

Visualization and Analysis of the Danish 2006 Highly Pathogenic Avian Influenza Virus H5N1 Wild Bird Surveillance Data by a Prototype Avian Influenza BioPortal

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SUMMARY. Infection with highly pathogenic avian influenza virus H5N1 occurred for the first time in Denmark in 2006 during the last part of the European epidemic that mainly affected migrating wild birds. The total number of Danish wild bird cases was 45, of which only one was found through active surveillance using fecal sampling from resting areas for migrating species, whereas passive surveillance of dead wild birds provided 44 cases. One backyard, mixed poultry flock also became infected late in the epidemic. This study describes the spatial and temporal distribution of cases, initially characterized by a spatial-temporal cluster of affected tufted ducks that led to further spread to other wild bird species in the vicinity. The surveillance data also indicate an apparent die-off of the regional epidemic. As a tool in visualizing the spatial and temporal development of the epidemic, a prototype avian influenza (AI) BioPortal was used to provide online web-based access to the data. The AI BioPortal tools include mapping, graphing, phylogenetic tree construction, playback scenarios, and visualization of results of temporal-spatial analyses. Several of the features of this surveillance system compare favorably to the design of existing national and international surveillance information systems, and the AI BioPortal may become a useful tool for disease surveillance and for decision support in the event of future AI epidemics, both at national and international levels.

RESUMEN. Visualización y análisis de datos obtenidos por el programa de vigilancia en aves silvestres de virus de influenza aviar de alta patogenicidad H5N1 en el año 2006 en Dinamarca, mediante un prototipo de sistema BioPortal.

La infección por el virus de la influenza aviar de alta patogenicidad H5N1 se produjo por primera vez en Dinamarca en el año 2006 durante la última parte de la epidemia europea que afectó principalmente la migración de aves silvestres. El número total de casos de aves silvestres afectadas en Dinamarca fue de 45, de las cuales sólo una se encontró a través de vigilancia activa en un muestreo de excretas en áreas de descanso para especies migratorias, mientras que la vigilancia pasiva de aves silvestres muertas contribuyó a la identificación de 44 casos. Un grupo de aves de traspacio incluyendo diversas especies también resultó infectado sobre el final de la epidemia. Este estudio describe la distribución espacial y temporal de casos, inicialmente caracterizada por un agrupamiento en espacio y tiempo de patos copetudos infectados que posteriormente afectaron a otras especies de aves silvestres en los alrededores. Los datos de vigilancia también sugieren la aparente extinción de la epidemia a nivel regional. Un prototipo de sistema BioPortal para la influenza aviar fue utilizado para proporcionar acceso a los datos vía Internet y como una herramienta para visualizar el desarrollo espacial y temporal de la epidemia. Las herramientas disponibles en el sistema BioPortal incluyen mapas, gráficos, construcción de árboles filogenéticos, simulación de escenarios y visualización de los resultados de los análisis espacio-temporales. Varias de las características de este sistema de vigilancia se comparan favorablemente con otros sistemas de información para vigilancia nacional e internacional por lo que el BioPortal puede convertirse en una herramienta útil para la vigilancia de enfermedades y para apoyar el proceso de decisión en caso de futuras epidemias de influenza aviar, tanto a nivel nacional como internacional.

Key words: avian influenza virus, HPAIV H5N1, wild birds, surveillance, temporal-spatial distribution, epidemic die-off

Abbreviations: FMD = foot-and-mouth disease; HPAIV = highly pathogenic avian influenza virus; LLR = log-likelihood ratio; MB-STM = multivariate Bernoulli space-time model; RR = relative risk; RT-PCR = reverse transcriptase-PCR; STV = spatial-temporal visualizer; STGV = spatial-temporal-genomic visualizer; UB-STM = univariate Bernoulli space-time model

During 2005–06 highly pathogenic avian influenza virus (HPAIV) H5N1 spread through Asia into Europe and Africa. Although movement of both poultry and migrating wild birds have been implicated in this spread (2,5,6,11,23), there is general agreement that migrating wild birds were the primary factor responsible for spreading the infection to and within Europe in 2005 and 2006 as part of a worldwide epidemic (6,8,10,11,17,22). Within Europe the infection spread from the southeast (Turkey) toward the northwest (Germany, Scandinavia), and the last part of the 2006 epidemic took place around the Baltic Sea between the middle of February and the end of April 2006. The regional epidemic in and around the Baltic Sea began on the German island of Rügen on February 12, 2006, during an unusually cold and harsh period. During that time, a large concentration of migrating wild

species congregated in relatively small areas around the island where water was not frozen (3,6,7,12,17,18,19). From there the infection spread along the coastline of Germany, into neighboring Poland and across the Baltic Sea to the southeast coast of Sweden. It took approximately 4 wk before the infection reached Danish coastal areas, even though the nearest coastline is just 40 km away across the Baltic Sea from Rügen.

The Danish epidemic has previously been documented in official reports from the Danish Veterinary and Food Administration (5) and from the European Commission (9). The particular spatial distribution of the infection within the country has been analyzed (21); phylogenetic data from eight of the Danish wild bird virus isolates have been published (3); and potential public health aspects have been described (16). In other reports the Danish data were combined with similar observations from other European countries (12,18).

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Previous epidemiologic studies of HPAIV H5N1 have primarily been concerned with the risk of international spread of avian influenza virus (AIV) infection to and within not previously affected countries and regions. Less emphasis has been placed on studying the factors associated with the natural course of HPAIV epidemics in infected countries. Such analyses would be relevant in order to document and understand stages and factors important in limiting the spread of the infection and to model the effects of strategies that may be implemented in the control of an epidemic. In retrospect, there are several indications that the regional Baltic Sea epidemic died out midway while moving across Denmark (7).

The objective of the present study was to apply descriptive and analytic procedures to the epidemic surveillance data in order to document the patterns of disease spread and the epidemic stages in the temporal-spatial distribution of the HPAIV H5N1 infection in wild birds within Denmark during the spring of 2006.

An additional objective of this study was to demonstrate and evaluate the recently developed prototype avian influenza (AI) BioPortal system as a tool in visualizing and analyzing the Danish data. Descriptive and analytic procedures are included in the BioPortal, which is a web-based, open access system intended for free use by all interested parties working with global or national surveillance data (20).

MATERIALS AND METHODS

Epidemiologic data. Surveillance data. The data for the present study were collected during the period of the regional epidemic from February 15 through May 31, 2006. They comprise 1011 observations from dead birds submitted to the laboratory for HPAIV H5N1 testing (passive surveillance). Of these, 951 (94%) had geocoordinates recorded for the location where they were found. The data are available in Excel and downloadable from the prototype AI BioPortal.

It is generally recognized by now that active surveillance of HPAIV H5N1 in wild birds is not as efficient a surveillance method as passive surveillance of dead birds (1,7). However, during February 15–May 31, 2006, some 1102 fecal samples were also collected from resting places of migrating birds according to a preplanned survey design specifying the sampling time and locations (active surveillance).

In order to avoid any distorting effects of the heterogeneity of the two surveillance samples if pooled during analysis, only data from the dead bird samples (the passive surveillance data) with geocoordinates are described and analyzed in the following sections.

Laboratory diagnosis. The laboratory methods used in testing samples collected from dead birds and fecal samples have been described elsewhere (3,5). In summary, reverse transcriptase–PCR (RT-PCR) was applied as screening tests for general influenza virus and for H5 subtypes with subsequent steps to confirm and characterize H5 and H7 subtypes for pathogenicity. Virus isolation in embryonated specific-pathogen-free eggs was pursued subsequently and positive samples were subjected to N-typing.

Some of the isolates have been sequenced with the results made available in GenBank (3) and transferred to the AI BioPortal. Because HPAIV H5 has never been found in Denmark outside of this epidemic, all HPAIV H5 positive dead wild birds were considered in the following to have been cases of HPAIV H5N1.

The prototype AI BioPortal. The BioPortal is an information technology system for real-time disease surveillance implemented through a web-based platform. The system allows secure or public data to be captured, shared, and securely routed, as per the user's preference. Multiple disparate databases can be analyzed together or individually. The BioPortal links data from various sources for mapping (including Google Earth displays), graphics, anomaly detection (using rule-based and model-based algorithms), phylogenetic models (trees), and spatiotemporal display and analysis (20).

An early prototype of the BioPortal was guided by specifications for a national infectious disease informatics infrastructure, created through an interagency working group. In 2006, with funding from the National Center for Medical Intelligence, and in collaboration with national and international partners, such as the World Foot-and-Mouth Disease (FMD) Laboratory at Pirbright and the University of Arizona, the FMD Lab at the University of California Davis began development of the global FMD BioPortal (<http://fmdbiportal.ucdavis.edu/>), which became fully operational in January 2007. The FMD BioPortal currently provides free access in near real time to publicly available FMD data from the World FMD Reference Lab in Pirbright, World Organization for Animal Health, GenBank, and FMD News, which is a global FMD news service of the FMD Lab (<http://fmd.ucdavis.edu/news/>). More than 349 users from 46 countries have signed in to the FMD BioPortal since its inception.

The system integrates several sources of information, including epidemiologic, demographic, spatial, and phylogenetic data, and different levels of analysis, which interact in real time from various locations. Despite its capabilities, the BioPortal computational requirements are quite simple: Internet Explorer 4.0 and above or Netscape 5 and above, or any equivalent web browser program and Java Runtime Environment 1.4 or above is required to run the system. The BioPortal system includes a generic visualization environment, referred to as the spatial-temporal visualizer (STV), that can be used to visualize surveillance data as they vary with time and space (see Fig. 3). Google Earth can also be used as a visualization tool in the BioPortal, and it allows for the inclusion of cluster analyses model results, as does the STV (see Fig. 4). Further, the BioPortal includes a spatial-temporal-genomic visualizer (STGV) containing a circular phylogenetic tree structure in which the relationship of sequenced virus isolates is shown, along with the STV map and color coding to show the locations of the isolates (see Fig. 5).

The general public may access the prototype AI BioPortal at <http://fmdbiportal.ucdavis.edu>, logging in with the username “Denmark” and using the password “avianflu.” After logging in, select “search data”; note that despite the legends referring to foot-and-mouth disease, this particular username directs the user to the AI BioPortal prototype.

Data analysis. Initial data exploration was performed using the AI BioPortal for visualizing and describing the temporal and spatial distribution of dead wild bird cases and noncases. Additional simple tables and graphs were constructed in Excel to describe the composition of dead bird samples, as well as the distribution of HPAIV H5N1 infections.

Cluster analysis. Quantitative procedures and statistical analysis. Space-time clusters of HPAIV H5N1 infections were identified by Bernoulli space-time models of the scan statistic test and by using SatScan v7.0.3TM (13). For the univariate Bernoulli space-time model (UB-STM): the technique centered a hypothetical space-time cylinder at the geospatial coordinates of the location of collection of each case and of each control, where negative samples were designated as controls ($n = 907$). The base and height of the cylinder, which represented the spatial and temporal dimensions for each potential cluster of cases and of controls, respectively, were allowed to vary up to a maximum size equivalent to the inclusion of 50% of the samples. Maximum spatial extension of clusters was set to a radius of 40 km based on applicability of the results to geographic conditions in Denmark. The model scanned for areas with high infection rates, with a time aggregation set to 7 days, based on the assumption that a cluster of cases among migrating bird flocks would develop within that time frame. The ratio of observed-to-expected number of cases and controls under the null hypothesis of a homogeneous space-time distribution of cases and controls, which is referred to as the observed-to-expected ratio in combination with estimated relative risks (RRs), was computed within each cylinder. The observed-to-expected ratio was used to estimate the log-likelihood ratio (LLR) that the cylinder actually represented a cluster of HPAIV H5N1 cases. For the multivariate Bernoulli space-time model (MB-STM), the observations were divided into two subpopulations: one for the tufted ducks and the other for all the other wild bird species, with their corresponding cases and controls. The model estimates a LLR for each

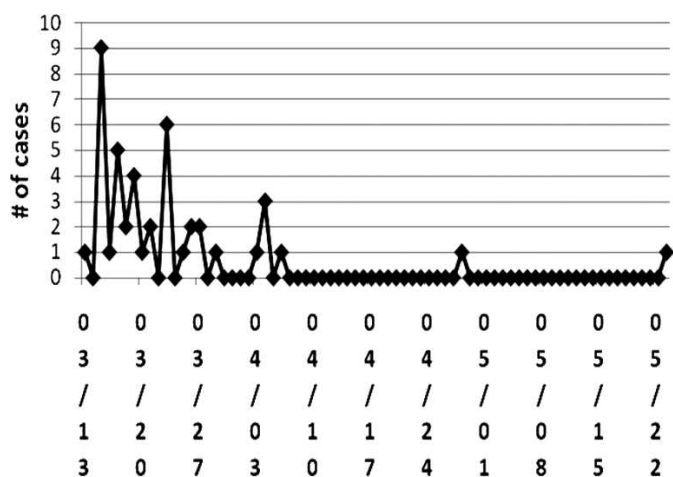


Fig. 1. Number of HPAIV H5N1 cases in dead wild birds by date of diagnosis, Denmark, February 15–May 31, 2006.

cylinder of each data set, and the maximum of all the summed LLRs, taken over all the cylinders, constitutes the most likely cluster for both data sets (14). Monte Carlo simulation was used in combination with the scan test to detect significant differences between observed and expected values per cluster.

RESULTS

Descriptive epidemiology. The first positive diagnosis of HPAIV H5N1 in Denmark was made March 14, 2006 in a common buzzard found dead on March 13 at Svinø Strand on the southwestern coast of Zealand. On March 15, a large number of sick tufted ducks were observed, and many dead ducks were collected around the island of Ærø south of Funen. Most of these birds were found positive for HPAIV H5N1 when tested. Subsequently, the infection spread inland to Zealand and Funen, affecting a variety of wild bird species.

Only one of the 1102 fecal samples examined (collected on March 17, 2006 from a herring gull [*Larus argentatus*]) was found to be positive for HPAIV H5 by RT-PCR (5,16).

Of the 951 dead birds in this study, 44 (4.6%) tested positive for HPAIV H5N1. The daily occurrence of HPAIV H5N1 cases among the dead birds is shown in Fig. 1, indicating the gradually waning epidemic during late March and early April. The last infected waterfowl was found on April 28. The maximum monthly prevalence observed was 6.1% for March (Table 1).

The prevalence of H5N1 was highest among ducks, whereas swans, birds of prey, and other bird species were affected to a much lesser degree (Table 2). Within the group of ducks, only tufted ducks (*Aythya fuligula*) were found to be infected. The high prevalence recorded for tufted ducks (72%) may have been influenced, however, by a high sampling rate from the heavily affected flock of tufted ducks on March 15–19.

Table 1. Dead birds tested for HPAI H5N1 by month, Denmark, February 15–May 31, 2006.

Month	Positive	Negative	Total	Prevalence (%)
February	0	133	133	0
March	37	571	608	6.1
April	6	166	172	3.5
May	1	37	38	2.6
Total	44	907	951	4.6

Table 2. Types/species of dead wild birds by HPAIV H5N1 status, Denmark, February 15–May 31, 2006.

Bird type/species	Positive	Negative	Total	Prevalence (%)
Birds of prey	8	390	398	2.0
Ducks	26	36	62	41.9
Tufted ducks (<i>Aythya fuligula</i>)	26	10	36	72.2
Mallards (<i>Anas platyrhynchos</i>)	0	22	22	0
Other duck spp.	0	4	4	0
Geese	1	3	4	25.0
Gulls and terns	0	86	86	0
Swans	7	162	169	4.1
Other water fowl	1	173	174	0.6
Other bird species	1	57	58	1.7
Total	44	907	951	4.6

On May 18, 2006, a backyard poultry flock on Funen was shown to be infected with HPAIV H5N1; detailed descriptions of the circumstances of this outbreak have been published previously (5,16). Protection and surveillance zones as well as A and B areas were immediately established on May 18, 2006, according to the European Commission's Decision 2006/135/EC on the basis of a positive PCR result from domestic poultry. On May 29, 2006, HPAIV H5N1 was also diagnosed in a dead magpie found on the affected poultry flock premise. The zones were lifted on June 30, 2006, following an extended screening of poultry and wild birds within the zones (5).

The source of the infection in the poultry flock was most likely wild birds, since the affected poultry had not been kept in net-covered enclosures in order to minimize contact with wild birds as prescribed in Danish legislation at that time. Furthermore, the premises were located within an established surveillance zone only approximately 5 km from where a positive dead wild bird had been found on April 4 (5).

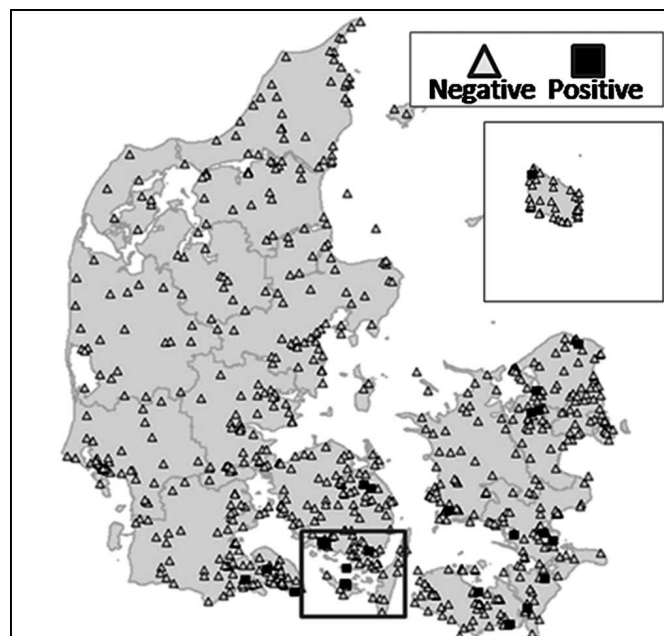


Fig. 2. Location and HPAIV H5N1 status of dead wild birds tested, Denmark, February 15–May 31, 2006. For details on the demarcated area, see Fig. 4. (Copyright released by the Danish Veterinary and Food Administration).

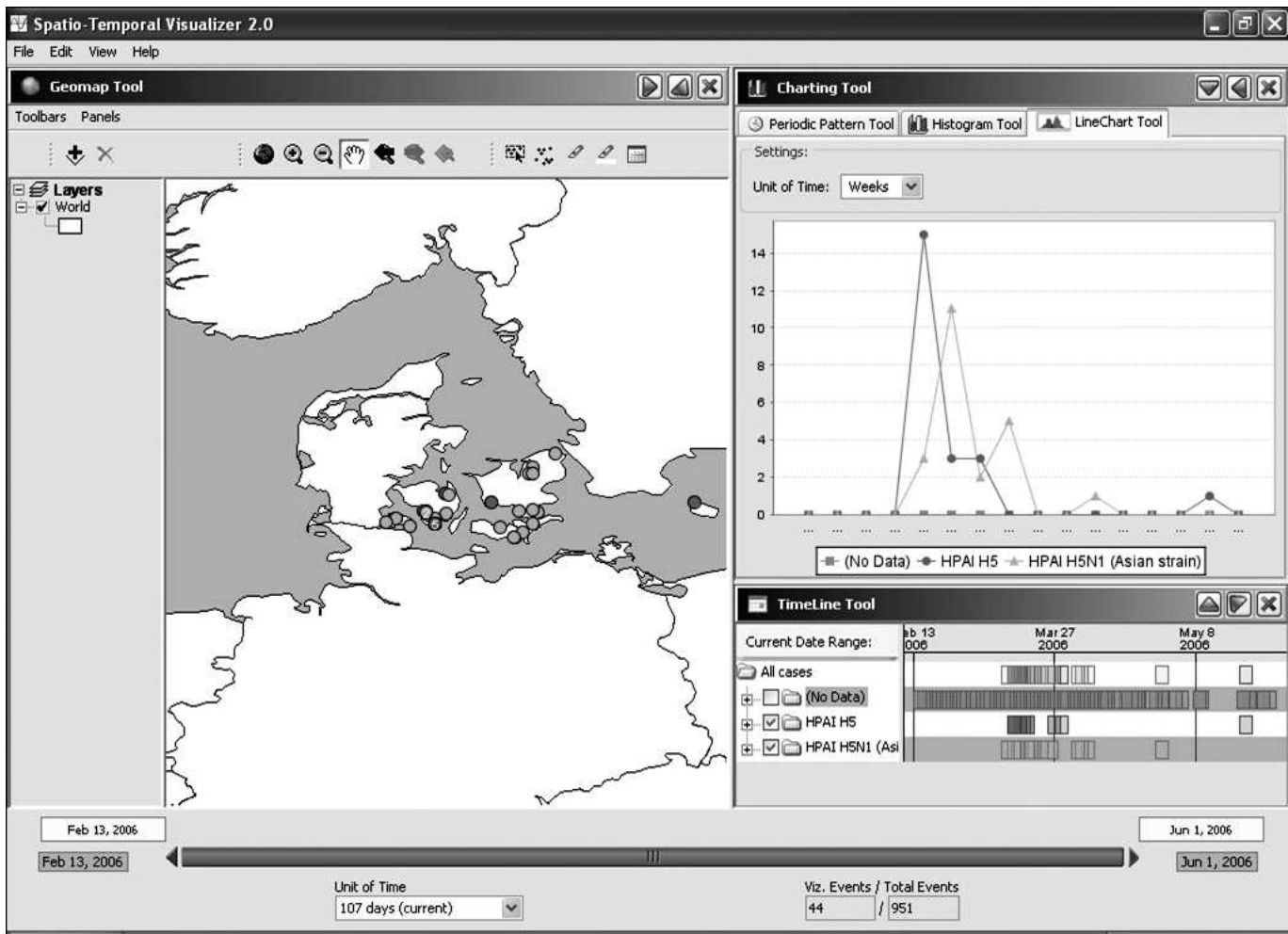


Fig. 3. Temporal and spatial relations of the 44 identified cases of HPAIV H5N1 among dead wild birds in Denmark, 2006, displayed by a screen-shot of the spatial-temporal visualizer (STV) available in the prototype AI BioPortal. The bar at the bottom of the screen is a time ruler, which during online sessions can be moved to play back the epidemic as it evolved. Note that the box “No data” (bottom right) has been unchecked, indicating that data on birds that tested negative are not eligible for inclusion in the map and in the graph. (Copyright released by the University of California).

Spatial distribution of the infection. Dead birds were collected for testing throughout Denmark during the spring of 2006 (Fig. 2), but infected birds were only found in the southeastern parts of the country. Most were located in coastal areas with a typically high seasonal (winter/early spring) density of water birds, migrating along the Baltic Sea and resting at shallow areas in the inner Danish waters (Fig. 3).

Adjusting for birds examined from counties not affected by the epidemic, the overall prevalence across affected counties was 7.0% of dead wild birds (data not shown).

Scan statistics. The results of the UB-STM and MB-STM appear in Table 3 and Fig. 4. The UB-STM identified a significant ($P < 0.001$) cluster of 20 birds (all cases) consisting of 19 tufted ducks and 1 peregrine falcon. The MB-STM using two data sets/subpopulations (“tufted ducks” and “all other species,” respectively) made it possible to identify a composite cluster structure consisting of a “tufted duck” partition with 20 birds (all cases), of which 19 were the same individuals as in the univariate cluster, while an additional 12 birds were identified to form a partition with four cases (two muted swans, one greylag goose, and the same peregrine falcon as in the UB-STM cluster) in the “other species” subpopulation. These two parts of the MB-STM cluster appeared

very close to each other in time, with only one day between the end of the tufted duck part and the beginning of the “other birds” part (March 15–19 and March 20–23, respectively; see Table 3). The UB-STM cluster, being 95% identical in composition to the tufted duck part of the MB-STM, was completely located within the MB-STM cluster perimeter (Fig. 4), since the radius of the UB-STM cluster perimeter was approximately half that of the MB-STM cluster and the locations of the two centers were very close (Table 3).

Phylogenetic visualization. Fig. 5 depicts the STGV tool showing the spatial and hemagglutinin gene data from the eight Danish virus isolates that have been sequenced until now. Little variation is seen among the eight isolates.

DISCUSSION

Most existing animal disease surveillance systems are limited in their scope and few contribute to global, real-time surveillance of emerging animal and zoonotic infections (15). The BioPortal was developed to fill the gap as a generic, globally accessible tool providing flexible input and output solutions (20). Focusing on a restricted geographic region, the present study applied some of its visualization tools, STV (Fig. 3), STGV (Fig. 5), and Google Earth,

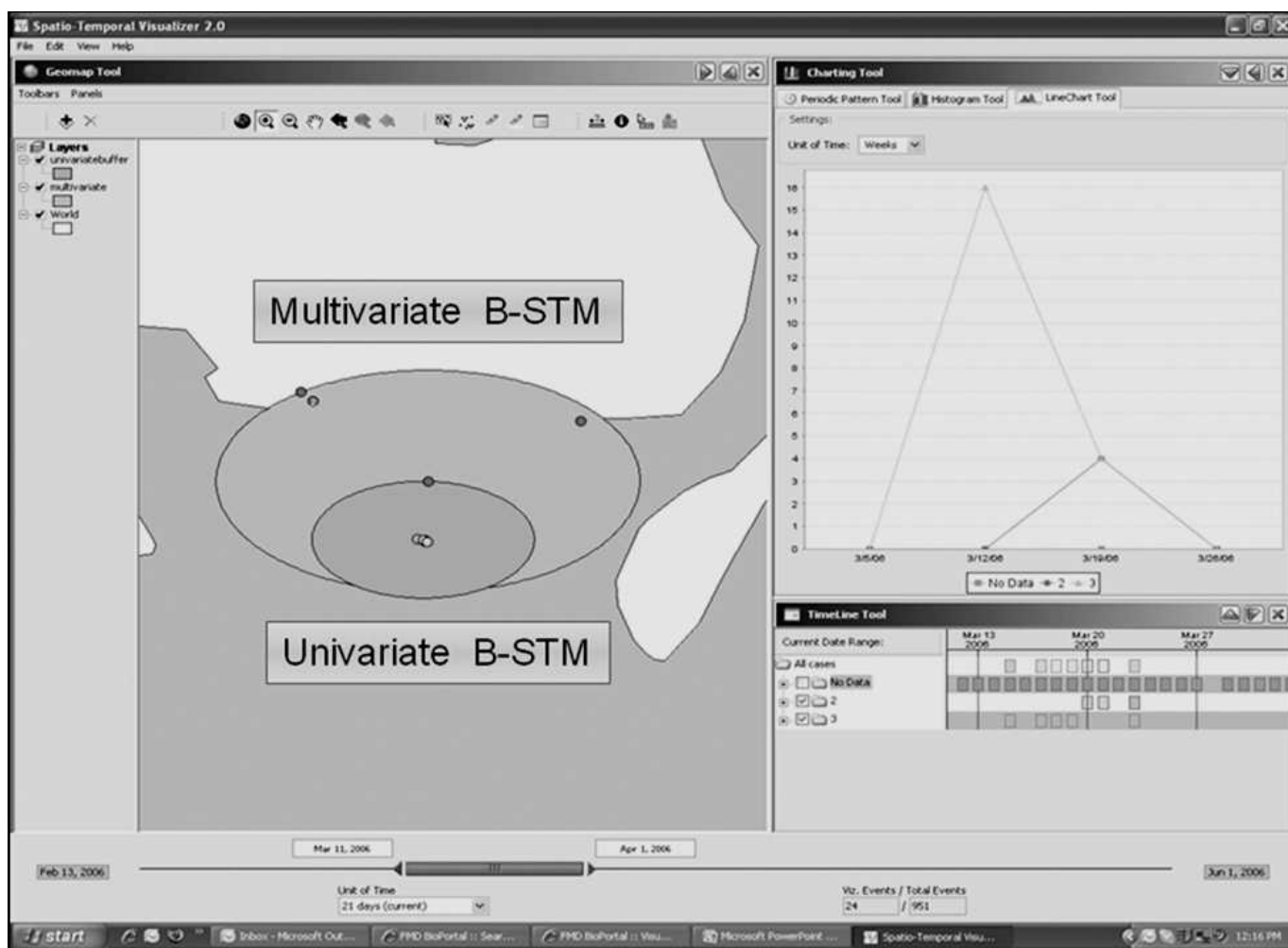


Fig. 4. Univariate and multivariate cluster perimeters with respective HPAIV H5N1 cases in Denmark, March 15–23, 2006, shown in a screenshot of the AI BioPortal STV. (Copyright released by the University of California).

while SatScan analysis was carried out separately (the members of clusters being identified in the database and cluster perimeters shown as overlays in STV; Fig. 4). Future versions will accommodate requests for SatScan analysis as an integral part of the online BioPortal access.

The descriptive epidemiologic observations, the location of the cases, the evolution of the regional epidemic, and the spatial-temporal distribution of the identified clusters indicate that the Danish HPAI H5N1 outbreak was linked to the migration of infectious birds into Danish waters adjacent to the Baltic Sea as has also been described for Germany (7). Initially a high proportion of tufted ducks was found to be infected. The epidemic propagated with a limited spread to other wild bird species, only to fade away before the larger parts of the country had become infected.

The detailed results of the MB-STM suggest that initially a heavily infected migrating flock of tufted ducks was likely transmitting HPAIV H5N1 infection to a variety of other bird species, including swans and raptors that were in close proximity to the infected tufted ducks. This is apparent from the extensions of the time-space cluster in the tufted duck subpopulation to the adjacent cluster in the “other species” subpopulation (Table 3; Fig. 4).

It is interesting to note that tufted ducks were also the species mostly affected in the Swedish epidemic (12), and they were also found heavily infected in the Rügen epidemic in Germany (7). It is plausible that neighboring countries sharing common waters were

exposed to members of a common large flock of infected tufted ducks that later dispersed in the Baltic Sea. Also, the tufted duck was found to be the most sensitive species in experimental HPAIV H5N1 infections carried out with six different duck species, and the tufted ducks, being affected with severe clinical signs, are likely to act as sentinels rather than long-distance vectors during epidemics (10).

Details of the Danish wild bird HPAIV H5N1 epidemic in 2006 have been published previously (3,5,12,16,21). Vigre *et al.* (21) reported on the spatial clustering of HPAIV H5N1 infected wild dead bird submissions (not individual birds as in the present study) in Denmark using data from the first 5 mo in 2006. The results showed that a single large space-time cluster covering the southeast parts of Denmark occurred, which could not have been caused by the geographic differences in the submission of dead birds to the laboratory. These results were generated using the default assumptions of the time-space SatScan scan statistics test, which set the scanning window to cover a maximum of 50% of the total population at risk without restricting the maximum spatial extension of clusters. By restricting the spatial extension of clusters of individual birds to a maximum radius of 40 km based on the size of the biologically relevant coastal biotopes in Denmark, the present study estimated the spatial extension of the space-time clusters from the two identified models UB-STM and MB-STM to be less than 10 km and 20 km, respectively (Table 3). It is, however, important to realize that analytic results using passive surveillance data obtained

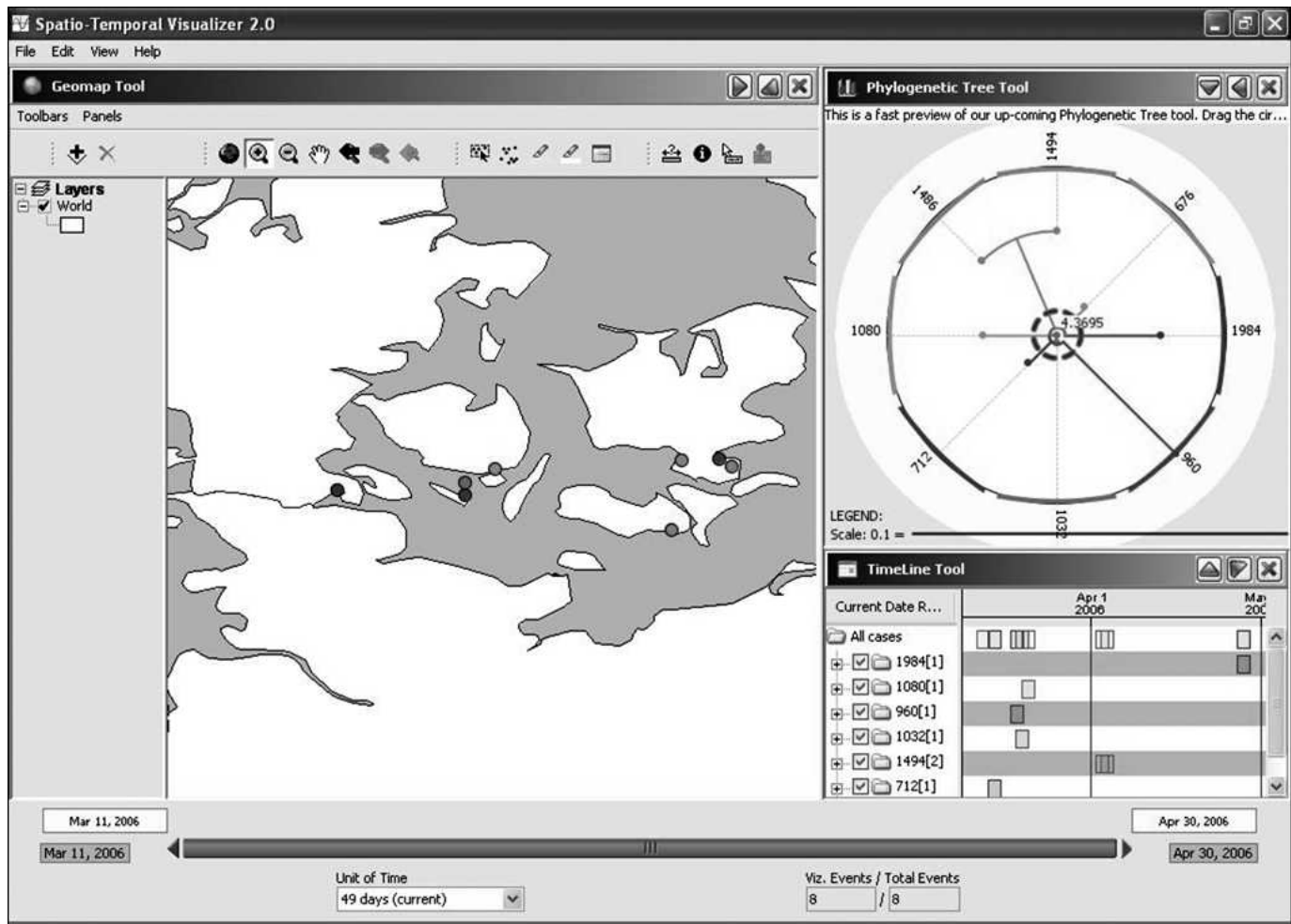


Fig. 5. Phylogenetic and spatial relations of eight HPAIV H5N1 sequenced viruses isolated from wild birds in Denmark, 2006, displayed by a screen-shot of the spatial-temporal-genomic visualizer (STGV) available in the prototype AI BioPortal. In this particular version of a phylogenetic tree (top right part), users may alter the diameter of the inner dotted circle that varies the genetic distance considered to represent “a true difference” in isolates. Thus changing the threshold for genetic difference will change the number of virus isolates within the circle. “Identical” isolates are shown in the same color in the tree and on the map, where different colors indicate genetically different isolates. The time ruler (see legend to Fig. 3) allows visualization of genomic changes that took place over time and geographic space. (Copyright released by the University of California).

by convenience sampling of bird carcasses could be significantly affected by selection bias.

Even so, it would also be important to consider which factors influenced the relatively rapid disappearance of the Danish epidemic (Fig. 1); thereby also limiting its spatial distribution within the country (Fig. 3). Preliminary observations suggest that the epidemic spread was already slowing down before the infection arrived to Denmark (7). The frontier of the infection through Denmark when extended in both directions produces a curvilinear delineation of all of the European 2006 wild bird cases to the south and east of this

line with just one or two potential outliers (5). Also, the observed HPAIV-associated mortality in wild birds was never very high in Denmark (Table 1), and the surviving birds would have been nonexposed, subclinically infected, or immune. Owing to the high pathogenicity of the HPAIV H5N1 for the tufted duck documented experimentally (10) and corroborated by epidemiologic studies ([7,12] and Table 3), subclinical or latent HPAIV infections with subsequent immunity is not likely to be significant for this species. However, a high degree of species variability in the pathogenicity is apparent (10). Not much is known about acquired AI immunity

Table 3. Space-time clusters detected by a univariate Bernoulli space-time model (UB-STM) and by a “species-specific” multivariate Bernoulli space-time model (MB-STM) of HPAIV H5N1 infection in dead wild birds (44 cases/907 controls), Denmark, February 15, 2006–May 31, 2006.

Space-time model	Cluster center		Radius (km)	Time frame		Cluster size	Cases			RR	LLR	P
	Longitude	Latitude		From	To		Obs.	Exp.	Obs/Exp			
UB-STM	10.4097	54.8930	8.99	10/03	23/03	20	20	0.93	21.61	38.8	66.7	0.001
MB-STM	10.4159	54.9738	17.08	10/03	23/03	—	—	—	—	—	19.4	0.001
Subpopulation												
Tufted ducks				15/03	19/03	20	20	14.4	1.385	2.67		
Other species				20/03	23/03	12	4	0.24	16.94	21.5		

from previous infection with related low pathogenicity avian influenza strains in wild birds, but this might play a role in the waning of epidemics (1). Seasonal decrease in exposure and spread could be associated with either dispersion away from migrating flocks by birds relocating for breeding or by increasing water temperatures throughout spring limiting the survival of the virus in the shallow waters (4). An alternative hypothesis for the rapidly waning Danish epidemic might be that the virus changed in a way that affected its pathogenicity, replication, or infectivity. The chance of detecting such changes would improve by having more of the 22 Danish isolates sequenced. Inclusion of additional national surveillance data from infected neighboring countries in the Baltic Sea region along with sequence data might also assist in further evaluating this and other hypotheses. Both options are being pursued at this time.

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