Surveillance for Swine Influenza Virus in the United States

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VETERINARY DIAGNOSTIC LAB

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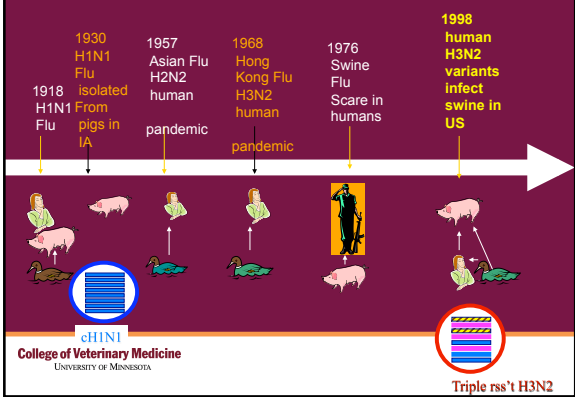
U.S. Swine Influenza Timeline



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Swine Influenza Virus Subtypes and Variants

Classic H1N1

- 1918 to date
- All 8 gene segments swine like
- Stable cH1N1 for almost 80 years
- Very little drift

1998 – H3N2

- Antigenic shift (H1 to H3)
- H3N2 – Double reassortant
  - 1998 to 1998
    - Hu HA, NA, PB1
    - Sw PB2, PA, NS, NP, M
- H3N2 – Triple reassortant
  - 1998 to date
    - Hu HA, NA, PB1
    - Av PB2, PA
    - Sw NS, NP, M
- H3N2 - Accelerated drift and continued reassortment

Triple Reassortant H3N2

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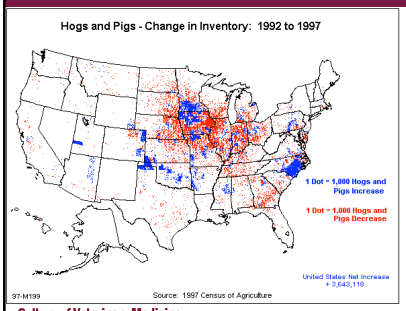
Changing Swine Geography 1997



- Move to "fringe corn-belt".
- Higher feed costs, lower other costs through production systems approach.
- N. Iowa and S. MN maintain advantage of "best natural hog region".
- Meat Packing and Processing Capacity is key to location shifts.

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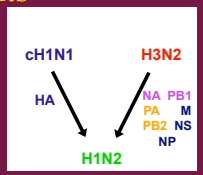
Swine Influenza Virus Subtypes and Variants

- H1N2
  - Emerged in 1999/2000
  - 7 genes from the H3N2 viruses
  - HA from the classical -swine H1N1 viruses



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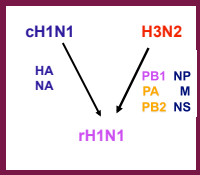
Swine Influenza Virus Subtypes and Variants

- rH1N1
  - **"reassortant H1N1"**
  - 2001/2002 to date
- Emerged in 2002
  - Surface of the H1N1 viruses
  - Internal gene components derived from the H3N2 viruses
  - Antigenic and genetic changes in the hemagglutinin gene between rH1N1 and cH1N1



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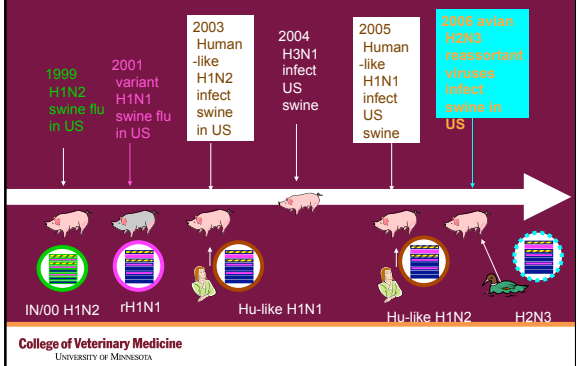

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H1N1, H1N2, and H3N2 viruses co-circulate in US swine

Each Virus Genetically and Antigenically Distinct

- A/Sw/MN/02 Reassortant H1N1-like (rH1N1)
- A/Sw/USA/00619/05 - Hu/Sw r'sst (Hu-like H1)
- A/Sw/IN/00 H1N2-like (IN/00 H1N2)
- A/Sw/IN/88 – classic-like (cH1N1)
- A/Sw/Ontario/05 Cluster IV H3N2-like (H3N2)

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Determining Genetic Characteristics of SIV in the US

- 2001 to date – 2510 HA gene sequences from swine influenza viruses detected at the U of MN–VDL
- Considerable variation in both H1 and H3 sequences are present
- Both reassortment and antigenic drift apparent

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H1 and H3 Phylogenetic Trees

- H1 and H3 hemagglutinin gene sequences of isolates from March and April 2003 and 2008
- Unrooted trees with 7 reference strains
- Scale is number of nucleotide differences per 100
- % prevalence of each HA gene sequence (IN /00-like, rH1N1-like, Human-like and Classic -like) does NOT include reference strains

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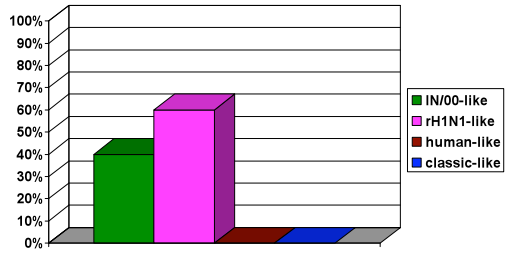
2003 H1s



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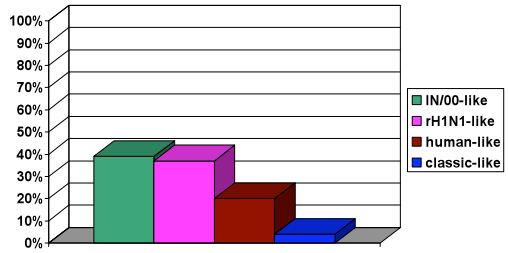
2008 H1s



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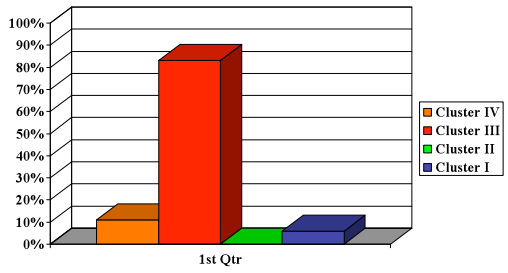
2003 H3s



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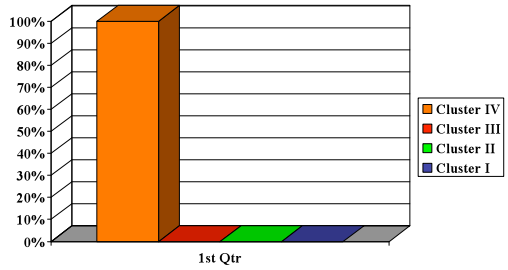
2008 H3s



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H1N1, H1N2, and H3N2 co-circulate in US swine

Each Virus Genetically and Antigenically Distinct

- A/Sw/MN/02 Reassortant H1N1-like (rH1N1)
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Determining Antigenic Characteristics of SIV in the U.S.

Serologic cross-reactions between reference strains and field isolates representing different genetic clusters of H1N1 and H3N2 swine influenza virus

AASV 2008

Marie Gramer[1], Al Ducommun[1], Carrie Wees[1], Michelle Trudeau-Spanjers[1], Shannon Devens[1], Tracy Seda[1], Greg Nitzel[2], Eric Wicklund[2], Jonathan Evans[2],Jeff Kula[2], Lucas Taylor[2], Jenifer Jeffers[2], Tracy Ricker[2], and Vicki J. Rapp-Gabrielson[2]

[1]University of Minnesota Veterinary Diagnostic Laboratory, College of Veterinary Medicine, Saint Paul, Minnesota; [2]Veterinary Medicine Research and Development, Pfizer Animal Health, Kalamazoo, Michigan

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Cross-HI Result Summary

- H1N1
  - Minimal cross-reactivity between newer human -like viruses and viruses representing the classic and older reassortant clusters
    - Consistent with low similarity (~ 70% - 75% identity) in HA gene sequences
- H3N2
  - Cluster 3 and 4 viruses showed different levels of HI cross-reactivity
    - Reflects both genetic and antigenic differences within these clusters

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Conclusions

- Genetic and serologic diversity exists in U.S. swine H3N2 and H1N1 viruses
  - Problematic for pigs when virus variants emerging that complicate diagnostic efforts and limit vaccination success
- Vaccination and challenge studies using genetically and serologically variant viruses are likely necessary to determine the effectiveness of the current SIV vaccines

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Implications of SIV Variability

- Genetic and antigenic variation exists
- Anecdotal reports of apparent vaccination failures with current commercial SIV bivalent vaccines containing A/SW/TX/98 -like vaccine virus
- SIV H3N2 variants are increasingly prevalent in swine farms
- Hypothesis
  - Immunity induced by current commercial vaccines may not be sufficient to protect against the H3N2 variants.
- Study completed to investigate whether USDA licensed, commercially available bivalent vaccines provide satisfactory protection against a H3N2 variant virus heterologous challenge

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Vaccination and Challenge Study

- Vaccination of pigs with commercially available vaccines in controlled experiments
- Challenge with heterologous virus
  - A/Sw/CO/2004 – H3N2
- Evaluation of Protection
  - Clinical signs, gross and microscopic lesions of pneumonia, virus shedding

Lee, Gramer, Joo. Can J Vet Res (2007) 71:207-212

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Article

Efficacy of swine influenza A virus vaccines against an H3N2 virus variant

Jee Hoon Lee, Marie René Gramer, Han Soo Joo

Abstract

We compared the efficacy of 3 commercial vaccines against swine influenza A virus (SIV) and an experimental homologous vaccine in young pigs that were subsequently challenged with a variant H3N2 SIV, A/Swine/Colorado/00294/2004, selected from a repository of serologically and genetically characterized H3N2 SIV isolates obtained from recent cases of swine respiratory disease. The experimental vaccine was prepared from the challenge virus. Four groups of 8 pigs each were vaccinated intramuscularly at both 4 and 6 wk of age with commercial or homologous vaccine. Two weeks after the 2nd vaccination, those 32 pigs and 8 nonvaccinated pigs were inoculated with the challenge virus by the deep intranasal route. Another 4 pigs served as nonvaccinated, nonchallenged controls. The serum antibody responses differed markedly between groups. After the 1st vaccination, the recipients of the homologous vaccine had hemagglutination inhibition (HI) titers of 1:640 to 1:2560 against the challenge (homologous) virus. In contrast, even after 2nd vaccination, the commercial-vaccine recipients had low titers or no detectable antibody against the challenge (heterologous) virus. After the 2nd vaccination, all the groups had high titers of antibody to the reference H3N2 virus A/Swine/Texas/4199-2/98. Vaccination reduced clinical signs and lung lesion scores; however, virus was isolated 1 to 5 d after challenge from the nasal swabs of most of the pigs vaccinated with a commercial product but from none of the pigs vaccinated with the experimental product. The efficacy of the commercial vaccines may need to be improved to provide sufficient protection against emerging H3N2 variants.

2007;71:207–212 The Canadian Journal of Veterinary Research 207

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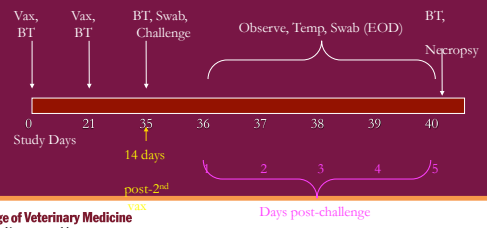
Experimental Design and Timeline

- 28 pigs, negative for SIV, PRRS, and Myco pre-trial
  - 8 in Homol. Vax/Chal Group
  - 8 in Heter. Vax/Chal Group
  - 8 in Non-vax/Chal Group
  - 4 in Control Group



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Virus Isolation Results - Nasal Swabs H3N2 study

Group	Study Day (Day Post-Challenge)			
	32 (0)	33 (1)	35 (3)	37(5)
Het vax	0/8+	8/8+	6/8+	3/8+
Non-vax	0/8+	8/8+	8/8+	7/8+
Homol-vax	0/8+	0/8+	0/8+	0/8+
Control	0/4+	0/4+	0/4+	0/4+

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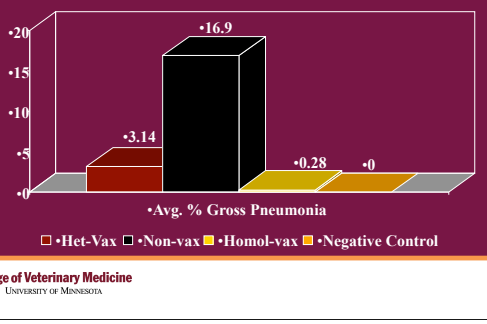
Protective Ability of Commercial (Heterologous) and Homologous Vaccine against Contemporary H3N2 SIV isolate



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Significance of differences?

- 2005 H3N2 vaccination and challenge study
  - challenge virus (CO/00294)
    - 92.6% nucleotide similarity,
    - 33 total a.a. differences and
    - 13 a.a. differences at the presumed antigenic sites
  - Outcome
    - Vaccinated pigs had reduced clinical signs and gross pneumonia when compared to non-vaccinated controls.

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SIV in the United States

- H1N1, H1N2, and H3N2 viruses co-circulate in US swine
- Complicate disease management and control
- Continued **reassortment** and change

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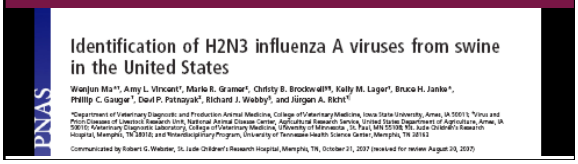
Isolation of reassortant H2N3 avian/swine influenza virus from pigs in the United States

Marie Gramer[1], Sagar Goyal[1], Devi Patnayak[1], Kelly Lager[2], Wenjun Ma[2,3], Amy Vincent[2], Juergen Richt[2], Richard Webby[4]

[1]Minnesota Veterinary Diagnostic Laboratory, Saint Paul, Minnesota, USA
[2]National Animal Disease Center, USDA-ARS, Ames, IA, USA
[3]Department of Veterinary Diagnostic and Production Animal Medicine, College of Veterinary Medicine, Iowa State University, Ames, Iowa, USA
[4]St. Jude Children's Research Hospital, Memphis, TN, USA



PNAS

Identification of H2N3 influenza A viruses from swine in the United States

Wenjun Ma*†, Amy L. Vincent‡, Marie R. Gramer§, Christy B. Brockwell¶‖, Kelly M. Lager‡, Bruce H. Janke‡, Phillip C. Gauger‡, Devi P. Patnayak§, Richard J. Webby§, and Jürgen A. Richt‡

*Department of Veterinary Diagnostic and Production Animal Medicine, College of Veterinary Medicine, Iowa State University, Ames, IA 50011; †Virus and Prion Diseases of Livestock Research Unit, National Animal Disease Center, Agricultural Research Service, United States Department of Agriculture, Ames, IA 50010; ‡Veterinary Diagnostic Laboratory, College of Veterinary Medicine, University of Minnesota, St. Paul, MN 55108; §St. Jude Children's Research Hospital, Memphis, TN 38105; and ¶Interdisciplinary Program, University of Tennessee Health Science Center, Memphis, TN 38163

Communicated by Robert G. Webster, St. Jude Children's Research Hospital, Memphis, TN, October 31, 2007 (received for review August 30, 2007)

Background

- April 2006 and September 2006
  - Outbreaks of respiratory disease in growing pigs
  - Gross lesions of bronchopneumonia
  - Two separate multi-site commercial swine farms
    - Farm A
    - Farm B.
      - 4 miles apart
      - did not share pigs, feed, personnel, or transportation.
  - Untypable influenza A viruses isolated from lungs with characteristic lesions

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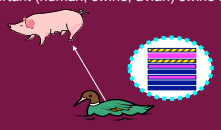
SIV Characterization

- **Full genome sequencing**
  - **H2N3 with avian origin HA and NA.**
  - Serotyping with ferret anti-duck H2N3 antisera was positive for both viruses.
  - Internal genes except PA similar to contemporary triple reassortant (human, swine, avian) swine influenza viruses



H2N3

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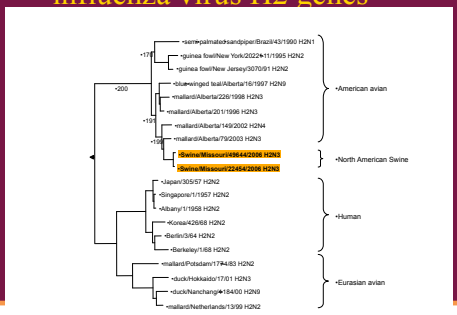
Phylogenetic trees of selected influenza virus H2 genes



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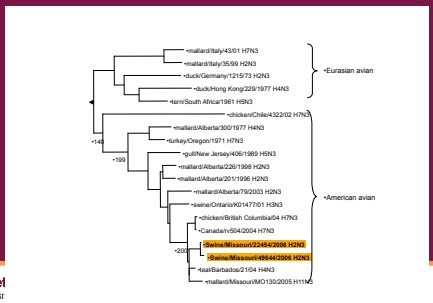
Phylogenetic trees of selected influenza virus N3 genes



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How did it get there?

- Use of surface (pond) water
- Ponds frequented by migrating waterfowl
  - Waterfowl = natural reservoir of flu viruses

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Continuing problem or one time event?

- Farms A and B had positive (>1:40) H2N3 antibody titers in sera from sows, gilts and weaned pigs collected 6 to 12 months after onset of clinical disease
- Farm B had positive titers in sera from old sows collected 18 months after onset of disease.
  - Farm B P1s and P2s (young sows on farm <15 months) are seronegative
- No human illness has been reported
  - Serological surveys conducted at Farm B
    - results pending

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Implications of new subtype H2N3

- **This is the first description of avian/swine H2N3 influenza virus isolated from pigs in the United States.**
- Virulent and mammalian adapted
  - Experimental infection of swine → severe lung lesions and seroconversion in sentinel contact pigs.
  - Experimental infection of mice → disease and death
  - Experimental infection of ferrets → transmitted to contact sentinel ferrets.

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Significance of H2 influenza viruses

- H2 viruses have not circulated in the human population since the late 1960s.
  - E. A. Govorkova, A. A. Kizina, V. F. Krylov, A. Smirnov Iu, *Zh Mikrobiol Epidemiol Immunobiol*, 58 (Nov-Dec, 1993).
- Unlike HPAI H5N1, the new H2N3 virus causes respiratory disease in pigs and mice and is readily transmitted among ferrets.
  - H. L. Yen *et al.*, *J Virol* (Apr 25, 2007).
- A new pandemic influenza strain is most likely to be of an H2, H5, H9, or H10 HA subtype and an N3 or N7 NA subtype.
  - M. R. Hilleman, *Vaccine* **20**, 3068 (Aug 19, 2002).
  - D. Shoham, *Virus Genes* **33**, 127 (Oct, 2006).

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Ongoing surveillance, questions

- Pigs
  - Purported mixing vessels for avian and human flu viruses
  - Pig tracheal epithelial cells have receptors for both human and avian flu
    - T. Ito *et al.*, *J Virol* **72**, 7367 (Sep, 1998).
- Other mixing vessels
  - humans
    - K. Shinya *et al.*, *Nature* **440**, 435 (Mar 23, 2006).
  - quail
    - H. Wan, D. R. Perez, *Virology* **346**, 278 (Mar 15, 2006).
- Previous pandemics
  - 1957 and 1968 = human-avian reassortants.
  - No direct evidence that 1957 and 1968 flu was generated in pigs
    - M. R. Castrucci *et al.*, *Virology* **193**, 503 (Mar, 1993).

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SIV – What does the future hold?

- Vaccination and challenge studies are useful tools for exploring in vitro observations such as antigenic and genetic differences
  - Expense may be limiting
- Gene sequencing and reverse genetics may be more helpful in determining significant differences in genes that may contribute to immune escape

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SIV – What does the future hold?

- Detection and characterization assays are in place to identify novel strains of influenza in pigs
  - H3N1 - 2004
  - Human-like H1N1 and H1N2 - 2003 to date
  - Avian/Swine H2N3 - 2006

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Summary and Conclusions

- Swine influenza virus surveillance is both prudent and practical
- Future surveillance efforts for novel swine influenza viruses should take place with avian and human influenza virus surveillance
  - especially in selected areas where interspecies transmission is likely to occur due to high densities of both avian and swine production

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Summary and Conclusions

- Data from increased and improved surveillance may be applied to timely disease control through:
  - vaccine and anti-viral pharmaceutical manufacturing,
  - biosecurity enhancements, and
  - detailed risk assessments.

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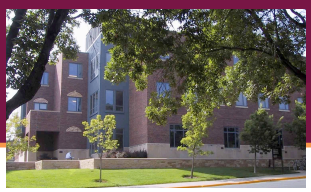
Acknowledgments

- The University of Minnesota Veterinary Diagnostic Laboratory, the entire staff and faculty



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