Evidence of Extrahepatic Sites of Replication of the Hepatitis E Virus in a Swine Model

by

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(ABSTRACT)

Hepatitis E virus (HEV) is the major cause of enterically transmitted non-A, non-B hepatitis in many developing countries, and is also endemic in many industrialized countries. Due to the lack of an effective cell culture system and a practical animal model, the mechanisms of HEV pathogenesis and replication are poorly understood. It has been speculated that HEV replicates in sites other than the liver. Since HEV is presumably fecal-orally transmitted it is unclear how the virus reaches the liver and extrahepatic replication could be a possible explanation. The recent identification of swine HEV from pigs affords us an opportunity to systematically study HEV replication in a swine model.

We experimentally infected specific-pathogen-free (SPF) pigs with two strains of HEV: swine HEV and the US-2 strain of human HEV. Eighteen pigs (group 1) were each inoculated intravenously with swine HEV, nineteen pigs (group 2) with the US-2 strain of human HEV, and seventeen pigs (group 3) as uninoculated controls. To identify the potential extrahepatic sites of HEV replication using the swine model, two pigs from each group were necropsied at 3, 7, 14, 20, 27, and 55 days post inoculation (DPI). Thirteen different types of tissues and organs were collected from each necropsied animal. Reverse transcriptase PCR (RT-PCR) was used to detect the presence of positive strand HEV RNA in each tissue collected during necropsy at different DPIs. A negative strand-specific RT-PCR was standardized and used to detect the replicative, negative-strand of HEV RNA from tissues that tested positive for the positive strand RNA. As expected, positive strand HEV RNA was detected in almost every type of tissue at some time point during viremic period between 3 and 27 DPI. Positive-strand HEV RNA was still detectable in some tissues in the absence of serum HEV RNA from both swine and human HEV inoculated pigs. However, replicative, negative strand of HEV RNA was detected primarily in the

small intestine, lymph nodes, colon, and liver. Our results demonstrate for the first time that HEV replicates in tissues other than the liver and that the gastrointestinal tract is also the target of virus infection. The data from this study may have important implications for HEV pathogenesis, xenotransplantation, and the development of an in vitro cell culture system for HEV.

This thesis is dedicated to my parents
David and Ramona Williams with thanks
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Abbreviations

ag	= attogram
bp	= Base Pairs
CPE	= Cytopathic Effect
DPI	= Days post inoculation
ELISA	= Enzyme-linked Immunosorbant Assay
ENANBH	= Entericaly-transmitted non-A, non-B Hepatitis
fg	= femtogram
HAV	= Hepatitis A Virus
HBV	= Hepatits B Virus
HEV	= Hepatitis E Virus
IEM	= Immune Electron Microscopy
IV	= Intravenous
kb	= kilobases
MID	= Monkey Infectious Dose
min	= Minute
NCR	= Non-coding Regions
ng	= nanogram
ORF	= Open Reading Frame
PCR	= Polymerase Chain Reaction
PID	= Pig Infectious Dose
pg	= picogram
PSK II	= pBluescript II SK (+) plasmid
RT-PCR	= Reverse Transcriptase PCR
SPF	= Specific Pathogen Free
µl	= microliter