

Research letters

Zoonotic transmission of hepatitis E virus from deer to human beings

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Zoonosis has been suggested for hepatitis E virus (HEV) infection, but so far is based only on indirect evidence. We experienced a series of cases of HEV infection among people who had eaten uncooked deer meat 6–7 weeks before. On testing, a left over portion of the deer meat, kept frozen to eat in the future, was positive for HEV RNA, whose nucleotide sequence was identical to those from the patients. Patients' family members who ate none or very little of the deer meat remained uninfected. These findings provide direct evidence for HEV infection to be a zoonosis.

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Hepatitis E virus (HEV) infections, which are endemic and frequently epidemic in developing countries, are seen also in developed countries, but generally in the form of sporadic acute or fulminant hepatitis. Some cases in developed countries occur in people who have travelled to endemic areas, but others are of domestic infection, for which the method of transmission remains obscure.^{1–3} Since HEV or like viruses and antibodies to HEV have been noted in a wide variety of animals, especially swine, a

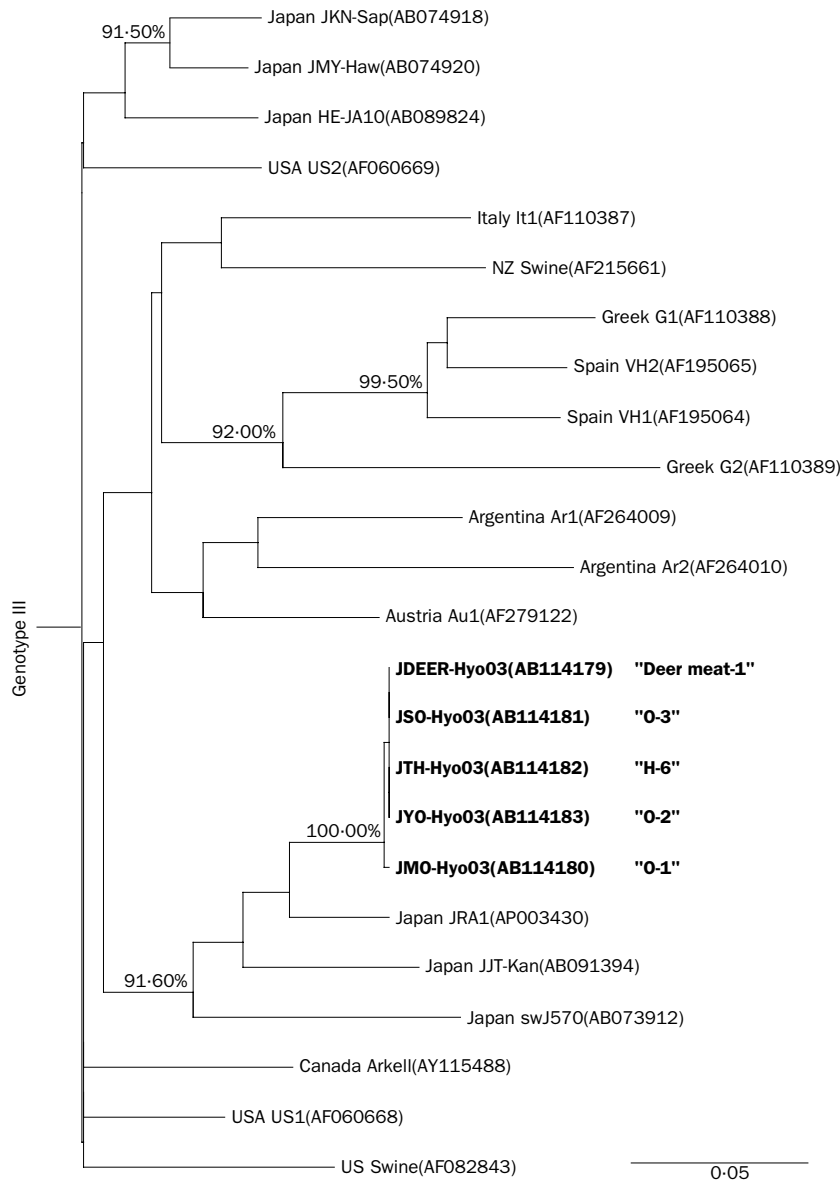
hypothesis has arisen that zoonosis is involved in the transmission of HEV, especially for the cases in non-endemic areas. This hypothesis is based on indirect evidence, such as the high frequency of antibodies to HEV in animal handlers and that the local pig strains are homologous to human strains in the same districts.⁴ We report direct evidence of zoonotic transmission.

A man aged 44 years (patient O-1, table) visited one of our hospitals on April 16, 2003, complaining of fever, nausea, and general malaise. Diagnosis of acute hepatitis was easily established based on raised liver enzymes and bilirubin. While he was recovering, his father (O-2) came to us on April 25 with symptoms and signs similar to his son's. Moreover, within 1 week after that, one of the brothers of the index patient (O-3) and his friend (H-6) also presented with hepatitis. All patients were negative for serological markers of hepatitis A, B, and C viruses when tested on admission. Later, however, all the serum samples obtained from these patients on April 29 were positive for HEV RNA and for IgM and IgG antibodies to HEV, leading to the diagnosis of hepatitis E.

	Family O					Family H	
	O-1	O-2	O-3	O-4	O-5	H-6	H-7
Age (years)/sex	44/male	69/male	42/male	38/male	35/female	61/male	30/male
Relation	Index patient	Father of O-1	Brother of O-1	Brother of O-1	Wife of O-4	Friend of O-3	Son of H-6
Ate deer meat							
Feb 22 (meat 1)	100 g	100 g	100 g	None	None	100 g	Very little
April 5 (meat 2)	100 g	100 g	100 g	100 g	100 g	100 g	100 g
April 13 (meat 3)	None	100 g	100 g	None	None	100 g	100 g
Time after admission of first patient							
April 16	Fever, nausea, malaise, ALT 2163 U/L, Bilirubin 54.7 mmol/L	Uneventful	Uneventful	Uneventful	Uneventful	Uneventful	Uneventful
April 25	ALT 394 U/L, bilirubin 29.1 mmol/L	Fever, nausea, malaise, ALT 3906 U/L, bilirubin 92.3 mmol/L	Uneventful	Uneventful	Uneventful	Uneventful	Uneventful
April 27	ALT 289 U/L, bilirubin 22.2 mmol/L	ALT 1605 U/L, bilirubin 90.6 mmol/L	Fever, malaise, arthralgia, ALT 666 U/L, bilirubin 10.3 mmol/L	Uneventful	Uneventful	Uneventful	Uneventful
April 29	ALT 201 U/L, bilirubin 18.8 mmol/L, anti-HEV IgM/G +/+, HEV RNA +	ALT 833 U/L, bilirubin 53.0 mmol/L, anti-HEV IgM/G +/+, HEV RNA +	ALT 455 U/L, bilirubin 8.6 mmol/L, anti-HEV IgM/G +/+, HEV RNA +	Uneventful, anti-HEV IgM/G -/-, HEV RNA -	Uneventful, anti-HEV IgM/G -/-, HEV RNA -	ALT 521 U/L, bilirubin 8.6 mmol/L, anti-HEV IgM/G +/+, HEV RNA +	Uneventful, anti-HEV IgM/G -/-, HEV RNA -
May 16	ALT 58 U/L, bilirubin 12.0 mmol/L	ALT 45 U/L, bilirubin 13.7 mmol/L	Uneventful	Uneventful	Uneventful	ALT 27 U/L, bilirubin 12.0 mmol/L	Uneventful

ALT=alanine amino transaminase.

Chronological description of cases



Phylogenetic tree (neighbour joining method) of the HEV genotype III isolates

A 326-nt sequence within the open reading frame 1 (corresponding to nt 124-449 of the prototype HEV strain Burma [M73218]) was compared between isolates of our study and known isolates. Only genotype III isolates shown. DDBJ/EMBL/GenBank accession numbers in parentheses. Bootstrap scores (1000 times) greater than 90% are shown.

Since the index patient, O-1, had travelled to China on February 15–17, 2003, we initially thought the virus was imported and had spread within the family. When we took the patients' histories, however, we were surprised to hear that they had eaten the meat of two wild-caught Japanese deer (Sika deer, *Cervus nippon nippon*), uncooked, like sashimi or sushi, three times during the 7-week period preceding their diseases. Thus a possibility of zoonotic transmission emerged.

The two families of which the present patients are members exchange or share a seasonal delicacies whenever they have them. They had eaten the deer meat three times during the period concerned (table). Fortunately, some left-over portions of deer meat had been kept frozen to eat in the future, labelled with the dates on which it was left over. We did PCR to detect HEV RNA, and found that the meat eaten on Feb 22 (deer meat 1) was positive but the

meat from another deer (deer meats 2 and 3), eaten on two occasions in April, were negative. This result was confirmed by another laboratory where HEV RT-PCR had never been done, to exclude the possibility of contamination. The titre of HEV RNA in deer meat 1 was about 10^5 copies/g.

We sequenced the HEV RNA from the deer meat and from the patients for 326 nucleotides within the open reading frame 1 by the methods described previously.⁵ The HEV sequence from the deer meat was 100% identical to those from patients O-2, O-3, and H-6, but not to that from O-1. Nevertheless, the nucleotide sequence between the HEV isolate from O-1 and the other three isolates differed by only one nucleotide (ie, 99.7% identity). As shown in the phylogenetic tree (figure), the sequences from our patients formed a mini-cluster in genotype III, neighbored by some Japanese isolates, yet with a notable genetic distance. These data suggested strongly that the four patients probably became infected with HEV by eating the deer meat on Feb 22, and prompted us to investigate the other members of the patients' families.

As shown in the table, three members (O-4, O-5, and H-7) in the two families were not infected with HEV. Among them, O-4 and O-5 did not eat the HEV-RNA-positive deer meat at all, although they ate the HEV-RNA-negative deer meats 2 and 3. In family H, the son (H-7) did eat the HEV-RNA-positive deer meat but did not become infected. He claims to have eaten only a very small portion, because his father (H-6, infected) ate most of deer meat 1. Thus hepatitis E developed only in the members of the two families who ate a notable amount of deer meat 1.

Our patients became infected with HEV by eating the raw meat of an infected deer. We know of no report that has described the presence of HEV RNA or antibodies in deer, whereas many have described its presence in swine, cows, goats, and rodents. We suggest the Sika deer and consumption of its raw meat be added to the list of foods with a risk of transmitting HEV.

Contributors

S Tei and N Kitajima had clinical responsibility for patients, and supplied the serum samples and other relevant materials. K Takahashi undertook virological analyses. S Mishiro analysed and interpreted the data and wrote the report.

Conflict of interest statement

None declared.

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Chromoendoscopy with methylene blue and associated DNA damage in Barrett's oesophagus

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Chromoendoscopy with methylene blue has been proposed to improve targeting of biopsies to specialised intestinal metaplasia and dysplasia in Barrett's oesophagus. However, methylene blue can induce oxidative damage of DNA when photosensitised by white light. We show that damage to DNA is increased in Barrett's mucosa after chromoendoscopy with methylene blue, an effect apparently dependent on presence of both methylene blue and endoscopic white light. Exposure of Barrett's mucosa to DNA damage during endoscopy warrants caution since it could accelerate carcinogenesis. This risk needs to be carefully balanced against the possible benefit of improved early detection of preneoplastic lesions with methylene blue chromoendoscopy.

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Chromoendoscopy with vital dyes is being applied more and more during routine endoscopic examinations to assist with visualisation of preneoplastic and neoplastic lesions. In particular, the thiazin dye methylene blue has been used in investigation of patients with Barrett's oesophagus who are undergoing surveillance because of a high risk of oesophageal adenocarcinoma. Methylene blue has been shown to selectively stain specialised intestinal metaplasia, referred to as Barrett's oesophagus, since it is specifically absorbed by the goblet cells that characterise this preneoplastic tissue type. Thus, methylene blue chromoendoscopy might improve endoscopic surveillance, by targeting biopsies either to focal regions of Barrett's oesophagus, or where stain is poorly absorbed within an area of Barrett's oesophagus, to potentially dysplastic lesions.¹

Results of experimental studies show that methylene blue binds directly to DNA, and can intercalate with purine nucleotides resulting in helical unwinding.² Furthermore, photosensitisation of methylene blue with white light stimulates formation of singlet oxygen species.³ In the

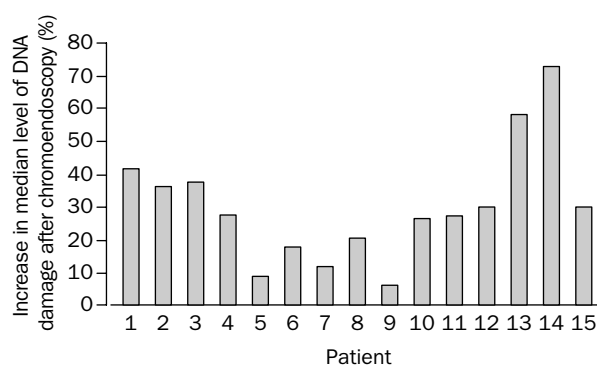


Figure 1: Effect of methylene blue chromoendoscopy on DNA damage in Barrett's oesophagus

Data are % increase in median level of DNA damage after chromoendoscopy for each patient.

presence of DNA, this process leads to generation of single-strand breaks and oxidative alterations to guanine residues, of which the promutagenic DNA adduct 8-hydroxydeoxyguanosine predominates.⁴ We postulated that application of methylene blue to the oesophagus followed by exposure to white light from the endoscope would stimulate high levels of DNA damage in Barrett's oesophagus, a preneoplastic tissue that is already genetically unstable.

After hospital ethics review and written informed consent, we took biopsy samples from 12 men and three women with Barrett's oesophagus (median age 62 years, range 34–76). The patients had histologically confirmed specialised intestinal metaplasia (median length of Barrett's oesophagus segment 5 cm, range 2–15) and were undergoing endoscopic surveillance at Pontefract General Infirmary. Chromoendoscopy with methylene blue (0.5% solution), preceded immediately by spraying with 10 mL N-acetyl cysteine (10% solution) to remove surface mucus, is used routinely at this hospital during surveillance of Barrett's oesophagus. Consequently, we could obtain biopsy samples from immediately adjacent sites at the midpoint of the segment of Barrett's oesophagus before and after exposure to methylene blue. After exposure to methylene blue and N-acetyl cysteine, we took biopsy samples clear from sites that showed any aberrant staining in the segment of Barrett's oesophagus. We used conventional alkaline single-strand gel electrophoresis (comet assay) to assess the level of strand breaks and alkali labile sites in DNA.⁵ DNA damage in individual cells was assessed by measuring the percentage of DNA in the tail of the comet. In addition, for all biopsy samples, we incorporated the enzyme Fapy-DNA glycosylase (FpG) at saturating concentrations into the comet assay as described

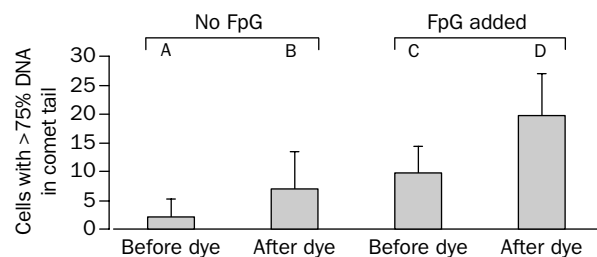


Figure 2: Effect of methylene blue chromoendoscopy on level of Fapy-DNA glycosylase-sensitive sites in Barrett's oesophagus

Data are median number of cells with >75% DNA in comet tail (n=15). Bars are 75th percentiles.