EPIDEMIOLOGICAL AND VIROLOGICAL SURVEILLANCE OF INFLUENZA AND INFLUENZA LIKE ILLNESS IN SLOVENIA

EPIDEMIOLOŠKI I VIROLOŠKI NADZOR INFLUENCE I INFLUENCI SLIČNE BOLESTI U SLOVENIJI

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Summary

The number of patients with acute respiratory infections rises sharply shortly after the influenza virus appears in population. Consequently, the hospitalization and mortality rates increase. Several indicators may be used to measure the burden-of-illness caused by the influenza virus: incidence rate of influenza-like illness (ILI) or acute respiratory illness (ARI), crude or cause-specific mortality rate, sick-live or monitoring the over the counter (OTC) medication sales. Two indicators have been measured in Slovenia since 1999: the ILI and ARI incidence rates in approximately 4% of the population.

Multiplex Reverse Transcription-Polymerase Chain Reaction (RT-PCR) was used as a screening method for the detection of influenza viruses, respiratory syncytial virus (RSV), adenoviruses and enteroviruses in nasal and/or throat swabs. All positive samples were further propagated in the corresponding cell culture line. RT-PCR was used for fast determination of hemaglutinins (H1, H3) and neuraminidases (N1, N2) of influenza A viruses. The antigenic subtype of the samples isolated on the cell culture was determined by means of a hemaglutination inhibition assay and confirmed in the WHO European Reference Centre for Influenza.

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In the 2004/2005 season, the ILI incidence rate was at highest in weeks 5, 6 and 7 of the year 2005, when it reached its peak value (392/100,000). The highest ARI incidence rate was recorded in the period from 31st January to 6th February, and remained elevated for the following two weeks. Samples were taken from 793 patients with clinical signs of ILI. The influenza A or B virus was detected in 15.2% of patients. One third of the influenza A cases were of the H1N1 subtype, while the others were H3N2. Enteroviral infection was relatively common in the past season (15.8% of patients). The influenza isolates in Slovenia were similar to those in other European countries and belonged to: A/H3N2/California/7/2004 (A/H3N2/Fujian/441/2002-like viruses), A/H1N1/New Caledonia/20/99 and B/Jiangsu/10/03 (B/Shaghai/361/2002-like viruses).

The dominant influenza virus in Europe and the USA was influenza A H3N2. The ARI and/or ILI incidence rates were higher in most European countries (including Slovenia) compared to previous seasons.

Key words: Influenza-like illness; Acute respiratory infections; Sentinel surveillance; Influenza A; Influenza B; Multiplex RT-PCR

INTRODUCTION

Influenza is a communicable disease that contributes significantly to the morbidity and mortality of the population, specially during winter period [1, 2]. Influenza pose a constant threat for the outbreak of pandemia [3]. The World Health Organization (WHO) established a network for the surveillance of influenza in 1949. WHO supplies reagents for the determination of influenza virus antigens to all national influenza centres (NIC) once yearly. The sentinel surveillance system of influenza-like illness were established in some European countries in early fifthies. The tendency towards connecting these systems into a common European network began to intensify in the 1980's and, through a number of preliminary projects, led to the establishment of the European Influenza Surveillance Scheme (EISS) in 1996. The principal objective of EISS and its members is to collect and disseminate defined epidemiological and virological data on influenza-like illnesses in the population. The participating primary health-care physicians report the number of patients with influenza-like illness and/or acute respiratory illness, and send nasal and throat swab samples to the designated laboratories. EISS provides professional assistance to its members in the area of virological diagnostics, checks the quality of testing, and supplies reagents [4]. In Slovenia, a network for the sentinel surveillance of influenza-like illness was established in 1999. Slovenia became a full member of EISS in the 1999/2000 season.

People contract influenza each year in the so-called influenza season, which in the Northern Hemisphere lasts from fall until spring of the following year. The circulation

of the influenza virus increases, to a larger or smaller extent, the incidence rate of all acute respiratory illnesses, resulting in the absence of pupils from school and employees from work [5, 6]. The number of hospitalizations increases due to secondary pneumonia, heart failure, worsening of chronic obstructive pulmonary disease and asthma [7, 8]. The overall mortality rate increases, in particular mortality due to pneumonia in elderly persons over 65 years of age and small children [2, 9].

The influenza viruses circulating in a season usually differ slightly in their antigenic composition from the viruses of the previous season. The incidence rate and most affected age groups may therefore vary from season to season.

In addition to influenza viruses, acute respiratory infections may be caused by many other microorganisms, and the clinical presentation can be very similar [10]. Respiratory syncytial viruses (RSV), adenoviruses and enteroviruses were folowed beside the surveillance of influenza A and B viruses.

The purpose of epidemiological surveillance is to obtain adequate and timely information on the impacts of the circulation of the influenza virus in population [11]. Several approaches are employed to obtain data.

In addition to its diagnostic value, the virological surveillance of influenza-like illnesses has many other purposes. These include the early detection of the circulation of influenza viruses and the timely implementation of adequate measures, contribution to the annual determination of strains to be included in the influenza vaccine, and the detection of new strains that could cause a major epidemic or pandemic. Clinical signs similar to influenza are caused by many other microorganisms e.g. rhinoviruses, adenovirus, RSV and others. The decision was taken to monitor not only influenza A and B virus but also to detect RSV, enteroviruses and adenoviruses from clinical sample.

In the paper the approach to the surveillance of influenza in Slovenia and the results of surveillance in the 2004/05 season are presented.

MATERIALS AND METHODS

Influenza and other acute respiratory infections were monitored through sentinel surveillance system set up in 1999. The physicians participating in the sentinel system send, on a weekly basis, the number of patient visiting their primary care physicians due to influenza-like illness and other acute respiratory infections. The patients are separated into five age groups: 0 to 3 years, 4 to 7 years, 8 to 14 years, 15 to 19 years, 20 to 64 years, and over 65 years, which enables the calculation of age-specific incidence rate of influenza-like illness (ILI) and acute respiratory illness (ARI). In the 2004/05 season, 41 primary health care physicians (general practitioners and family physicians, pediatricians and specialists in school medicine) from all nine Slovene regions participated in

the network, thus ensuring proportionate geographic coverage. The physicians cover health care services to approximately 80,000 persons.

Before the beginning of the influenza season, the sentinel physicians received materials for sampling and dispatch of infectious materials from the upper respiratory tract (nose and throat swabs). Samples were taken from a patient when, in their opinion, his/her clinical presentation resembled influenza – fever, severe malaise, pain in the muscles and joints, dry cough. A short questionnaire, completed by physicians, providing the basic demographic and clinical data on patients was enclosed with each sample. To ensure the quality of epidemiological or virological surveillance of influenza, the samples of hospitalized patients should also be obtained in addition to the infectious materials of primary health-care patients. Most of the samples of hospitalized patients were from the Clinic for Infectious Diseases, Clinical Centre, Ljubljana.

When swabs were taken, they were stored in an EMEM transport medium (Eagle's minimum essential medium) and sent to our laboratory. Ribonucleic acid (RNA) was immediately extracted from each $200 \, \mu l$ sample. The High Pure Viral Nucleic Acid Kit (Roche diagnostic GmbH, Germany) was used according to the manufacturer's instructions. Antibiotics were added to a part of the sample and stored for inoculation in the cell culture, if the viruses being sought were found using the screening method. Reverse transcription polymerase chain reaction (RT-PCR) was used as a screening method. RT-PCR was performed on the same or the following day. The employed RT-PCR method combines, in a single reaction, five pairs of primers which enable the simultaneous amplification of target sequences of the genome of five different viruses (multiplex PCR). Primers for detecting the RNA of influenza A and B viruses, RSV, adenoviruses and enteroviruses cover highly conserved regions of the genome, allowing us to detect a broad range of viruses from these groups. The primers used, the reaction mixture and the temperature conditions for reverse transcription and PCR were previously described [12].

PCR products were analysed by electrophoresis in a 2% agarose gel using an UV transiluminator. In RT-PCR, the reaction products were labeled with a dioxigeninom-11-dUTP. This enabled the further confirmation of positive samples with a microwell hybridization analysis using biotinylated capture probes for the respective antigene [12].

In the 2003/04 season, we began to use the Multiplex RT-PCR method to determine the types of heamglutinins and neuraminidases in samples where the nucleic acid of influenza A was found, and to determine the RSV subtype when RSV nucleic acid was found. For type determination, the extracted RNA was first transcribed in the copy DNA (cDNA) with random hexamers, then the obtained cDNA was amplified in a PCR multiplex reaction, first with outer and then with inner pairs of primers. By this reaction

we determined a type of influenza B and two subtypes of influenza A (H1 and H3) [13]. To determine the influenza A subtypes with respect to neuraminidase, initial oligonucleotides were used in the multiplex PCR reaction, thus enabling the multiplication of a specific part of the genome of two influenza A subtypes (N1 and N2) [14]. PCR products were analysed by electrophoresis in agarose gel, as described in the previous paragraph.

In order to prevent cross contamination, the RNA extraction procedures, preparation of the master mix for RT-PCR, and work with the PCR products were conducted in separate rooms in conformity with applicable safety measures. All reactions were controled by the use of positive and negative controls. All the samples that gave positive RT-PCR results were inoculated into the continuous cell lines: the MDCK (Madin-Darby canine kidney), which is the recommended culture for propagating influenza viruses [15], the Hep2 (human epithelial pharynx cell line) for RSV, and the Hep2, RD (rabdomyosarcoma cell line) and GMK (Green monkey kidney cell line) for adenoviruses or enteroviruses [16]. The isolation of viruses was checked by observing the cytopathogenic effect on the cell culture, by direct immunofluorescence with monoclonal antibodies, and by repetitions of the RT-PCR reaction.

The titer of isolated influenza viruses were determined with a hemaglutination assay (HA) using chicken erythrocites. The antigenic determination of influenza viruses was carried out by inhibition of the hemagluination assay (IHA) using chicken erythrocites and WHO-provided antisera [17].

Selected isolates of influenza viruses were sent for confirmation and additional with and antigenic determination to the WHO reference laboratory in London.

All results were reported on a weekly basis to WHO and EISS, UK.

RESULTS

The influenza season begins on the first day of the 40th week, which is in the first days of October. In October, November and December, the number of patient visits to their chosen physicians due to acute respiratory infections was low (incidence rate from 1100 to 1400/100,000). In January, however, the number of patients with ARI began to increase rapidly, attaining its peak in the 5th week (from 31 January to 6th February 2005), when the incidence rate was 3120/100,000. The incidence rate remained elevated (over 3000/100,000) for another two weeks (6th and 7th week, from 7th February to 20th February 2005). After the 8th week, the number of patients began to decrease sharply, dropping to only 1240/100,000 in the 10th week (Fig. 1). As expected, the highest ARI incidence rate in the entire season was recorded in small children (around 4000/100,000 children under four years). In this age group, the number of patients rose steeply even before the
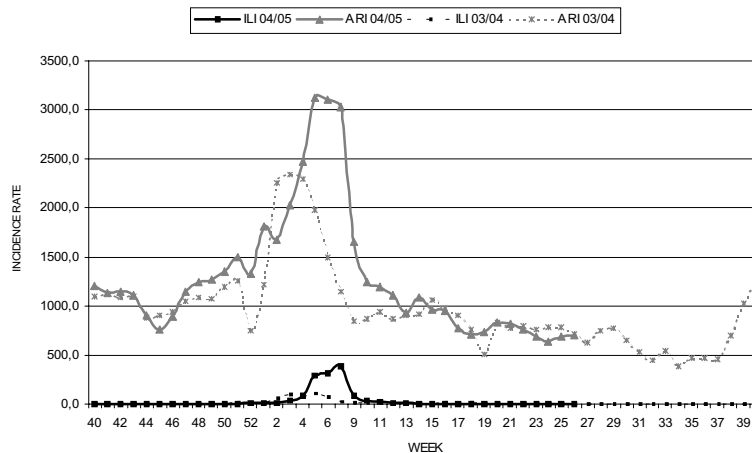
Fig. 1. ILI and ARI incidence rate – season 2003/04 and 2004/05

occurrence of influenza in the population, primarily on account of the intensive circulation of RSV. In weeks 5, 6 and 7, the incidence rate in all age groups increased 3 to 5 times in comparison to those weeks at the beginning or end of the season (i.e. at the beginning of October or in the second half of May).

The incidence rate of influenza-like illness in the 2004/05 season was highest in week 7 (392/100,000), and was only slightly higher than in weeks 5 and 6. When the influenza reached its peak, the highest incidence rate was registered in elementary and pre-school children.

In this season, we received throath and/or nasal swabs from 793 patients. It is recommanded to take the nose and throath swabs simultaneously in each patient, as this increases the possibility of isolating the influenza virus. This year, we received 486 paired swabs (nose + throat), 44 nose swabs and 263 throat swabs. The sentinel physicians took samples from 476 patients, while other primary health care physicians sent us 27 samples (mostly from the Ljubljana region). The specimens of 290 patients were received from hospitals, mostly from the Clinic for Infectious Diseases in Ljubljana (swabs of 271 patients). The samples of 18 patients were received from the Izola General Hospital and one from the Trbovlje General Hospital.

The specimens of 524 patients were negative for influenza A and B viruses, RSV, adenovirus and enterovirus. In the remaining patients (269 patients, 33.9%), the presence of one of the following viruses was confirmed by RT-PCR: 50 cases of influenza A virus (6.3%), 71 cases of influenza B virus (8.9%), 23 cases of RSV (2.9%), 18 cases of adenovirus (2.3%), and as many as 125 cases of enterovirus (15.7%). Two viruses were found simultaneously in 18 patients: influenza A and enterovirus in five patients, influ-
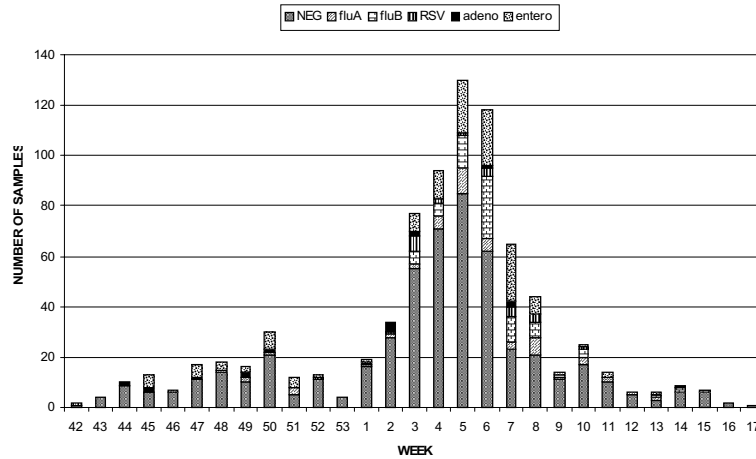
Fig. 2. The number of confirmed influenza A, influenza B, RSV, adenoviral and enteroviral infections during 2004/05 season

enza A virus and RSV in two patients, influenza B virus and enterovirus in eight patients, influenza B virus and RSV in one patient, and RSV and enterovirus (Figure 2) in two patients. In the previous season (2003/04), RT-PCR was used to prove the presence of influenza A virus in 9.0%, influenza B in 0.2%, adenovirus in 4.5% and enterovirus in 5.3% of samples, while RSV was not found in any sample (Fig. 3).

With regard of the time needed for samples to arrive in the laboratory, we didn't observed essential drop of positiveness rate obtained with RT-PCR. The longest time
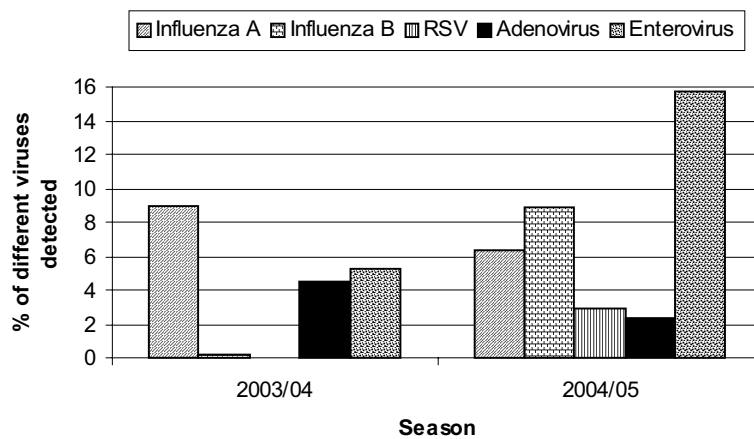

Fig. 3. Percentage of different viruses detected with RT-PCR in two seasons

period between the swab was taken and arrived in the laboratory was 4 days. The dealay of swab delivery has much bigger impact on the isolation in the cell culture, where each day more of the travel results in lower possibility of successful isolation.

In the 2004/05 season, the Multiplex RT-PCR method was used to determine the subtype of 35 samples that were influenza A positive. 11 samples were the H1N1 subtype, and 24 were the H3N2 subtype. Using an IHA and WHO antiserums, we found that the strains were similar to those included in the vaccine, both in influenza A and influenza B. This was also confirmed in the WHO reference laboratory by means of antigenic and genetic analyses. The strains found corresponded mostly to A/H1N1/New Caledonia/20/1999 and A/H3N2/California, which belongs to the A/H3N2/Fujian/441/2002-like group of strains. From influenza B subtype B/Jiangsu/10/2003 that belongs to the B/Sanghai/361/2002-like group of strains, was determined. In the previous season (2003/04), the multiplex RT-PCR method was used to determine the subtype of 37 samples that were influenza A positive. All were of the subtype H3N2. Using the IHA and the WHO antisera, we found that the strains were similar to those included in the vaccine (A/H3N2/Fujian/441/2002).

Data on previous influenza vaccinations was available for 643 patients, of whom 18 had been vaccinated. The presence of the influenza A virus was confirmed in one vaccinated patient, and the presence of the influenza B virus was confirmed in three vaccinated patients.

DISCUSSION

The principal task in the epidemiological and virological surveillance of influenza is the early detection of the influenza virus and the evaluation of its impact on human health. The incidence rate of influenza illness varies from season to season and depends on the type of circulating virus. The incidence rate is generally higher if the A H3N2 influenza virus is dominant in comparison with the influenza B virus or influenza A H1N1 [15].

The burden-of-illness caused by the influenza virus can be evaluated in different ways. In the USA, the collection of data for evaluating the impact of influenza in a particular season is directed towards virological surveillance and the surveillance of cause-specific mortality (pneumonia and influenza as causes of death) and specific mortality in a particular age group (mortality in persons under 18 years of age), as well as towards the incidence rate of hospitalizations of children under four years of age. A specific number of physicians send weekly information on the number of patient visits due to influenza-like illness [18]. In Europe, sentinel surveillance by physicians sending weekly data and collecting swabs in the influenza season was begun in England and Wales as early as in the 1960's, and later in France and in the Netherlands [19, 20]. In the

1990's, many EC countries introduced sentinel surveillance, among them also Slovenia in the late nineties [21]. In contrast to the USA, less emphasis is given in Europe to the surveillance of mortality by week, which is otherwise a good indicator of how serious the influenza process is. Owing to the current method of collecting data on deceased persons in Slovenia, we are unable to observe the impact of influenza on mortality because the information is collected and entered in the data base with a delay of at least one month. It would by all means prove interesting to obtain information on the number of small children (under four years of age) admitted to the hospitals because of acute respiratory illness or worsening asthma. To interpret such data, we would require a system of collecting data on cases of acute respiratory illness caused by RSV, as this is a frequent cause of hospitalization in this age group. Because RSV infections are not reported, we do not dispose with any epidemiological data. This will be changed in the new law on communicable diseases currently being prepared.

The Slovene system monitors the incidence rate of influenza-like illness separately from incidence rate of other acute respiratory infections. Such a division is far from ideal, as the clinical presentation of influenza is nonspecific and can easily be exchanged for any acute infections. Some European countries monitor only ILI, while others are of the opinion that influenza cannot be separated from other ARI and therefore monitor only ARI [21]. According to study reports, sudden fever and cough in adults offers the best positive predictive value for influenza, particularly in periods when there are numerous influenza cases in the community. According to the data of two studies involving patients over 12 years of age, the presence of throat pain had a negative predictive value [22, 23]. In both studies, the dominant isolate was the influenza A H3N2 virus. In a French study aimed at detecting clinical signs and symptoms forecasting a specific subtype of the influenza A virus, it was found by means of logistic regression that a temperature exceeding 38 °C, myalgia, rhinorrhea and coughing predict influenza A H3N2, while fatigue, conjunctivitis and absence of muscle pain predict influenza A H1N1 [24]. The study covered only one influenza season (1995/1996). It is even more difficult to define the typical clinical presentation of influenza in small children and elderly persons, who frequently do not have a very high temperature [25, 26]. For this reason we decided to conduct the surveillance of all acute respiratory infections through our network. Theoretically, this will allow us to detect increased number of patients with any type of respiratory infection. The sentinel system does not enable identification of the first cases of a new illness, e.g. the emergence of an imported case of avian influenza, as data is only taken from approximately 4% of the population and are aggregated. In case of a pandemic threat, the detection of the first cases will by all means be based on the obligatory reporting of each patient, and only later, when the pandemic has spread, on a sample of the population.

Last year, a one-month epidemic of acute respiratory infections was declared (from 11 February to 11 March 2005) because the incidence rate was the highest of all years in which ARI and ILI surveillance have been conducted in this way in Slovenia. It is not easy to define the epidemic threshold. In countries with many years of experience in influenza surveillance, the so-called baseline of influenza activity was determined. The baseline designates the incidence rate when there is no influenza in the community. The baseline incidence rate in Great Britain ranges from 0 to 30/100,000, the normal seasonal incidence rate of influenza is from 30-200/100,000, and the epidemic threshold is over 220/100,000 [27]. These limits are lower in Portugal, where the epidemic threshold has been set at 120/100,000 (28). It is still too early to say what the baseline in the Slovene system is, as we have only been conducting sentinel surveillance for a relatively short time.

In the 2004/05 season samples were obtained from 793 patiens and 15.2% were positive for the influenza A and B viruses altogether, yet considerable differences were found between the hospital samples and the samples received from sentinel physicians. The later reported a 19.3% patients positive to influenza A and B, while those patients hospitalized at the Clinic for Infectious Diseases were positive in 8.8%. A possible reason for the lower percentage of positive samples in hospitalized patients is that samples are taken in a later stage of illness. Patients are usually sent for an examination and hospital admission in a later stage and not on the first or second day of their illness, when the probability that the influenza virus will be confirmed is the highest. The age structure of patients of the Clinic for Infectious Diseases from whom we received infectious materials was different than in sentinel patients. 53.4% of sentinel patients were aged 4-19 years, while only 22.5% of patients from the Clinic for Infectious Diseases were in this age group. More samples were taken from hospitalized patients who were over 65 years of age and infants.

In this season, more influenza B virus infections were confirmed than influenza A infections. In Europe, the influenza A virus was dominant primarily in western countries, whereas a substantial number of cases of the influenza B virus was confirmed particularly in Poland, Romania, Latvia and Slovakia. According to EISS data, the influenza virus was confirmed in 14183 patients, who mostly had the influenza A virus (84%). Approximately three quarters of influenza A viruses were subtyped. Predominant among the subtyped viruses was the influenza H3N2 virus [29].

The dominant virus in the USA was influenza A, mostly the subtype H3N2 [30]. The highest percentage of influenza-positive samples was registered in the first week of February 2005 (27%). The dominant virus in Japan was influenza B [30]. It is difficult to explain what is the reason for difference found. It is possible that one subtype of the influenza virus is predominant in a specific geographic area. Beyond any doubt, the

method of collecting samples differs slightly from country to country (e.g. more samples from a specific age group, more samples of hospitalized patients, taking samples only in specific clinical cases, etc.), which renders difficult, if not enables , the comparison of virological results. More important than the percentage of individual types of influenza virus in a certain country is the detection, by means of virological surveillance, of those viruses for which a vaccine must be prepared in the upcoming season.

In the viruses isolated from each year epidemics showed strain differences when compared in the IHA ie. although the viruses belong to the same subtype, they do not cross react completely. These lesser antigenic changes are known as antigenic drift. Antigenic drift is thought to arise through natural mutation, and selection of new strains takes place by antibody pressure in an immune or partially immune population. Epidemics due to new virus strains arising due to antigenic drift is not as great as for those showing antigenic shift, since partial immunity is present in persons with cross- reacting antibody induced by previous infection. Large pandemics are caused due to antigenic shifts for influenza A viruses. This may occure whith reasortment when two different strains of influenza combine to form a new subtype having a mixture of the surface antigens of the two original strains.

With regard to the constant threat of the emergence of a new, highly transformed strain of the influenza virus, it is important to determine the subtypes of those samples found to be positive for the influenza A by the RT-PCR screening method. By proving subtypes H1 and H3, we eliminate other subtypes which are otherwise characteristic for the infections of other hosts (birds, pigs, horses), yet may be very dangerous for humans. If a sample that is positive to influenza A failed to give a positive result for H1 or H3, it would immediately be tested for the presence of all other hemaglutinins (H1 to H15, except H1 and H3, which had already been eliminated). The same applies for the testing of neuraminidase, where, in the event of a negative result of tests for N1 and N2, the sample would also be tested for neuraminidase from N3 to N9.

Antigenic determination of viruses is more time-consuming, as it initially requires the acquisition of a good isolate. WHO offers a range of reference antiserums that react with those viruses known to be circulating in the human population in past years and are also included in the vaccine. If we were to obtain an isolate of the influenza virus which did not react to any of the reference antiserums, this could indicates the existence of a new virus strain which could be highly transformed. In such cases, the WHO reference laboratory is immediately informed and the isolate is promptly sent for further analysis in a previously agreed way. The analysis of antigenic characteristics of viruses is of exceptional importance, as genetic changes do not always means changes in antigenic characteristics. However, quick molecular methods provide assistance and orientation and constant attention to any changes. Molecular methods also less sensitive to

the quality of samples (delay of the samples delivery to the laboratory) and enable detection of the viral nucleid acids as well in the samples from which isolation on cell culture wolud be impossible.

In addition to influenza viruses, acute respiratory infections may be caused by many other microorganisms, and the clinical presentation can be very similar (10). Using the RT-PCR method, RSV, adenoviruses and enteroviruses were detected beside the surveillance of influenza A and B viruses. It was found that the above-mentioned viruses often cause acute respiratory infections during the circulation of influenza viruses, and that the frequency of their occurrence in individual seasons varies considerably. For example, in the 2003/04 season RSV was not detected in any sample, while in the 2004/05 season it was proved in 2.9% of samples (Fig. 3). The percentage of samples with proven RSV was undoubtedly strongly influenced by the age structure of patients from whom swabs were taken, as it is known that infections are considerably more frequent among small children with RSV than in adults [31]. A higher percentage of RSV positives was observed in seasons when a large number of samples have been received from hospitals, as these normally send more swabs of small children. The percentage of adenovirus infections was 4.5% in the 2003/04 season and 2.3% in the 2004/05 season, while the percentage of enterovirus infections was 5.3% in the 2003/04 season and 15.7% in the 2004/05 season (Fig. 3). The specter of potential etiological agents of influenza-like illness is, of course, even wider. A clinical presentation of ILI may also be caused by parainfluenza viruses, human coronaviruses, human rhinoviruses, human metapneumoviruses, and even bacteria such as *Mycoplasma pneumoniae* and *Chlamydia pneumoniae* [12, 10, 32]. It would be of great interest to include the above-mentioned potential etiological agents in the annual virological surveillance of ILI, but it would, of course entails high costs.

Regardless of how well-collected epidemiological and virological data are, these have no real significance if they are not analyzed and sent in due time to professional institutions and the general public. When acute respiratory infections are most numerous, a short press release is prepared at the Centre for Communicable Diseases and posted on the web pages of National Institute of Public Health (http//www.gov.si/ivz/). Our data on influenza are accessible on the web pages of EISS (European Influenza Surveillance Scheme: http//www.eiss.org) and on the pages of WHO-FluNet, which are designed for influenza surveillance.

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Sažetak

Broj pacijenata s akutnom dišnom zarazom naglo se povećava nakon što se u populaciji počinje javljati virus influence. Kao posljedica toga javlja se povećana stopa hospitalizacije i mortaliteta. Nekoliko pokazatelja može se rabiti za mjerenje pojave popratnih bolesti vezanih uz influencu: stopa pojavnosti influenci slične bolesti ili akutne dišne bolesti, stopa nespecifičnog i specifičnog mortaliteta, bolovanje ili promatranje povećane potrošnje lijekova. Dva su pokazatelja mjerena u Sloveniji od 1999.: pojava bolesti slične influenci i stopa pojave akutne dišne bolesti na oko 4% populacije.

Lančana reakcija polimerazom uz prethodnu reverznu transkripciju (RT-PCR) bila je rabljena kao pregledna metoda za dokaz virusa influence, respiratornog sincicijskog virusa, adenovirusa i enterovirusa u obriscima nosa i/ili žrijela. Svi pozitivni uzorci bili su zatim nacijepljeni na odgovarajuće stanične linije. RT-PCR je bio upotrijebljen za brzo određivanje hemaglutinina (H1, H3) i neuraminidaze (N1, N2) virusa influence A. Antigenski podtipovi izdvojenih virusa na staničnim kulturama određeni su pomoću testa inhibicije hemaglutinacije te potvrđeni u Europskom referentnom centru za influencu Svjetske zdravstvene organizacije.

U sezoni 2004./2005. stopa pojavnosti bolesti slične influenci bila je najveća u 5., 6. i 7. tjednu 2005., kada je dostigla svoj vrhunac (392/100000). Najviša incidencija akutne dišne bolesti ustanovljena je u razdoblju od 31. siječnja do 6. veljače i na toj je razini ostala sljedeća dva tjedna. Uzorci su bili uzeti od 793 bolesnika s kliničkim znakovima bolesti slične influenci. Virus influence A i B bio je dokazan u 15,2% pacijenata. Trećina slučajeva influence bila je uzrokovana podtipom H1N1 dok su ostali bili uzrokovani podtipom H3N2. Enterovirusne infekcije bile su relativno česte u prošloj sezoni (u 15,8% pacijenata). Izolati virusa influence bili su slični onima izdvojenima u drugim europskim zemljama i pripadali su serotipovima A/H3N2/California/7/2004 (A/H3N2/Fujian/441/2002-slični virusi), A/H1N1/New Caledonia/20/99 and B/Jiangsu/10/03 (B/Shanghai/361/2002-slični virusi).

Dominantni virus influence u Europi i SAD-u bio je podtip A H3N2. Pojavnost akutne dišne bolesti i/ili influenci slične bolesti bila je veća u usporedbi s prethodnom sezonom u većini europskih zemalja (uključujući i Sloveniju).

Ključne riječi: Bolest slična influenci; Akutne dišne infekcije; Influenca A; Influenca B; RT-PCR