMAN SPECIMENS FOR THE PRESENCE OF HIGHLY PATHOGENIC ZOONOTIC AVIAN INFLUENZA VIRUS A(H5N1) IN POLAND IN 2006–2008 – JUSTIFIED OR UNNECESSARY STEPS?

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Abstract: Since 1997, human infections with highly pathogenic zoonotic avian influenza viruses have shown that the risk of influenza pandemic is significant. In Europe, infections caused by the highly pathogenic avian influenza A(H7N7) virus were confirmed in the human population in 2003 in the Netherlands. Moreover, outbreaks of A(H5N1) infections were observed in wild and farm birds in different European regions, including Poland in 2006–2008. This study presents 16 patients in Poland from whom clinical specimens were collected and tested for A(H5N1) highly pathogenic avian influenza. This article shows the results of laboratory tests and discusses the legitimacy of the collection and testing of the specimens. All patients were negative for A(H5N1) infection. Nevertheless, only two patients met clinical and epidemiological criteria from the avian influenza case definition. The conclusion is that there is still a strong necessity for increasing the awareness of medical and laboratory staff, as well as the awareness of some occupational groups about human infections with avian influenza viruses, including the importance of seasonal influenza vaccination. It should also be emphasized that in the case of patients suspected of being infected with avian influenza, the information about clinical symptoms is insufficient and must be accompanied by a wide epidemiological investigation.

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INTRODUCTION

Between May 1997–14 January 2009 a total number of 414 human infections caused by the A(H5N1) highly pathogenic avian influenza (HPAI) were laboratory confirmed, including 255 cases which resulted in death (61.6%) [18, 19]. These cases occurred in 16 countries: Indonesia (33.6% of cases), Viet Nam (25.8%), Egypt (12.6%), China (7.5%), Thailand (6%), Hong Kong (4.8%), Turkey (2.9%), Azerbaijan (1.9%), Cambodia (1.9%), Iraq (0.72%), Pakistan (0.72%), Lao People's Democratic Republic (0.5%), Nigeria (0.24%), Djibouti (0.24%), Myanmar (0.24%) and Bangladesh (0.24%) [18, 19]. Only in Djibouti, Myanmar and Bangladesh no fatal cases were confirmed. In Europe, excluding Turkey, no human infections with A(H5N1) were registered.

Nevertheless, human infections with other highly pathogenic avian influenza virus of subtype A(H7N7) were confirmed in 89 patients in 2003 in the Netherlands, including one fatal case [7]. Moreover, outbreaks of A(H5N1) HPAI were observed in wild birds or poultry kept on commercial farms or backyards in different parts of Europe, including, e.g. Albania, Austria, Bosnia and Herzegovina, Bulgaria, Croatia, Czech Republic, Denmark, France, Germany,

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Greece, Hungary, Italy, Romania, Serbia and Montenegro, Slovakia, Slovenia, Spain, Sweden, Switzerland, Ukraine, and the United Kingdom [20]. In 2006, 2007 and 2008, infections with A(H5N1) HPAI also occurred in birds in Poland [20]. Between March–May 2006 there were confirmed infections in wild birds, i.e. swans (*Cygnus olor*) and gossanders (*Mergus merganser*) in 4 of the 16 administrative regions (voivodships) of Poland: Łódzkie (swans), Kujawsko-Pomorskie (swans), Lubuskie (swans) and Zachodniopomorskie (gossanders) [20]. Then, between November 2007–January 2008, infections with A(H5N1) HPAI occurred in poultry (laying hens, turkeys, ducks, geese) and in wild birds (stork and 2 buzzards) in 2 voivodships of Poland: Mazowieckie and Warmińsko-Mazurskie [20]. Since 1997, human infections with HPAI viruses, as well as many outbreaks observed in wild birds and birds kept on farms, show that the risk of a new influenza pandemic outbreak is significant. Common travelling of people, the role of birds – especially migratory birds – as the reservoir of influenza A viruses of different subtypes, together with the unstable nature of the influenza virus, make it imperative that influenza pandemic preparedness should be one of the most important priorities in the area of public health [3, 9].

Similarly, in recent times, the upsurge in the influenza H1N1 human cases and the declaration of H1N1 influenza pandemic by the WHO, affirmed that influenza studies and research should continue unabated. This also means that influenza surveillance cannot be performed only during the epidemic season, but authorities at all levels should be ready to monitor and identify infections that may occur at other periods of the year, which may be caused by typical avian influenza virus of subtypes, including those that do not normally occur in humans.

In the present study, 16 human patients from Poland were presented from whom clinical specimens were collected and sent to the National Influenza Centre (NIC), National Institute of Public Health – National Institute of Hygiene (NIPH–NIH), Warsaw, in order to test for the presence of A(H5N1) HPAI virus.

MATERIALS AND METHODS

Patients and clinical specimens. Between March 2006–February 2008 NIC, NIPH–NIH, Warsaw received clinical specimens collected from 16 patients living in different parts of the country with the information that they might have had contacts with A(H5N1) HPAI, and therefore these specimens should be tested for the presence of this virus (Fig. 1). Information on the type of specimens collected and diagnostic methods used is presented in Table 1.

Specimens were collected by the laboratory staff (specimen No. 1, 3–13), hospital staff (specimen No. 15), or from not well defined sample collectors (specimen No. 2, 14, 16). From all patients nasal and throat swabs were collected and placed in viral transport medium (specimen No. 3–13) or PBS or physiological saline (other samples). In 3 patients, bronchoalveolar liquid and serum were additionally obtained. All samples were collected between 5–15 days post symptoms (mean: 8 days).

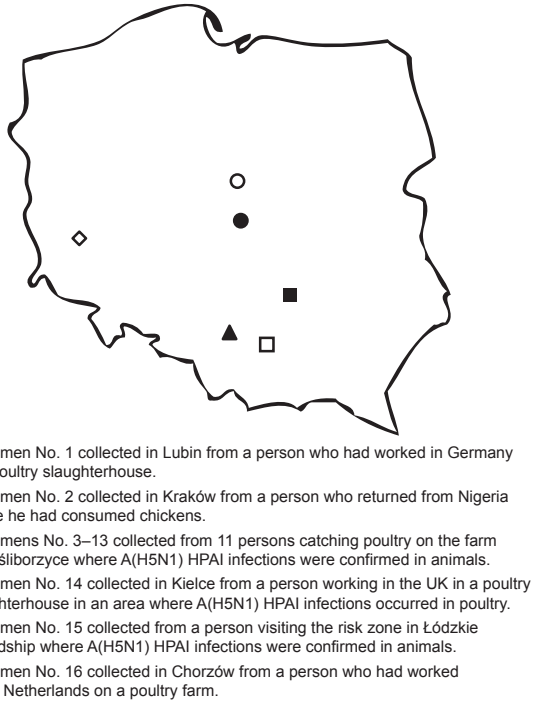

Figure 1. Map of Poland showing areas where clinical specimens were collected.

Direct immunofluorescence assay (DIF). As the preliminary screening, DIF was performed on the combined nasal and throat swabs of all 16 patients by using commercial kits *IMAGEN* (DakoCytomation Ltd., Glostrup, Denmark). Briefly described, monoclonal antibodies conjugated to fluorescein isothiocyanate (FITC) were directed against specific viral proteins of influenza A, influenza B, RSV, all serotypes of human adenovirus, parainfluenza type 1, parainfluenza type 2 and parainfluenza type 3. This assay was performed at NIC, NIPH–NIH, Warsaw (specimen No. 1, 14, 15, 16) or at the local laboratories of Voivodship Sanitary Epidemiological Stations (specimen No. 2, 3–13, 16).

Near patient tests. The specimens collected from 3 patients (No. 1, 14, 16) were additionally tested by near patient tests: *QuickVue Influenza Test* (Quidel, San Diego, USA), *BD Directigen Flu A+B* (Becton Dickinson, Franklin Lakes, USA), *BD Directigen EZ Flu A+B* (Becton Dickinson, Franklin Lakes, USA) or *Actim Influenza A&B* (Medix Biochemica, Kauniainen, Finland). All the above tests, excluding *QuickVue Influenza Test*, enable to differentiate the infections between influenza A and influenza B.

RT-PCR. Pooled nasal and throat swabs collected from all 16 patients, and the bronchoalveolar liquid collected from patient No. 1, were tested at NIC, NIPH–NIH by RT-PCR to detect RNA specific for hemagglutinin H5 and

Table 1. Types of specimens collected and diagnostic methods used.

No.	Date of specimen collection	No. of days between symptoms onset and specimen collection	Type of specimen collected	Method of testing/target
1.	21.03.2006	7	nasal swabs, throat swab, bronchoalveolar liquid, single serum	– near patient test: *QuickVue Influenza Test*/influenza antigens; – DIF[a]/antigens of seven respiratory viruses[b]; – RT-PCR/hemagglutinin H1, H3, HB; – RT-PCR/hemagglutinin H5, neuraminidase N1 (specific for H5 HPAI); – HAI[c]/antihemagglutinin antibodies against influenza strains: A/New Caldonia/20/99(H1N1), A/California/7/04(H3N2), B/Shanghai/361/02
2.	not known; specimens received on 20.06.2006	5 (?)	nasal swabs, throat swab	– DIF[a]/antigens of influenza A, antigens of influenza B; – RT-PCR/hemagglutinin H5, neuraminidase N1 (specific for H5 HPAI)
3.	05.12.2007	n.a.	nasal swabs, throat swab	see above
4.	05.12.2007	n.a.	nasal swabs, throat swab	see above
5.	05.12.2007	n.a.	nasal swabs, throat swab	see above
6.	05.12.2007	n.a.	nasal swabs, throat swab	see above
7.	05.12.2007	n.a.	nasal swabs, throat swab	see above
8.	05.12.2007	n.a.	nasal swabs, throat swab	see above
9.	05.12.2007	n.a.	nasal swabs, throat swab	see above
10.	05.12.2007	n.a.	nasal swabs, throat swab	see above
11.	05.12.2007	n.a.	nasal swabs, throat swab	see above
12.	05.12.2007	n.a.	nasal swabs, throat swab	see above
13.	05.12.2007	n.a.	nasal swabs, throat swab	see above
14.	27.12.2007 28.12.2007	6, 7	nasal swabs, throat swab; nasal swabs, throat swab; single serum	– near patient test: *QuickVue Influenza Test*/influenza antigens, *BD Directigen Flu A+B*/antigens of influenza A, antigens of influenza B; – DIF[a]/antigens of 7 respiratory viruses[b]; – RT-PCR/hemagglutinin H5, neuraminidase N1 (specific for H5 HPAI); – HAI[c]/antihemagglutinin antibodies against influenza strains: A/Solomon Islands/3/2006(H1N1), A/Wisconsin/67/2005(H3N2), B/Malaysia/2506/2004
15.	04.01.2008	15	nasal swabs, throat swab	– DIF[a]/antigens of 7 respiratory viruses[b]; – RT-PCR/hemagglutinin H5, neuraminidase N1 (specific for H5 HPAI)
16.	06.02.2008, 11.02.2008	7, 10	nasal swabs, throat swab, single serum; nasal swabs, throat swab	– near patient test: *BD Directigen EZ Flu A+B*/antigens of influenza A, antigens of influenza B, *Actim Influenza A&B*/antigens of influenza A, antigens of influenza B; – DIF[a]/antigens of 7 respiratory viruses[b]; – RT-PCR/hemagglutinin H5, neuraminidase N1 (specific for H5 HPAI); – Real time PCR/hemagglutinin H5, neuraminidase N1 (specific for H5 HPAI); – HAI[c]/antihemagglutinin antibodies against influenza strains: A/Solomon Islands/3/2006(H1N1), A/Wisconsin/67/2005(H3N2), B/Malaysia/2506/2004

[a] direct immunofluorescence assay
[b] influenza A, influenza B, RSV, adenovirus, parainfluenza type 1, parainfluenza type 2, parainfluenza type 3
[c] hemagglutination inhibition test

Table 2. Characteristics of patients from whom clinical specimens were collected to confirm or exclude infection with A/H5N1/ HPAI.

No.	Patient's age (years)	Sex	Date of symptoms onset	Symptoms
1.	39	female	14.03.2006	fever up to 40°C, shortness of breath, cardiac arrest (on 21.03.2008)
2.	53	male	15.06.2006	fever up to 40°C
3.	52	male	no symptoms	n.a.
4.	39	male	no symptoms	n.a.
5.	19	male	no symptoms	n.a.
6.	22	male	no symptoms	n.a.
7.	50	male	no symptoms	n.a.
8.	29	male	no symptoms	n.a.
9.	49	male	no symptoms	n.a.
10.	27	male	no symptoms	n.a.
11.	49	male	no symptoms	n.a.
12.	36	male	no symptoms	n.a.
13.	24	male	no symptoms	n.a.
14.	29	male	21.12.2007	fever ≥39°C, chills, headache, malaise, vomiting, cough, shortness of breath
15.	44	male	20.12.2007	fever, muscular weakness of lower limbs, diarrhoea, retention of urine, cough, shortness of breath
16.	24	male	30.01.2008 05.02.2008	on 30.01.2008., during a stay in the Netherlands, fever up to 40°C, lymphadenitis, muscular weakness of lower limbs, sore throat, cough (regression of symptoms after irregular taking antibiotic of unknown name in the Netherlands); since 05.02.2008 fever 37.6°C up to 38°C, general weakness, muscular weakness and rash on lower limbs, myalgia, lymphadenopathy, polyneuropathy

neuraminidase N1 of A/H5N1 HPAI. In patient No. 1, RT-PCR to detect RNA specific for hemagglutinin H1, H3 and hemagglutinin of influenza B (HB) was also performed. Briefly described, RNA was extracted by using QIAamp Mini Elute Virus Spin Kit (Qiagen, Valencia, CA, USA). RT-PCR was performed by using One Step RT-PCR Kit (Qiagen, Valencia, CA, USA) and the following primers: H5-1: 5`GCC ATT CCA CAA CAT ACA CCC 3`, H5-3: 5` CTC CCC TGC TCA TTG CTA TG 3`, N1-1: 5` TTG CTT GGT CGG CAA GTG C 3`, N1-2: 5` CCA GTC CAC CCA TTT GGA TCC 3` [17]. Primer sequences used for hemagglutinin H1, H3 and HB were obtained from World Health Organization Collaborating Centre (WHO CC), London. RNA of A/Duck/Vietnam/TG24-01/05 inactivated strain (Robert Koch Institute, Germany) was used as positive control for A(H5N1). Positive control for hemagglutinin H1, H3, HB was RNA of an appropriate influenza reference strain of subtype A(H1N1), A(H3N2) and type B, respectively. The following cycling conditions were used in the RT-PCR reactions: reverse transcription at 50°C for 30 min., polymerase activation at 95°C for 15 min., denaturation (95°C for 15 sec.), annealing (50°C for 15 sec.) and extension (72°C for 2 min.). 45 cycles were observed for H5 and 35 cycles for H1, H3, HB. Final extensions were done at 72°C for 10 min. Electrophoresis of RT-PCR products was performed on 2% agarose gel with ethidium bromide (10 mg/ml) in TAE buffer at 65V–70V for 30–45 min., and viewed by ultraviolet (UV) transillumination.

Real-time PCR. This reaction was performed at NIC, NIPH–NIH on the pooled nasal and throat swabs from patient No. 16 to detect RNA specific for hemagglutinin

Exposure to A/H5N1/ HPAI	Seasonal influenza vaccination in a current epidemic season	Hospitalization; treatment with antivirals
exposure not confirmed; patient returned from Germany, where she worked in a poultry slaughterhouse	no	yes; no antiviral treatment
exposure not confirmed; patient returned from Nigeria, where he consumed cooked chickens	?	yes; no antiviral treatment
exposure confirmed; person caught poultry on 28/29 November 2007 on a poultry farm in Myśliborzyce (Mazowieckie voivodship, Poland), where infections of animals with A/H5N1/ were confirmed	no	no; no antiviral treatment
see above	no	no; no antiviral treatment
see above	yes	no; no antiviral treatment
see above	no	no; no antiviral treatment
see above	no	no; no antiviral treatment
see above	no	no; no antiviral treatment
see above	no	no; no antiviral treatment
see above	no	no; no antiviral treatment
see above	no	no; no antiviral treatment
see above	no	no; no antiviral treatment
see above	no	no; no antiviral treatment
see above	no	no; no antiviral treatment
exposure possible; patient returned from UK, where he worked in a poultry slaughterhouse in an area designated as a Restricted Zone (until 19.12.2008) due to A/H5N1 infections in poultry (Ipswich District)	no	yes; treatment with oseltamivir since 28.12.2007
exposure not confirmed; patient patrolled for 1 hour on 10–12 December 2007 the risk zone in Łódzkie voivodship (Poland), where infections of animals with A/H5N1/HPAI were confirmed; no contact with animals, patient stayed all this time in the car	no	yes; treatment with oseltamivir since 02.01.2008
exposure not confirmed; patient returned from the Netherlands, where he worked on a poultry farm	no	yes; treatment with oseltamivir since 06.02.2008

H5 and neuraminidase N1 of A(H5N1) HPAI. RNA was extracted by using QIAamp Mini Elute Virus Spin Kit (Qiagen, Valencia, CA, USA). Transcriptor First Strand cDNA Synthesis Kit (Roche, Indianapolis, IN, USA) was used for the reverse transcription that was performed at 50°C for 60 min., and then at 85°C for 2 min. Real-time PCR was performed on the Lightcycler 2.0 (Roche) with the Lightcycler Taqman Master (Roche, Indianapolis, IN, USA). Sequences of the primers and probes as well as the temperature conditions were obtained from the procedure available for the members of the European Influenza Surveillance Scheme in the context of the Community Network of Reference Laboratories for Human Influenza in Europe, 29 August 2007. RNA of A/Duck/Vietnam/TG24-01/05 inactivated strain (Robert Koch Institute, Germany) was used as positive control.

Hemagglutination inhibition test (HAI). Serum specimens from the patients No. 1, 14 and 16 were tested at NIC, NIPH–NIH by the HAI test to detect antihemagglutinin (anti-HA) antibodies against seasonal influenza strains A(H1N1), A(H3N2) and B, circulating in a given epidemic season [8].

RESULTS

Data collected through the *Specimen forms* showed that the age of the patients ranged between 19–53 years (mean: 36.6 years). One or more clinical symptoms occurred in only 5 patients (31%), and all of them were hospitalized; the other 11 patients (69%) had no symptoms. Only one of the 16 patients was vaccinated against seasonal influenza in the current epidemic season. Detailed information on the

patients: their age, sex, symptoms, history of exposure to A(H5N1) HPAI, vaccination status, hospitalization as well as antiviral treatment is presented in Table 2.

Information included in the *Specimen forms* were analyzed according to influenza case definition proposed by the European Commission (EC) in 2002, and EC avian influenza case definition from 2008 [1, 2]. In this study, clinical criteria included in the influenza case definition proposed by EC were met in 4 patients (No. 1, 14, 15, 16), i.e. 25% of all patients under investigation (Tab. 2) [1, 2]. According to case classification proposed by EC, a possible case of avian influenza A/H5 or A/H5N1/ in humans is a case meeting the clinical and epidemiological criteria [2, 6, 17]. Therefore, epidemiological information available on the patients was also analyzed in this study. In 11 patients (No. 3–13) epidemiological criteria were met (69%), in 3 patients (No. 2, 15, 16) these criteria were not met (19%), while in 2 patients (No. 1, 14) information included in the Specimen forms was insufficient to make such assumption (12%) (Tab. 2) [2]. Clinical criteria, together with epidemiological criteria of avian influenza infection, were possibly met only in 2 patients (No. 1, 14), but this is questionable as the information about these 2 patients and their possible exposure to HPAI was insufficient to make a reliable assessment of the infection risk.

The results of near patient tests, DIF assay, RT-PCR and real-time PCR were negative for all tested specimens. In the case of patient No. 14, a weak band on the gel for H5 was noted; however, weaker than the band observed for the control amplification of influenza A/Duck/Vietnam/TG24-01/05 (H5N1). Considering the above finding and according to WHO criteria for accepting positive PCR test results of H5 infections in humans, the original specimen of patient No. 14 was sent to the WHO Collaborating Centre for Reference and Research on Influenza, London, UK, as well as to the National Veterinary Institute in Puławy, Poland [16]. The results of testing performed in Puławy (real-time PCR for H5 and N1 specific for H5N1 HPAI) and in London (real-time PCR for M gene of influenza A, NS gene of influenza B, H1, H3, H5, H7, H9, N1 specific for H5N1 HPAI; RT-PCR for H5 and N1, isolation of virus on chicken embryos), were negative.

Anti-HA antibody titers measured by HAI test in the patients No. 1 and 16 were low (HAI titer amounting to 10) or not detectable (HAI titer <10). In the case of patient No. 14, HAI titers amounted to: 40 for hemagglutinin H1, 80 for hemagglutinin H3 and 20 for hemagglutinin HB.

DISCUSSION

In 1996, influenza virus A(H5N1) HPAI was isolated from a farm goose in Guangdong Province, China. Soon, in 1997, human infections with A(H5N1) HPAI were confirmed in Hong Kong [18]. From then on, HPAI outbreaks in poultry and wild birds, as well as infections in humans, have been registered every year in different parts of the world. Until 2006, Poland did not experience any infections caused by A(H5N1) HPAI in animals or humans. All decisions and measures taken by the veterinary sector during the outbreaks of A(H5N1) HPAI in poultry and wild birds between May 2006–January 2008 in Poland enabled rapid control of this situation and the effective prevention of further spreading the infection. In November 2005, the Chief Sanitary Inspector implemented a regulation containing standard proceedings for health protection in a case of A(H5N1) HPAI appearance in Poland. This regulation also included guidelines for the collection of human specimens for laboratory diagnosis of avian influenza infection prepared by the NIC, NIPH–NIH, Warsaw, on the basis of a WHO document from January 2005 [11]. The information, received by the NIC, NIPH–NIH on the clinical specimens collected from 16 patients presented in this paper, showed that the knowledge of the above guidelines was insufficient. The reason is that the specimens (nasal and throat swabs) were collected 5–15 days (mean: 8) after the onset of symptoms, while the first 3 days are the best optimal time for this type of material, although virus detection is theoretically possible until the end of the second week after the onset of symptoms [11, 14]. Moreover, some of these specimens, excluding patients No. 3–13, were collected and placed in PBS or physiological saline, and not on appropriate viral transport medium that should ensure the stability of the virions and viral proteins. Similarly, there are some reservations about the sera as only single and not paired (acute and convalescent phase) samples were collected in the acute phase of the disease (7 days after onset of symptoms) [11]. This rendered the reliable interpretation of the results of HAI test almost impossible. Single serum samples would be useful, but only if collected in the convalescent phase, i.e. 3–4 weeks after onset of symptoms [14]. As a matter of fact, HAI test was not performed to measure antibodies against A(H5N1) HPAI, as the NIC, NIPH–NIH has no capacity to do that by any method. Nevertheless, it should be emphasized that a lack of detectable anti-HA antibodies or low antibody titers (<10, 10) observed in sera of 2 patients without the information about the second serum sample, do not exclude infection caused by seasonal influenza. The inappropriate collection of the clinical samples described above calls for concern since there were prepared guidelines according to a WHO document on the collection of specimens for laboratory diagnosis of avian influenza, and these rules are also applicable to specimens collected for diagnosis of seasonal influenza (e.g. collection of nasal and throat swabs no later than 5 days after the onset of symptoms, collection of 2 serum samples). Similarly important is the fact that the negative results may not be unconnected with antiviral therapy. Specimens collected after 3 days of treatment may be negative [14]. Among the 16 patients described in this article, 3 of them (19%) received oseltamivir, including patient No. 15 who was swabbed 2 days after the onset of antiviral therapy. The other 2 patients received oseltamivir immediately after the collection of specimens.

In general, some of the cases described in this article clearly show that the epidemiological information of suspected patients was lacking in precision and details, and these limit the assessment of the risk of infection with HPAI. Although 69% of the cases (No. 3–13) met the epidemiological criteria for risk of avian influenza, these individuals wore protective personal equipment (PPE), hence the risk of infection with HPAI may be considered as low [2]. In 19% of the patients (No. 2, 15, 16), no epidemiological link indicating the possible contact with HPAI was found [2]. One of these patients (No. 2) consumed chickens in Nigeria, but it is known that the meat was cooked. Thus, the possibility of contacting avian influenza infection through this means could be excluded [5]. The other argument is that in 2006 A(H5N1) HPAI outbreaks in Nigeria were registered in wild birds and poultry farms only between January–March, while the symptoms occurred in patient No. 2 in June 2006 [20]. The other patient (No. 15) was present in the area where A(H5N1) HPAI infections in birds were confirmed. Nevertheless, the information received by NIC, together with the specimens, suggested that this patient did not have any direct and close contact with the sick, dead animals, or other contaminated materials. Similarly in patient No. 16 there was no risk of infection with HPAI. On the one hand, close contact with birds occurred as this patient worked on a poultry farm in the Netherlands in 2008; but on the other hand, there had not been any HPAI outbreaks in that country since the A(H7N7) HPAI infections in 2003 [20]. In 12% of the patients (No. 1, 14), the reliable assumption of the risk of infection with HPAI could not be made. In one of these patients (No. 1), it was only known that this person worked in a poultry slaughterhouse in Germany, and the symptoms appeared on 14 March 2006. According to the OIE, it is known that between February–March 2006 there were A(H5N1) HPAI outbreaks in wild birds in Germany [20]. Thus, some risk of human infection theoretically existed. Nevertheless, there is no detailed information where this patient exactly worked and lived during his stay in Germany. Similarly, information about the other patient (No. 14) did not allow for reliable assessment of the risk of infection with HPAI. This patient was present in the Restricted Zone due to A(H5N1) infections in poultry, but it is not known whether there is any possibility of close contact with sick or dead birds, their faeces, secretions, body fluids, feathers or contaminated surfaces. If there were no contacts of this type, then the risk of infection should be considered as very low as almost all human infections with A(H5N1) HPAI registered so far resulted from close contact with infected birds [5, 14]. Actually, since there is insufficient epidemiological information on these 2 patients (No. 1, 14) they are the only cases among the 16 patients which could be considered as cases meeting both clinical and epidemiological criteria of possible avian influenza infection.

It should be emphasized that avian influenza case definition as proposed by the EC was intended to be used for the purposes of surveillance and reporting of cases to the Community network, and not necessarily for diagnosis as it was done in the case of the patients described in this article [2]. Nevertheless, such definition may be helpful to track and identify the cases and decide from whom clinical material for laboratory testing should be collected [6, 13].

The events described in this article may be considered as a kind of exercise and experience. They enabled the identification of weak links in the actions that should be taken in the situation when human infections with HPAI are suspected.

In the cases presented in this paper, actions taken by physicians and staff of the local laboratories showed their readiness for quick response in the case of A(H5N1) or other HPAI threat to human population. On the one hand, the legitimacy of the collection of clinical material from some of the patients was questionable and the risk of HPAI infection seemed to be overestimated. On the other hand, taking into consideration the fact that bird infections with A(H5N1) HPAI in Poland was a relatively new phenomenon occurring sporadically to date, collection of the clinical samples for laboratory diagnosis of avian A(H5N1) influenza may be considered as the correct decision, showing that the danger of avian influenza to human is well known in this environment. Nevertheless, the described cases clearly show that the level of preparedness of physicians and laboratories for such events is still an area that should necessarily be improved. Therefore, in December 2007, NIC at NIPH-NIH, Warsaw, prepared instructions on the collection of human clinical specimens from patients suspected to be infected with avian influenza HPAI, together with a form for clinical and epidemiological information about the patient that should be completed by a physician and laboratory. These guidelines were distributed by the Chief Sanitary Inspector to all Voivodship Sanitary-Epidemiological Stations. In these instructions, it was emphasized that it is difficult to determine clinical symptoms that could be specific only for A(H5N1) HPAI infection. Therefore, when taking a decision on the performance of laboratory diagnostic tests for avian influenza infection it should be considered that the clinical picture of the disease may be different from typical influenza and avian influenza cases registered so far. During a A(H7N7) HPAI outbreak in 2003 in the Netherlands the most characteristic, and sometimes the only symptom, was conjunctivitis. For this reason, keeping exactly to the clinical symptoms mentioned in the definitions of influenza and avian influenza may be inappropriate. Therefore, some flexibility is required as the new emerging virological, clinical and epidemiological data may impose a necessity for modification of the existing case definition [12].

A question arose about why NIC at NIPH-NIH, Warsaw, agreed to perform laboratory diagnostic tests for avian influenza in the cases where information in the *Specimen forms* allowed exclusion of the possibility of A(H5N1) HPAI infection. The reason was that epidemiological

information on the patients was incompleted or insufficient, thus the reliable risk assessment of HPAI infection was difficult. Nevertheless, NIC decided to perform laboratory testing to avoid any negative impressions from the medical staff and patients, especially in the situation where their awareness of avian influenza was limited to the fact that the infection in humans is extremely dangerous and may result in death. This was confirmed in a study performed in Poland between September–November 2005, where 61% of the respondents thought that they were likely or very likely to become infected with avian influenza if an outbreak occurred in Poland [4]. This was the highest level of risk perception among 5 European countries and 3 East Asian areas where the study was performed [4]. Nevertheless, it should be stressed that the collection of clinical material and laboratory diagnostic testing for avian influenza should be conducted only in cases that are justified by the given clinical symptoms and epidemiological reasons. Otherwise, unnecessary costs, the burden on laboratories/NIC, increased risk perception in the community, aroused the interest of the media and may consequently generate negative effects.

CONCLUSIONS

The lessons learned from the cases described in this paper and conclusions are as follows:

- it is necessary to increase the awareness of medical staff, laboratory staff, and associated occupational groups about avian influenza viruses with regards to human infections: possible routes of infections, knowledge of "at risk" individuals, and consequently field surveillance, transportation of samples and laboratory diagnosis [6, 11, 15];
- should doubt arise in sample collection, the medical and laboratory staff should consult with the necessary authorities (NIC);
- details and more precise epidemiological information should be mandatorily collected by all medical and laboratory staff [15];
- it is necessary to emphasize the importance of seasonal influenza vaccination and field surveillance for people who are at high risk of infection with avian influenza virus. This will reduce the possibility of dual infections with seasonal influenza strain and avian influenza strain and possible reassortment that may result in the emergence of a novel influenza virus with a pandemic potential [5, 10].

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