HRVATSKA AKADEMIJA
ZNANOSTI I UMJETNOSTI

Razred za medicinske znanosti
Odbor za animalnu i komparativnu patologiju

Simpozij

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EPIDEMIOLOGICAL ASPECTS OF INFLUENZA IN HUMANS AND ANIMALS

Sažetci

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AVIAN INFLUENZA - PAST, PRESENT AND FUTURE CHALLENGES

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Avian influenza is an OIE listed disease, which has become a disease of great importance both for animal and for human health. Until 1999, AI was considered a sporadic disease with only 18 outbreaks occurring in domestic poultry world-wide since 1959. The total number of birds involved in all outbreaks over this 40-year period was approximately 23 million. From 1999 onwards, AI infections cannot be considered sporadic any longer. Including estimations of the ongoing Asian H5N1 epidemic, in five years over 200 million birds have been affected by this disease. Some outbreaks have maintained the characteristic of minor relevance but others, such as the Italian 1999-2000, the Dutch 2003, the Canadian 2004 and the Asian 2003-2004 have lead to devastating consequences for the poultry industry, negative repercussions on public opinion and in some countries it has even impacted the national economy. In addition, it has created significant human health issues, including the risk of generating a new pandemic virus for humans via the avian-human link, and thus represents one of the major challenges the veterinary community will have to face.

The increased relevance of AI in the fields of animal and human health, has highlighted the lack of scientific information on several aspects of the disease, which has hampered the adequate management of some of the recent crises thus resulting in millions of dead animals and concern over loss of human lives and over management of the pandemic potential.

The main issues that need to be resolved include legislative and regulatory aspects such as the inclusion of Low Pathogenicity Avian Influenza (LPAI) viruses in the definition of AI since it has been shown that LPAI viruses are the progenitors of Highly Pathogenic Avian Influenza (HPAI). Given the increase in the numbers of isolations of LPAI viruses of the H5 and H7 subtypes, this represents a crucial aspect for prevention and control of future outbreaks.

Recent outbreaks in Europe, North and South America, the Republic of South Africa and the unprecedented ongoing Asian H5N1 outbreak have raised the necessity of developing alternative control strategies to the stamping out policy which had traditionally been used to control this disease. It is clear, that given the human health implications of AI, regardless of the strategy chosen for control, it must aim at the eradication of infection.

It is likely that the international effort that is being carried out in the medical and veterinary scientific communities in analysing data from recent outbreaks will generate significant amounts of information in the short-medium term which will broaden our current knowledge on AI, and be instrumental to the development of novel prevention and control strategies.

However, given the current situation, it is imperative that poultry veterinarians collaborate actively with public health officials to gather and analyse field and laboratory data on this disease. The poultry sector has a pivotal role in the AI crisis management, and should make active support to the international AI crisis a global priority of the industry.

MOLECULAR APPROACHES FOR THE IDENTIFICATION OF AVIAN INFLUENZA VIRUSES

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The recently raised awareness of the threat of a new influenza pandemic has stimulated the interest in the detection of influenza A viruses in the secretions of a wide variety of birds and mammals. Influenza A viruses are subtyped conventionally according to the characteristics of the external gycoproteins, haemagglutinin (HA) and neuraminidase (NA). All 16 HA and 9 NA subtypes have been isolated from aquatic birds that are believed to be a natural host and reservoir for influenza viruses. Poultry, specifically chickens and turkeys, are not considered to be a normal host for the virus, although transmission from wild birds to poultry occurs routinely. Although most HA subtypes have been found in poultry, particular emphasis is placed on the H5 and H7 HA subtypes of avian influenza virus because only these subtypes are known to cause highly pathogenic avian influenza (HPAI) in poultry. Recently developed molecular methods enable fast, accurate and reliable approach to identify influenza virus and its subtypes.

AVIAN INFLUENZA IN ITALY - EXPERIENCES IN MANAGEMENT OF MULTIPLE INTRODUCTIONS

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Since 1997 Italy has been affected by seven epidemic waves of avian influenza caused by viruses of the H5 or H7 subtypes. These epidemics were caused by H5N2 HPAI, H7N1 (HPAI and LPAI), H7N3 (LPAI) and, very recently, by H5N2 LPAI.

The most severe episode was observed during 1999-2001, in which Italy was affected by four epidemic waves of avian influenza (AI). The first epidemic wave was caused by a low pathogenicity avian influenza (LPAI) virus of the H7N1 subtype that subsequently mutated into a highly pathogenic avian influenza (HPAI) virus. Following the eradication of the HPAI virus, that caused death or culling of millions birds on infected and at risk farms, the H7N1 LPAI virus re-emerged twice in the same densely populated poultry area (DPPA) of the Po Valley.

Following the eradication of the H7N1 virus, a LPAI H7N3 virus was introduced in the industrial poultry population of Northern Italy. The H7N3 LPAI strain rapidly spread among poultry flocks located in the DPPA which had been affected by the H7N1 epidemic in 1999-2001. From October 2002 to October 2003 the virus was able to infect a total of 388 poultry holdings. About one year after the elimination of the last H7N3 affected poultry flock the virus re-emerged in the same area and the last affected farm was depopulated in December 2004.

The re-emergence or the introduction of AI viruses in the same DPPA resulted in the implementation of an emergency vaccination

programme based on the "DIVA" (Differentiating Infected from Vaccinated Animals) strategy. By enabling the detection of field exposure in vaccinated animals the application of this system, in conjunction to a monitoring programme and a well defined territorial strategy has resulted in the eradication of H7N1 and H7N3 epidemics which occurred between 2000 and 2004.

Field and laboratory evidence indicate that vaccinated animals are more resistant to challenge and shed lower amounts of virus, thus vaccination can be applied both as a tool for control and for prevention limiting the impact of AI infections.

Taking into account the high risk of AI virus introduction and spread in the identified DPPA, since October 2004 a bivalent H5/H7 pilot prophylactic vaccination programme has been implemented in a limited geographical area with the highest poultry population densities. The rationale behind the use of prophylactic vaccination is that it should be able to generate a minimal level of protective immunity in the population at risk. The immune response may be boosted if there is evidence of the introduction of a field virus.

In conclusion, Italy has been challenged with several subsequent epidemics of AI and has developed and applied a vaccination programme which has resulted in eradication of infection and given the high risk of re-introduction from the wild bird reservoir, is currently being used for the first time ever as a prevention tool.

AVIAN INFLUENZA SURVEILLANCE IN BACKYARD FLOCKS AND MIGRATORY BIRDS IN SLOVENIA

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Blood samples and cloacal swabs were taken from free living migratory birds and backyard flock birds. Enzyme linked immunosorbent assays (ELISA) and haemaglutination inhibition test (IH) was used to screen free living migratory birds and backyard flocks for antibodies against avian influenza viruses (AIV). Pools of cloacal swabs were used for the attempt of isolation of AIV. Samples were inoculated of 9 to 11 day-old embryos. Amnioallantoic fluid were collected and analyzed for haemaglutination activity. Pools of cloacal swabs were used for the detection of AIV by RT-PCR. Sera samples of four out of twenty three geese were positive on presence of specific antibodies against AIV. All attempts of virus isolation were negative. No AIV nucleic acid was detected by RT-PCR.

NEPOUZDANOST KOMERCIJALNOG ELISA KITA ZA SEROLOŠKI MONITORING INFLUENCE PTICA

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Pretražena su 342 uzorka seruma iz ekstenzivnih uzgoja kokoši i 184 uzorka iz intenzivnih uzgoja purana uzeta u razdoblju od 27. prosinca 2004. do 9. rujna 2005. komercijalnim ELISA kitom na protutijela za virus influence ptica (IP). Nađeno je 45,6% pozitivnih uzoraka seruma kokoši i svega 0,5% pozitivnih uzoraka seruma purana. Serumi koji su dali pozitivnu ili sumnjivu reakciju ELISA testom pretraženi su i imunodifuzijom u gelu na nazočnost protutijela za virus IP. Svi pretraženi uzorci dali su negativan rezultat. Provjerom specifičnosti komercijalnog ELISA testa dobivene su pozitivne reakcije i s monovalentim standardnim serumima za viruse newcastleske bolesti i CELO, dok su serumi za PMV-2 i PMV-3 dali sumnjivu reakciju pa se može zaključiti da je korišteni komercijalni kit nepouzdan u serološkom monitoringu IP u peradi. S obzirom na proširenost visokopatogene IP u svijetu i opasnost koju predstavlja visokopatogeni virus podtipa H5N1 za perad, ali i ljude, korištenjem komercijalnog kita bez provjere pozitivnih rezultata klasičnim serološkim metodama mogu se donositi krivi zaključci s nesagledivim posljedicama.

INFLUENZA EPIDEMIOLOGY IN CROATIA AND PREPARATIONS FOR POTENTIAL PANDEMIC

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Surveillance of influenza in Croatia is based on notification of disease/death due to communicable diseases that are reported within 24 hours (during outbreak weekly) to the epidemiologic service of Croatian National Institute of Public Health (CNIPH), clusters field investigation and CNIPH National laboratory for influenza. The registered influenza morbidity in Croatia amounts to up to 191,000 cases annually, oscillating, in the interpandemic period, from several thousand to over 190,000. Overage annual notification rate during last five years was 168/10000 inhabitants. In the season 1957/58, Asian influenza pandemic A/Singapore/H2N2 caused illness in more than 500,000 people; whom did the Health Service register. The Hong Kong pandemic influenza (A/Hong Kong/H3N2) was registered in Croatia in the 1967/68 and 1969/70 season. Total number of notified with influenza during that period was 582,000 people (14% of inhabitants). During the Hong Kong influenza pandemic morbidity higher than 1,000/10,000 inhabitants was recorded both in children aged 7-19 years and in persons aged 20+ years. The Preparedness Plan for Pandemic influenza is based on historical morbidity data in previous pandemic influenza outbreaks in the country. The brief overview of preparedness plan was given using the phases of interpandemic and pandemic period according to the latest agreed definitions published by the WHO.

EPIDEMIOLOGICAL AND VIROLOGICAL SURVEILLANCE OF INFLUENZA LIKE ILLNESS IN SLOVENIA

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The number of patients with acute respiratory infections usually increases during the circulation of influenza virus in the population. The hospitalization rate and mortality rate also increase. There are several indicators which can be used as a measure of the burden-of-illness caused by influenza virus: incidence rate of influenza-like illness (ILI) or acute respiratory illness (ARI), crude or cause-specific mortality rate, sick-live or monitoring OTC medication sales. Two indicators are measured in Slovenia from year 1999 on: ILI and ARI incidence rate in approximately 4% of the population.

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

In season 2004/2005 ILI incidence rate was at highest in week 5, 6 and 7, when it reached the peak value (392/100 000). The highest

ARI incidence rate was recorded from 31st January to 6th February and remained elevated for 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. Influeza B was detected more frequently than influenza A. From all positives 18,5% was influenza A, one third were H1N1 subtype, others were H3N2. Influenza B, RSV, adenoviruses and enteroviruses were detected in 26,4%, 8,6%, 6,7%, and 46,7% from positive samples respectively. In the past season, enteroviral infection was relatively commonly found (in 15,8% of all tested patients). In samples from 18 patients double infections were detected: in 5 influenza A and enterovirus, in 2 influenza A and RSV and in 2 RSV and enterovirus. Influenza isolates in Slovenia were similar to other European ones 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 USA was influenza A H3N2. The ARI and/or ILI incidence rate were higher in most European countries (including Slovenia) compared to previous seasons.

OSOBITOSTI IZOLATA VIRUSA INFLUENCE IZDVOJENIH IZ LJUDI U HRVATSKOJ

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Nacionalni influenca centar (NIC) osnovan je 1949. godine u suradnji Zavoda za javno zdravstvo Republike Hrvatske i Medicinskog fakulteta u Zagrebu. Virus influence prvi put je izdvojen u Hrvatskoj 1950. Do 1977. izdvojen je po jedan tip, a od tada uvijek više tipova virusa influence po sezoni. Godine 1992. u dijagnostiku je uvedena stanična kultura MDCK, a od 1995. brze dijagnostičke metode s monoklonskim protutijelima. Hrvatska je preko svog Nacionalnog centra za praćenje i dijagnostiku influence uključena u mrežu od 110 laboratorija Svjetske zdravstvene organizacija (SZO) pa kao dugogodišnji član prima najnovija dnevna izvješća i sve najvažnije činjenice prenosi u javnost. Praćenje virusa gripe A/H5N1/ u Nacionalnom centru za influencu provodi se od 1998. godine i dosada u našoj zemlji nije zabilježen nijedan slučaj infekcije čovjeka tim tipom virusa. Od 1999.-2005. u cirkulaciji su bili sljedeći serotipovi virusa influence: A/H3N2/Sydney/5/97, B/Beijing/184/93, A/H3N2/Moscow/10/99, A/H1N1/NewCaledonia/20/99, A/H3N2/Ireland/10586/20/99, B/Sichuan/379/99, A/H1N1/Madagascar/57794/00, A/H3N2/Panama/2007/99, B/Hong1Kong/330/01, A/H3N2/Thessaloniki/1/95, A/H3N2/Poland/15/91 i B/Shangdong/7/97.

EPIDEMIOLOGY OF EQUINE INFLUENZA VIRUSES: PATHOGENICITY AND TRANSMISSIBILITY

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A large-scale outbreak caused by equine influenza virus of the H3N8 subtype has occurred in each decade since an H3N8 virus was first isolated from horses in 1963. Each epidemic, and some minor outbreaks, has influenced equine influenza surveillance and vaccination policies in the UK and elsewhere. The latest widespread epidemic of equine influenza occurred in 2003 affecting Europe, America and South Africa. The possible reasons for infection of horses despite intensive vaccination are currently being investigated and may shed new light on the epidemiology of equine influenza.

AN OUTBREAK OF EQUINE INFLUENZA (H3N8) IN CROATIA IN 2004 CAUSED BY SOUTH AMERICAN LINEAGE

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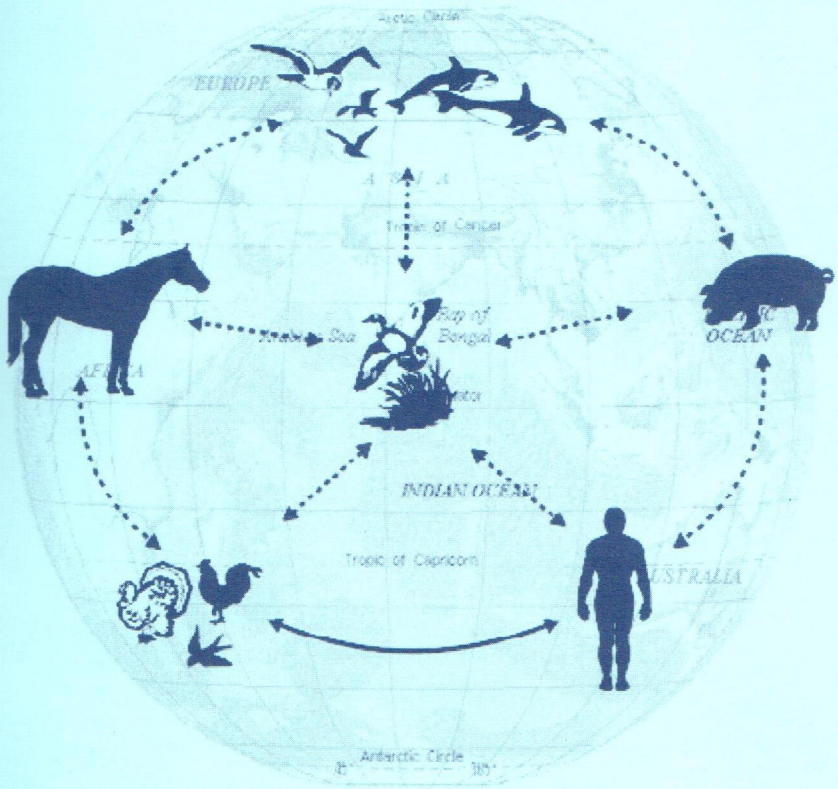
Several epizootics of equine influenza have been reported in Croatia in the past. Both subtypes, A/equine-1 (H7N7) and A/equine-2 (H3N8) were isolated. Antibodies against H7N7 subtype were detected in unvaccinated horses in Croatia suggesting that it continues to circulate in a subclinical form. An outbreak of equine influenza in 2004, in racehorses on Zagreb hippodrome is described. Both unvaccinated and vaccinated horses showed clinical signs of respiratory disease. By cross HI test and serological examinations of paired sera of apparently affected horses equine influenza (H3N8) virus was detected. Antigenic and genetic analysis of isolated viruses revealed the occurrence of South American sublineage of equine-2 (H3N8) influenza virus. The results presented in this work demonstrated that the HA1 gene sequence of the 6 isolates that were sequenced had only a single, non-coding nucleotide substitution among them. When the amino acid sequences of the Croatian isolates were aligned with a virus isolated in the UK during a widespread outbreak in the spring of 2003 (Newmarket/5/03), only a single amino acid substitution in the signal sequence was seen. Further surveillance of equine population grazed in the fields is needed.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