

Detection and Genetic Analysis of Swine Hepatitis E Virus in Farm Waste

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Hepatitis E

- ï First described in a large outbreak in India that was not caused by hepatitis A virus
- ï Outbreaks frequently associated with contaminated water, few secondary cases occur
- ï Infects mainly young adults, higher mortality in pregnant women-up to 25%

Hepatitis E and Seroprevalence of anti-HEV in Humans Worldwide

- i Endemic areas: India, Pakistan, Nepal, China, Middle Asia**
 - Epidemics reported**
 - High seroprevalence of anti-HEV**
- ii Non-endemic areas: US, Western Europe**
 - No epidemics, low seroprevalence**
 - Usually reported in travelers**
 - Rare domestically-acquired cases identified in US, United Kingdom, Greece, Austria, New Zealand**

Serologic Evidence of Non-human HEV Infection Worldwide

ï Non-domestic animals

Rats

Rhesus monkey

ï Domestic animals

Chickens

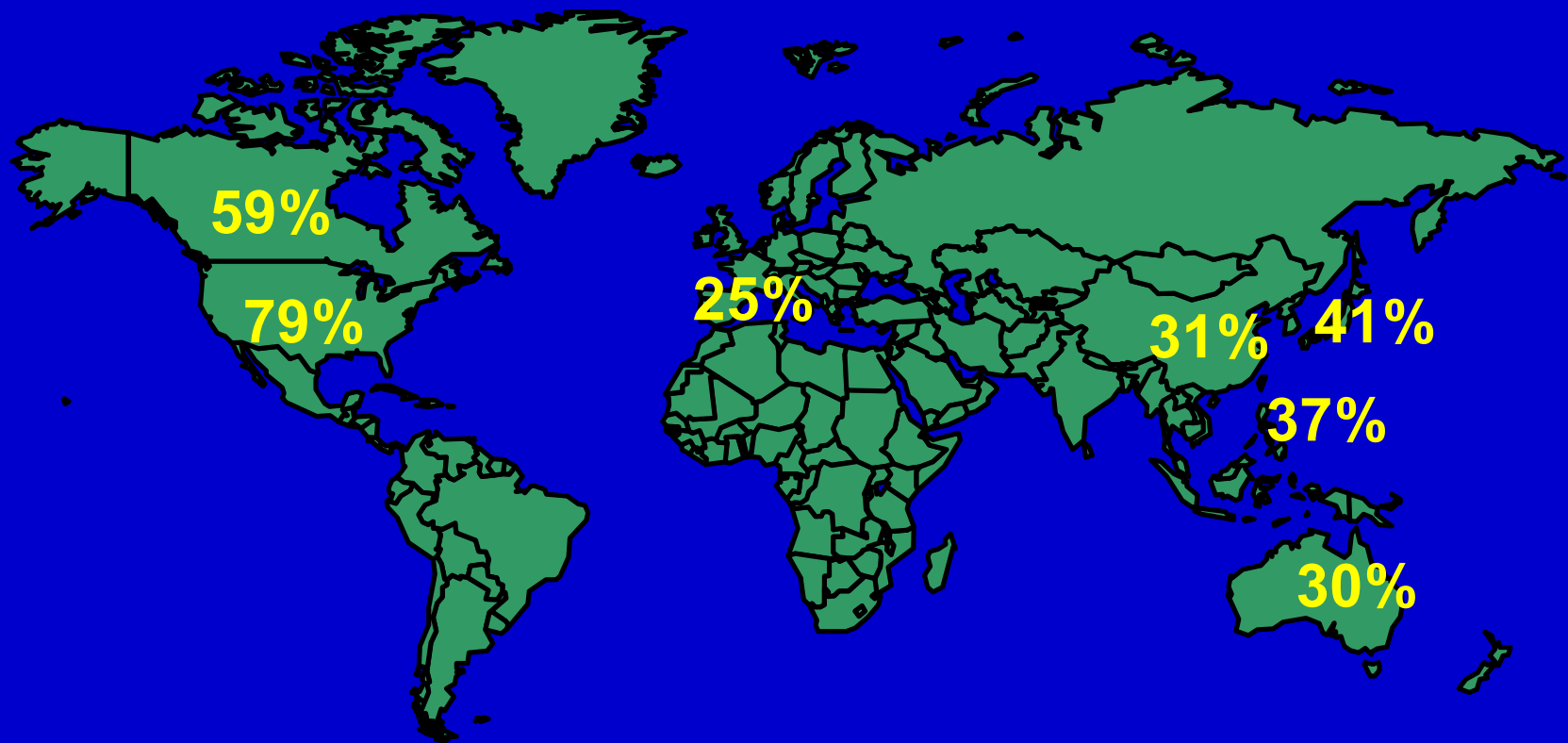
Dogs

Sheep and goats

Cattle

Swine

Worldwide Seroprevalence of HEV Antibodies in Swine



Indication of Potential Cross Species Transmission of HEV

- ï Cross-sectional studies: increased prevalence of anti-HEV in persons who work with swine in Moldova and the US*
- ï Anti-HEV among 2-20% U.S. blood donors
- ï Non-human primates infected with US-1 swine HEV
- ï Specific-pathogen-free pigs were infected with US-2 (a swine-like human isolate)

*Drobeniuc et.al, 2001, JID 184:1594, Meng et al., 2001, J.Clin. Micro. 40:117, and Wither et al., 2002, Am J. Trop Med. Hyg. Accepted

HEV Genotypes Identified Worldwide

	I	II	III	IV	
Genotype I Burma		76	74	75	Nucleic Acid Similarity (%) Full genome
Genotype II Mexico	83		75	74	
Genotype III US 1 Swine	83	82		75	
Genotype IV China-new	83	82	85		
	Amino acid Identity (%) ORF 1 region				

Objective

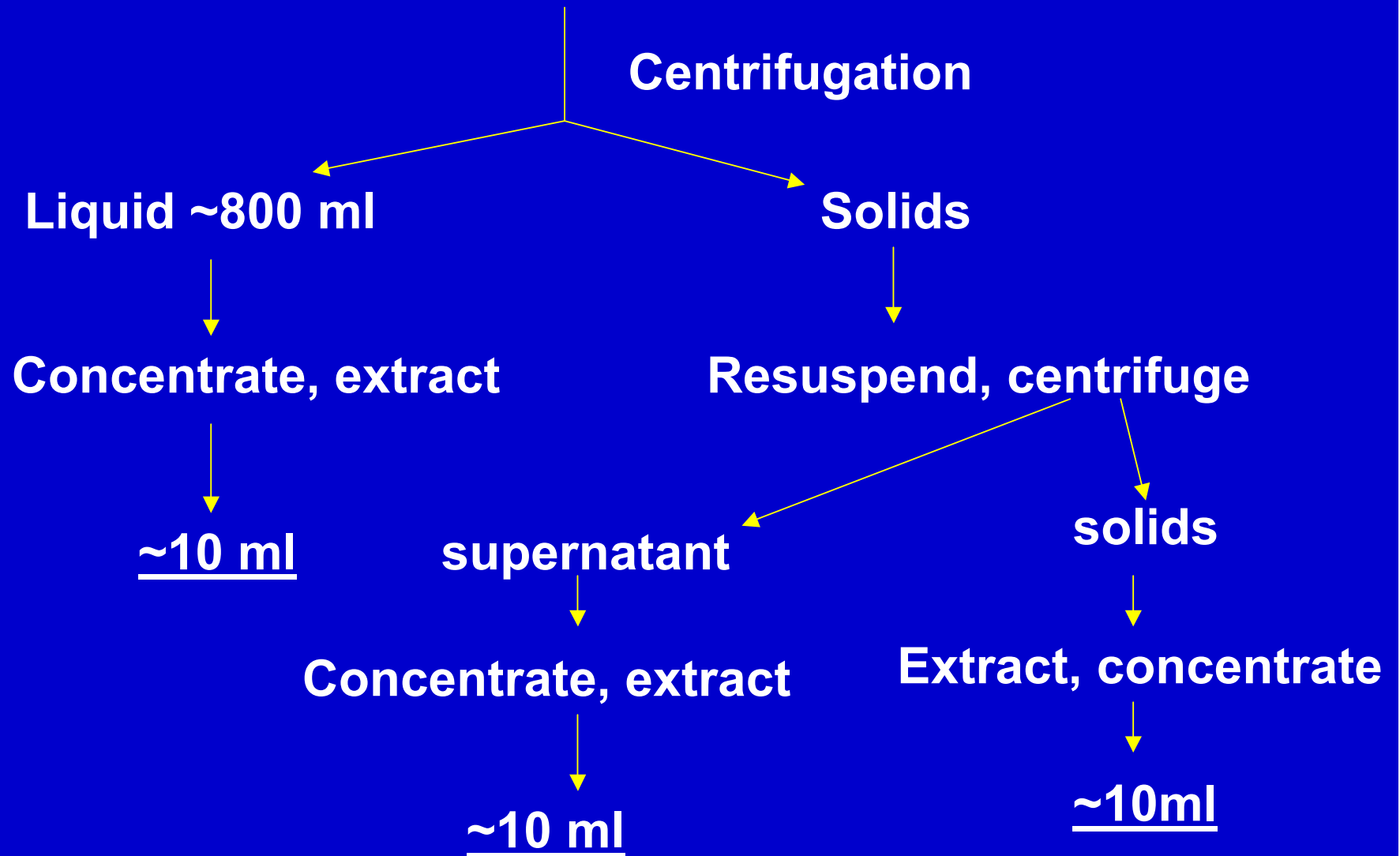
**Isolate and characterize swine HEV
from combined waste found in
pits/lagoons on swine farms**



Summary of Waste Samples Processed and Analyzed by RT-PCR

- ï Obtained from Midwestern farm where animals arrive (~50 lbs) and grow to ~200 lb**
- ï Sample of liquid manure taken from pit waste at time of field application**
- ï Five one liter samples:
 - Fall: 2 liters, pre- and post-application**
 - Spring: 3 liters, one pre-application and two post-application****
- ï Fall and spring samples represent collection from two different groups of animals**

Concentration of Virus from One Liter of Manure



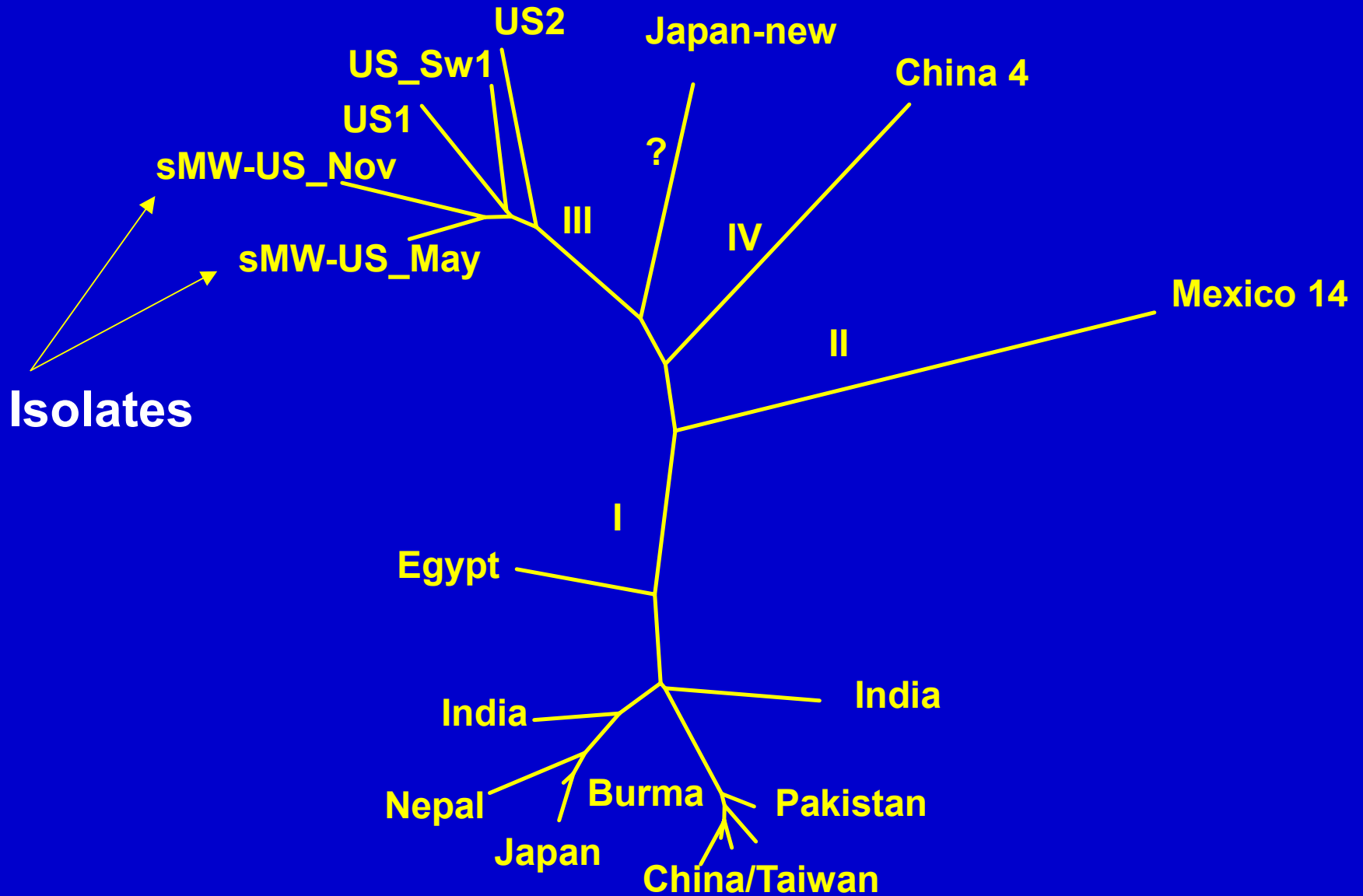
Evaluation of Concentrates

- ï Tested 140 ul sample of each 10 ml concentrate by RT-PCR for presence of swHEV ñprimers capsid region, swine specific, Meng et al. 1998
- ï End-point titration of each concentrate by serial ten-fold dilution
- ï $\sim 2 \times 10^6$ to 2×10^7 RT-PCR units (genome equivalents) per liter
- ï Direct sequence analysis of amplicons

Genome Organization of HEV



Phylogenetic Analysis of HEV Isolates



Summary

- ï Swine HEV was isolated from samples of pooled liquid manure
- ï Sequence analysis of one region (220 bp) of the genome yielded two unique, but related, sequences obtained at different time points
- ï Different time points represent different groups of animals
- ï These sequences are most similar to swUS-1 and two human US isolates US-1 and US-2 (Genotype III)
- ï Further epidemiological and environmental studies are needed

