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# APPLICATION OF QUANTITATIVE METHODS AND MOLECULAR BIOLOGICAL TECHNIQUES TO INVESTIGATION OF THE SPREAD OF AUJESZKY'S DISEASE VIRUS

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*Des isolements du Virus de la Maladie d'Aujesky (ADV) ont été récoltés pendant une année à partir de 15 cas de mortalité chez les : porcs, bovins, ovins, chiens et raton laveur provenant de 13 élevages d'une zone où cette maladie est endémique. Chaque échantillon d'ADV a subi la digestion de 4 enzymes de restriction (Alw 44I, BamH1, Sal I, et Xho 1), puis les fragments marqués ont subi une électrophorèse sur gel d'agarose. La taille des paires des fragments de base a été estimée à partir de la distance de migration en les comparant avec des fragments d'λ DNA de molécules de poids connus. Un algorithme a permis de comparer les prélèvements d'ADV tout en respectant la distribution des fragments de restriction (RFPs). La similarité génomique a été estimée à partir de la distance euclidienne en 4 dimensions en utilisant les mesures de similarité de la RFP provenant des 4 enzymes de restriction. Une analyse de regroupement hiérarchique a été utilisée afin d'identifier les groupes d'ADV isolés génétiquement similaires. Ce groupage a ensuite été comparé à celui des regroupements d'élevages basé sur leur distance géographique.*

*Les échantillons qui étaient génétiquement similaires provenaient généralement du même groupe géographique. En utilisant cette information sur la similarité génétique et les distances géographiques conjointement avec le moment de la découverte du foyer, il est possible de reconstruire un scénario probable de la transmission de l'ADV entre les élevages.*

## INTRODUCTION

Restriction endonuclease analysis (REA) has been used repeatedly to compare Aujeszky's Disease virus (ADV) samples with regard to their genomic similarity (Pirtle et al., 1984; Jestin et al., 1990; Christensen & Soerensen, 1991). Thus, in epidemiologic investigations REA has been used as a tool to identify sources of ADV infection for swine farms (Christensen et al., 1988, 1995). These investigations have been limited in scope and have not been used to trace the path of transmission over the course of an epidemic. Comparison of restriction fragment patterns (RFPs) has also consisted primarily of matching fragments on molecular weight and identifying matches at specific fragment sizes. We have developed an objective quantitative algorithm for comparison of restriction fragment patterns based on the overall similarity in the distribution of fragment base pair sizes (Weigel & Scherba, 1997). This method has been applied here in the analysis of an outbreak of ADV among swine farms in a geographic region where ADV has been endemic.

## METHODS

An outbreak of Aujeszky's disease had occurred in a region where ADV infection had been endemic. Over a 10 month period, there were 14 swine farms with clinical signs in swine or other domestic or wild mammalian species, from which it was possible to obtain ADV isolates from a total of 16 animals. An epidemiologic investigation had been conducted on each farm. In several cases, ADV vaccines were suspected as the cause of death in nonporcine species. The DNA of 16 ADV field isolates and 2 ADV vaccine strains[2,3] were digested using 4 restriction enzymes (Alw 44I, BamH1, Sal I, Xho I). Fragments were electrophoresed in agerose gel. Base pair size marker fragments of lamda DNA which were run in the outermost lanes of each gel. The base pair sizes of ADV restriction fragments were estimated using the linear regression relationship between base pair size and migration distance for the lamda DNA fragments. For each restriction enzyme, the genomic similarity between each pair of samples was estimated using a quantitative algorithm developed by Weigel and Scherba (1997), which bases the similarity on a comparison of the distribution of fragment base pair sizes. This measure of genomic similarity was standardized across enzymes, and an overall measure of genomic similarity based on Euclidean distance was calculated. Hierarchical cluster analysis used the data from this distance matrix to identify ADV samples that were genetically similar. A complete linkage algorithm was used to produce clusters with elements that were all closely related to each other. The dendrogram indicating clusters of genetically similar ADV isolates was used in conjunction with information on geographic distance between farms and time of disease outbreak to reconstruct a hypothetical scenario of the routes of transmission of ADV between farms over the course of the epidemic.

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[3] : PRV/Marker Blue, SyntroVet Inc. Lenexa, KS.

## RESULTS AND DISCUSSION

The cluster analysis identified 4 clusters of ADV isolates which had similar RFP distributions:

Cluster #1: C1(**1**) C2(**1**) C3(**1**) C4(**1**) D1a(**1**) D1b(**1**) A1(**2**) A2(**2**) B1(**3**) D3(**4**)
Cluster #2: A3(**1**) B4(**4**) B2(**4**) B3(**4**)
Cluster #3: D2(**1**) No
Cluster #4: E1(**10**) Sb

where the letter designating each isolate indicates the geographic cluster to which a farm belonged, and the number in parentheses indicating the month is which ADV disease signs were first observed. Cluster #1 consists of farms that mostly had the initial disease outbreak in the first 2 months of the epidemic. These farms were distributed over 4 geographic clusters of farms. The second cluster consisted mostly of farms from a single geographic cluster (B) that had disease signs in the fourth month of the epidemic. Cluster #3 contains an isolate (D2) obtained from a bovine death, linked with the PR-Vac ADV vaccine (No). This vaccine was used on swine farms in this region. Cluster #4 consisted of a geographically isolated sample (E1) obtained from a dog that had been vaccinated with Marker Blue vaccine (Sb), and was linked with an independent sample of this vaccine.

Using the information available on genomic similarity, geographic proximity, and time of appearance of clinical signs, it was possible to reconstruct a hypothetical scenario for the course of the epidemic. Due to genomic differences, it was assumed that the two clusters #1 and #2 represent different patterns of spread of ADV. The accompanying figure displays the 2 routes of between herd spread. Farm C1 had the earliest observed clinical signs. The second outbreak was on Farm C4, which is most closely linked genetically with C1, and thus C1 is considered the source of ADV for C4. Farms C2 and C3, also having ADV disease signs in the first month, were also closely linked genetically with C1, their probable source of infection. Also linked closely genetically and in time of outbreak with these geographically clustered farms were the 2 isolates obtained on farm D2. The closest genetic relationship between any 2 samples were between 2 canine isolates (D1 and C2). This links C2 as a probable source of infection for farm D1. Genetic relatedness, geographic proximity and time of outbreak also indicate farm D1 as a probable source of ADV for farms D3 and A2, and A2 is considered a probable source of ADV infection for farms A1 and B1. The second outbreak cluster has earliest outbreak on A3 in month 1 as the hypothesized source of ADV infection for farms B4, B3, and B2, with similar RFPs. (See figure.)

The combination of molecular biological techniques, statistical methodology, and epidemiologic data can provide considerable insight into the patterns of spread of infectious pathogens for which REA is feasible.

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