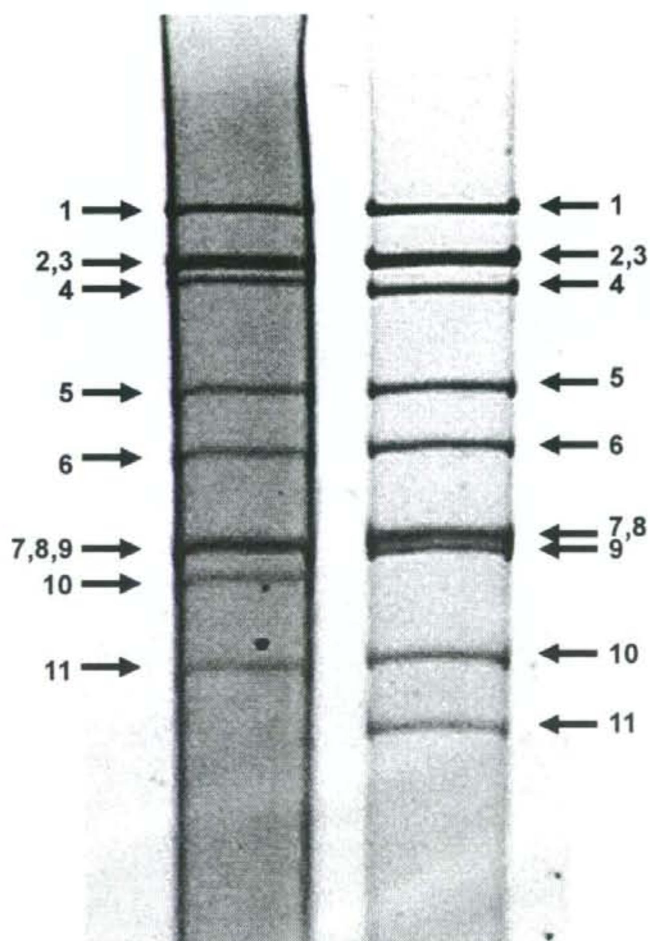

図3　ウシロタウイルス2株のPAGEにおけるRNA泳動パターン

両株の抽出RNAの10%ポリアクリルアミド電気泳動像

→ RNA分節のバンド

図4 GB12-22株とKY2565株の各遺伝子の相同性および系統樹解析結果

VP7遺伝子は72.6%と低い相同性を示し、異なるG遺伝子型だった。

系統解析における各分節の近縁の株の由来を示した。それぞれ :ウシ :サル :ヒトを表す。

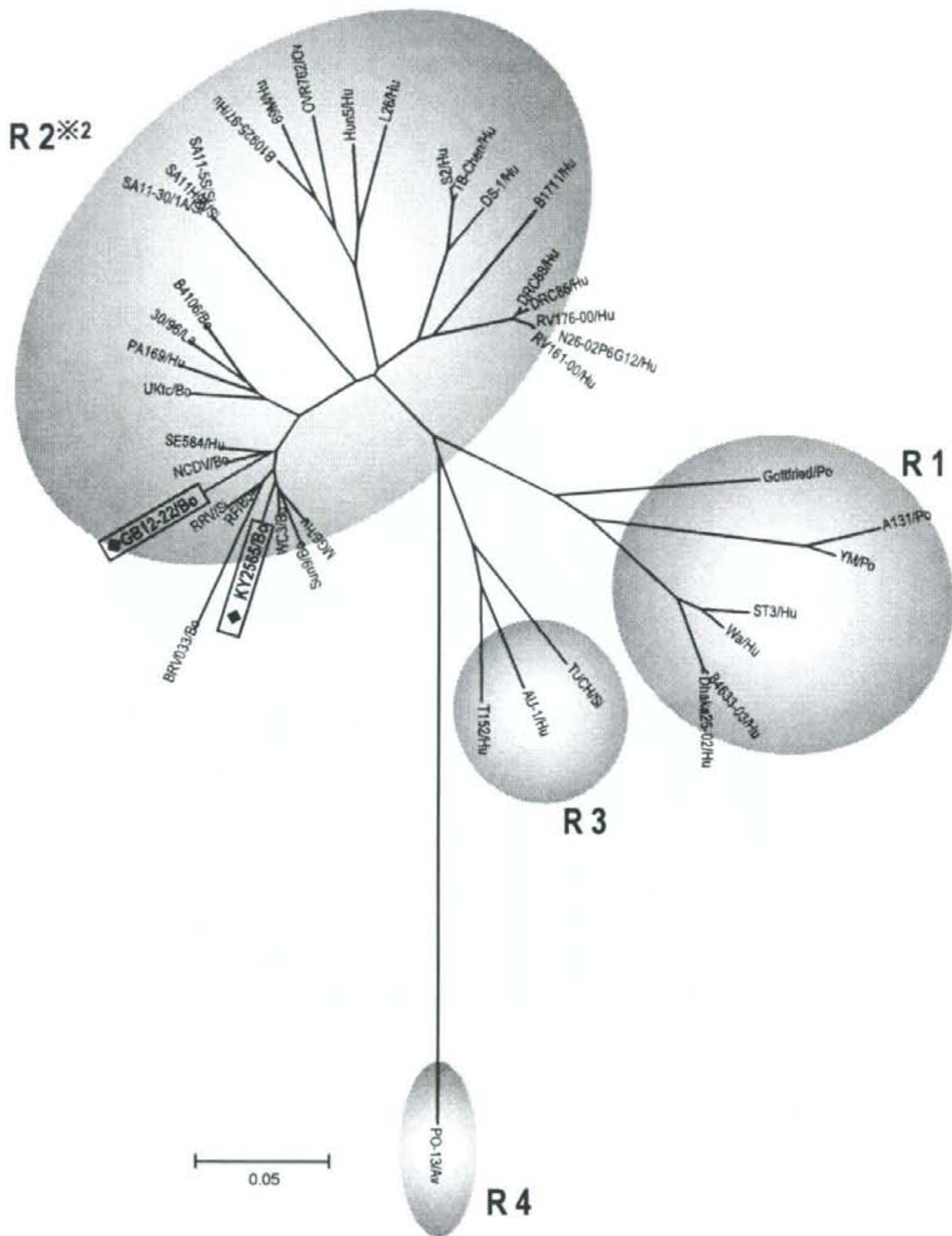

図5 GB12-22株とKY2565株および各種ロタウイルスの

VP1遺伝子に基づく系統樹

※1 株名/由来(Hu:ヒト、Bo:ウシ、Si:サル、Po:ブタ、Ov:ヒツジ、

La:ウサギ、Av:家禽)

※2 遺伝子型

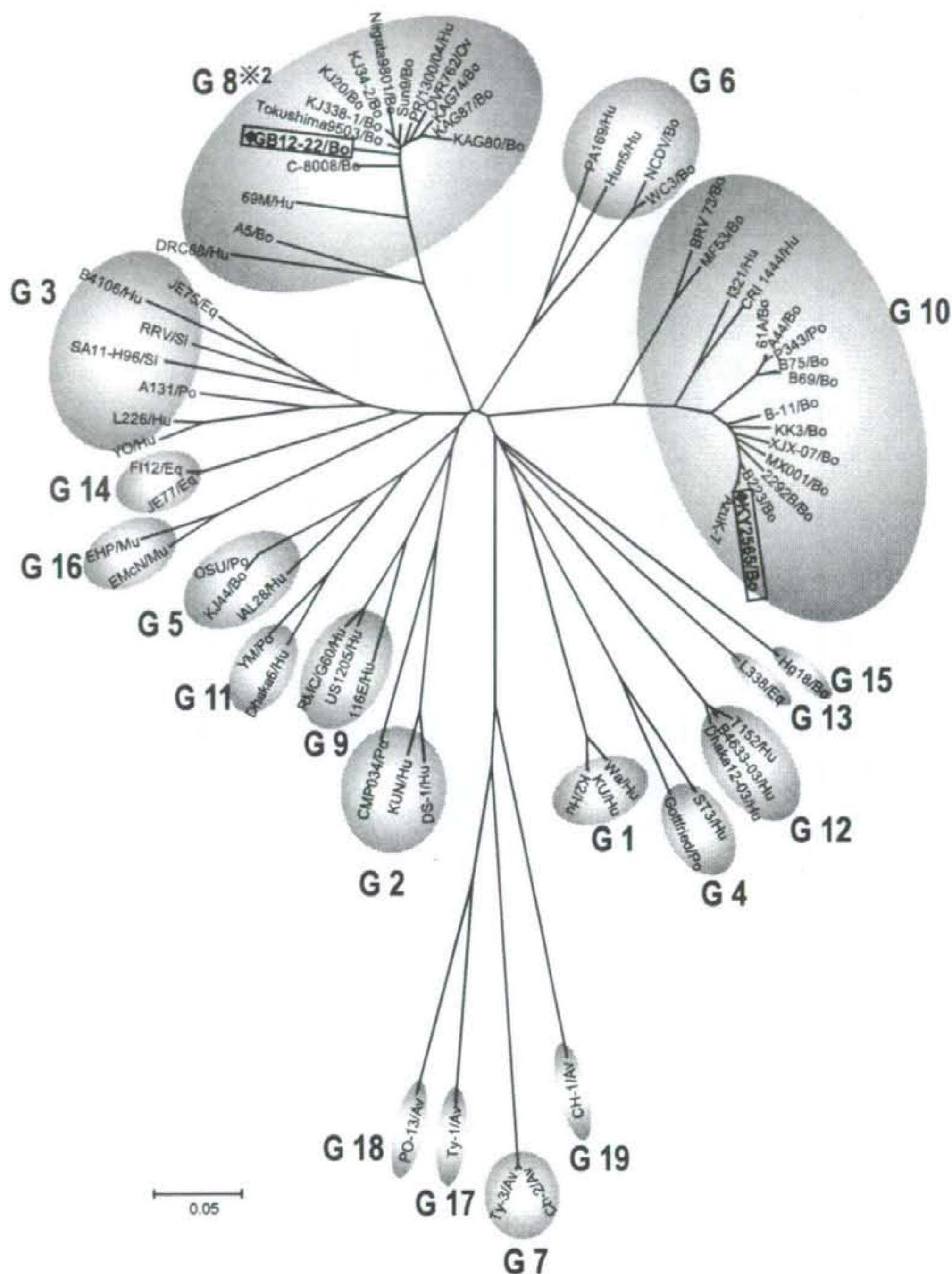

図6 GB12-22株とKY2565株および各種ロタウイルスの

VP7遺伝子に基づく系統樹

※1 株名/由来(Hu :ヒト、Bo:ウシ、Si:サル、Po:ブタ、Eq:ウマ、Ov:ヒツジ、

Mu:ネズミ、Av:家禽)

※2 遺伝子型

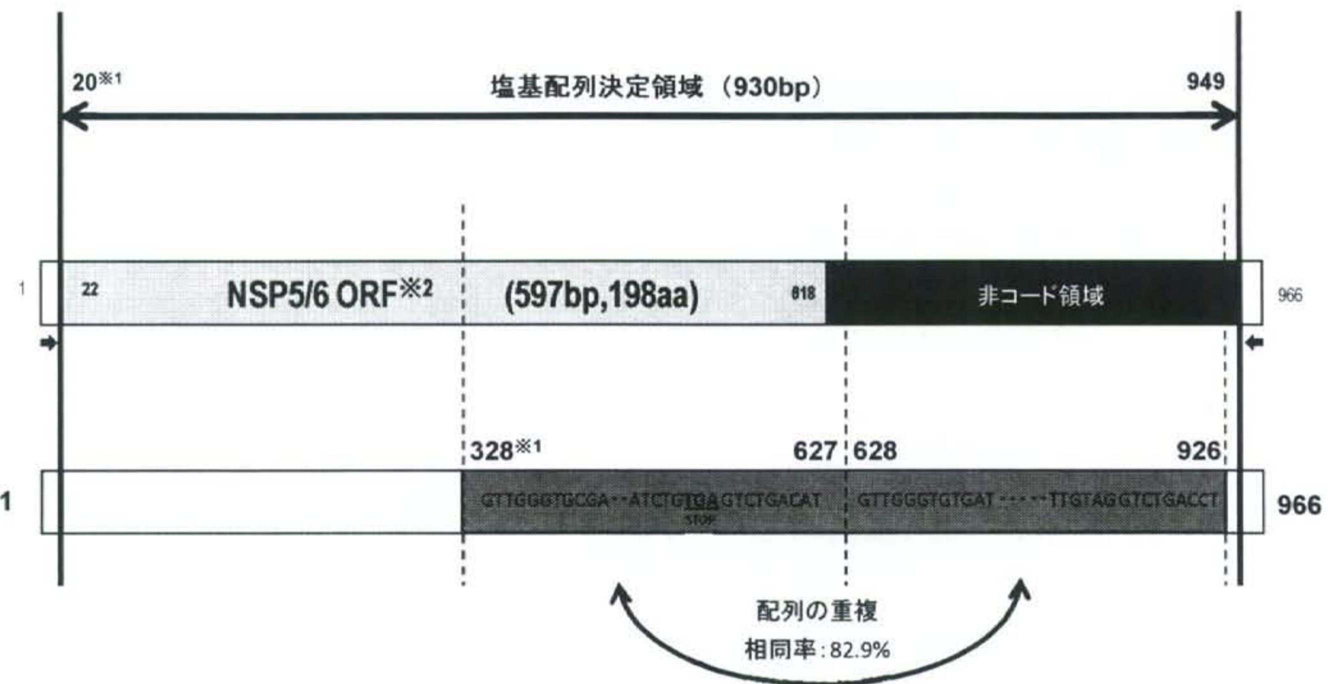

図7　GB12-22株 GB12-22　リアレンジメントNSP5/6遺伝子

■：重複領域　　□：NSP5/6 ORF

➡：プライマー（GEN_NSP5F、表1）　⬅：プライマー（GEN_NSP5R）

※1　ウシロタウイルスVMRI株に基づく塩基配列番号

厚生労働省科学研究費補助金（食品の安心・安全確保推進研究事業）
分担研究報告書

エゾシカにおけるＥ型肝炎ウイルスの疫学調査

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研究協力者：大澤宜明、井上恵美（北海道医療大学薬学部）

研究要旨：　2007年4月～2008年1月、北海道日高地区で捕獲されたエゾシカ320頭の血清からRNAを抽出し、E型肝炎ウイルス（HEV）ORF2領域を標的としたRT-PCRによりHEV遺伝子の検出を試みた。その結果、1検体（0.3%）でHEV遺伝子断片の増幅が認められた。その塩基配列から本ウイルスは北海道で分離されたブタのHEVに近縁であり、Genotype 3に属するものと考えられた。エゾシカがHEVに感染していることが確認されたことから、HEV感染を防ぐためエゾシカ肉の調理には十分な加熱をする必要がある。

A. 研究目的

Ｅ型肝炎ウイルス（HEV）は感染者の糞便中に排泄され、それに汚染された水などが主な感染源となり、東南アジアや中米、アフリカなど熱帯・亜熱帯地域で散発的に流行してきた。日本を始めとする先進国でのＥ型肝炎発生例の大部分は発展途上国で感染を受けた輸入感染であるが、近年、日本や米国などで海外渡航歴の無いＥ型肝炎の散発的な発生例が報告されている。このような中、兵庫県において野生シカの生肉を感染源とするＥ型肝炎患者が報告された。さらに、北海道で市販されていた豚レバーからHEV遺伝子が検出され、食肉が日本における散発例の原因である可能性が示された。

エゾシカはニホンジカの１亜種で、本州で見られる「ホンシュウジカ」とは同じ種に属する。近年、北海道ではエゾシカによる食害や衝突事故が多発し、エゾシカ有効活用推進事業の一環として肉の普及が図られている。エゾシカ肉は、ルイベ（凍結生肉の刺し身）あるいはカルパッチョとして生で食される機会も少なくなく、エゾシカにおけるHEVの感染状況の解明は急務である。

そこで、我々は日高地区で捕獲されたエゾシカについて血清疫学調査を実施し、当該地域のエゾシカの10～30%が抗HEVIgG抗体を保有していることを示した。ヒトではIgM抗体あるいはウイルスRNAの検出をもってHEV感染が診断されることから、本年度は抗体調査に供したエゾシカ血清からの HEV 遺伝子の検出を行い、エゾシカのHEV感染を証明することを試みた。

B. 研究方法

1. 血清およびHEV：2007年4～2007年12月、北海道日高地方で捕獲されたエゾシカ320頭の血清を試験に供した。年齢は、0歳：23頭、1歳：25頭、2歳：87頭、3歳：61頭、4歳以上：123頭、不明：1頭であった。年齢はエゾシカを捕獲した猟師が判断した推定年齢に従った。4歳以上では年齢の推定が困難なため、一群としてまとめた。陽性対照として、北海道のブタから分離されたGenotype 3 HEVswJB-M8株に実験感染したブタの糞便から精製したHEV粒子を用いた。これは酪農学園大学萩原克郎博士から分与を受けた。

2. RNA抽出：FUJIFILMのQuickGene RNA tissue kit S IIおよびQuickGene-Mini 80を用いた。

3. nested PCR：HEV ORF2領域に相補的なプライマー RV1 (5'-CCYTTATCYTGGTGNGCRTTCTC -3')およびSuperScript IIを用いてcDNAを得た。これを鋳型にプライマー対 FW1 (5'-AATTAYGCYCAGTAYCGBGTKG-3')/RV1 およびTaq DNA polymeraseを用いてPCRを行った。nested PCRでは、プライマー対 Fw2(5'-GTHATGCTYTGYATYCATGGVT-3')/Rv2 (5'-YGCCGACGAAATCAATTCTGTGTC-3')を用いた。反応条件は、何れも95℃で7分間加熱後、94℃ 1分、56℃ 1分、72℃ 2分を35サイクル行った後、72℃に7分間放置とした。

4. サザンハイブリダイゼーション：Terminal deoxynucleotidyl transferaseを用い、陽性対照から増幅されたDNA断片をDIGで標識した。nested PCRの産物をアガロースゲル電気泳動で分画し、アルカリ変性後、キャピラリートランスファーによってHybond N+膜に転写した。DIG標識プローブと反応させ、洗浄後、AP標識抗DIG抗体を用いてプローブを検出した。

5. 塩基配列の決定：RT-PCR産物をリン酸化し、pBluescript II KS (+) の*Eco*RV切断部位にクローニングした。複数のクローンについてT7およびT3プロモータープライマーを用いて挿入断片の塩基配列を決定し、2本鎖両鎖の配列が完全に一致することを確認した。

C. 研究結果

1. エゾシカ血清からのHEV遺伝子の検出：調査期間中捕獲したエゾシカ320頭中1頭 (0.3%) の血清から陽性対照と同位置に泳動されるDNA断片が検出された。サザンハイブリダイゼーションで陽性シグナルを示したことからHEV遺伝子と考えられた(図1)。

2. エゾシカ由来HEV遺伝子の塩基配列：図2に示すように、北海道のブタから分離されたHEVswJB-M8株とは320塩基中2塩基の置換が認められた（図2）。塩基ホモロジーは99.4%であり、アミノ酸の置換は予想されなかった。系統進化解析の結果、エゾシカ由来HEVはGenotype 3に属するものと考えられた。

D. 考察

北海道日高地方で捕獲されたエゾシカの血清中にHEV遺伝子を見出した。陽性対照として用いた北海道のブタ由来HEVとは、調べた320塩基中2塩基の置換しか認められなかった。検体との混交を否定するため、検体および陽性対照について複数クローンの解析を行い、全く同一の塩基置換を確認した。本研究で標的としたORF2領域はHEV株間でよ

く保存されており、5年を隔ててヒトおよびブタから分離された株間の塩基配列が完全に一致した例も報告されている。本調査域内には放牧養豚場が点在している。国内の養豚場のほとんどでHEV感染豚が認められることならびにウイルス感染豚は3-4週に渡り大量のウイルスを排泄することから、エゾシカは放牧養豚場のブタから感染した可能性が極めて高い。

エゾシカ血清のウイルス遺伝子陽性率は0.3%であった。これまでの抗体調査の結果、日高地方のエゾシカの 10〜30%が抗 HEV IgG抗体を保有することが分かっている。また、抗体陽性率は加齢とともに上昇する傾向にある。一方、国内のブタの抗HEV IgG抗体保有率および血中のウイルス遺伝子陽性率は、各々平均で58%および10%とされている。ブタは1歳未満の肥育豚の比率が大きいためウイルス陽性率は高くなるが、エゾシカでは3歳以上の個体が約 60%を占めていたため遺伝子陽性率は低くなるものと考えられる。

E. 結論

日高地方のエゾシカがHEV遺伝子を保有していることがわかった。HEV の感染源となる危険性があることから、エゾシカ肉の生食は厳に慎むべきである。

F．健康危険情報

特になし

G．研究発表

1．論文発表

Tomiyama, D., Inoue, E., Osawa,Y., and Okazaki, K: Serological evidence of infection with hepatitis E virus among wild Yezo-deer, Cervus nippon yesoensis, in Hokkaido, Japan. J. Viral Hepat. (in press)

2. 学会発表

1) 川口紘史、井上恵美、大澤宜明、岡崎克則　エゾシカ血清からのE型肝炎ウイルスRNAの検出　第56回日本ウイルス学会　岡山市　2008年10月

2) 井上恵美、浅野逸郎、川口紘史、松村佳子、室内友恵、大澤宜明、岡崎克則　A型インフルエンザウイルス共通プライマーを用いたHAおよびNA亜型遺伝子型別法の開発　第 56 回日本ウイルス学会　岡山市　2008年10月

H．知的財産権の出願・登録状況

1．特許取得　なし

2．実用新案登録　なし

3．その他　なし

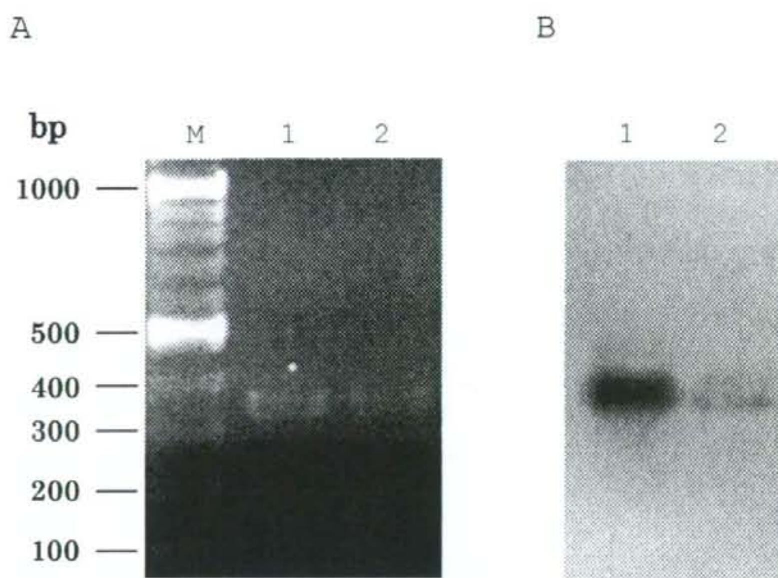

図1. nested PCRによるHEV遺伝子の増幅 (A) とサザンハイブリダイゼーションによる相同性の検出 (B). レーン1, ブタ由来HEV swJB-M8株; 2, エゾシカ血清#50831。MはDNAサイズマーカーを示す。

```
#50831     1  CCCCTGTTAACTCCTACACTAATACACCTTATACTGGTGCATTGGGGCTCCTTGATTTTG   60
swJB-M8       ------------------------------------------------------------
HE-JI3C       ----------T--------------------C---------C------------------

#50831    61  CATTGGAACTTGAATTTAGAAACTTGACACCCGGGAATACCAACACCCGTGTCTCCCGGT  120
swJB-M8       ------------------------------------------------------------
HE-JI3C       ------------------------------------------------------------

#50831   121  ATACTAGCACAGCTCGCCACCGGCTGCGTCGCGGTGCTGATGGGACAGCAGAGCTTACCG  180
swJB-M8       -----------------------------------------------------------A
HE-JI3C       -------------C-------------------------------------T---C---A

#50831   181  CCACAGCAGCCACACGTTTCATGAAAGATCTGCATTTTACTGGCACGGACGGTGTTGGTG  240
swJB-M8       -----------------------------------------------A------------
HE-JI3C       -----------------------------------------------A------------

#50831   241  AGGTGGGTCGTGGTATTGCCCTGACACTGTTTAATCTTGCTGACACGCTTCTTGGCGGTT  300
swJB-M8       ------------------------------------------------------------
HE-JI3C       ------------------------------------------------------------

#50831   301  TACC                                                          304
swJB-M8       ----
HE-JI3C       ----
```

図2. HEV ORF2 領域塩基配列の比較. エゾシカ血清由来#50831 と陽性対照として用いたブタ由来 swJB-M8 株および日本のヒト由来 HE-JI3C 株の塩基配列を比較した。

Ⅲ．研究成果の刊行に関する一覧表

研究成果の刊行に関する一覧表

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Yoshii M, Okinaga T, Miyazaki A, Kato K, Ikeda H, Tsunemitsu H	Genetic polymorphism of the nsp2 gene in North American type--porcine reproductive and respiratory syndrome virus.	Arch Virol	153 (7)	1323-1334.	2008
Tomiyama D, Inoue E, Osawa Y, and Okazaki, K	Serological evidence of infection with hepatitis E virus among wild Yezo-deer, *Cervus nippon yesoensis*, in Hokkaido, Japan.	J Viral Hepat			in press
Ito T	Outbreaks of highly pathogenic avian influenza in Japan.	Glob Env Res	12 (1)	15-20.	2008
Motoike K, Hirano S, Yamana H, Onda T, Maeda T, Ito T, and Hayakawa M	Antiviral activities of heated dolomite power.	Biocontrol Sci	13 (4)	131-138	2008

Ⅳ. 研究成果の刊行物・別刷

Journal of Viral Hepatitis, 2009 doi:10.1111/j.1365-2893.2009.01107.x

Serological evidence of infection with hepatitis E virus among wild Yezo-deer, *Cervus nippon yesoensis*, in Hokkaido, Japan

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Received April 2008; accepted for publication December 2008

SUMMARY. In this study, 520 serum samples from Yezo-deer in the Hidaka district, Hokkaido, Japan were examined by enzyme-linked immunosorbent assay to investigate whether the animals were infected with hepatitis E virus (HEV). The distribution of optical density values showed a bimodal pattern and 181 samples (34.8%) were deemed to be antibody-positive against HEV. At least five (2.8%) of the positive sera gave specific bands by Western blot analysis. An age-dependent increase in prevalence of the antibodies was found among the animals. These findings indicate that Yezo-deer are a possible host for HEV infection. To avoid the risk of becoming HEV infected, the consumption of raw Yezo-deer meat must be prohibited.

Keywords: hepatitis E virus, seroprevalence, Yezo-deer.

INTRODUCTION

Hepatitis E virus (HEV) is the causative agent of acute self-limiting hepatitis in humans [17] and the sole member of the genus *Hepevirus* in the provisionally proposed family *Hepeviridae* [4]. The virus is thought to comprise a single serotype [17], although it is divided into at least four genotypes (genotypes 1–4) [19]. The majority of infections in several countries in Asia and Africa [8,25] are caused by genotype 1 and in Mexico and Nigeria by genotype 2 [3,26]. Genotype 3 HEV is widely distributed and found in the USA, Europe and Asia [7,16,18,21,23], while genotype 4 is largely confined to Asia [23,27].

Hepatitis E occurs sporadically following transmission by the fecal–oral route through sewage-contaminated water supplies in developing countries [1]. In addition, zoonotic food-borne transmission by rare meat from pig and boar has been reported in industrialized countries [5,11,28]. In Japan, direct evidence of HEV transmission from rare meat from wild deer (*Cervus nippon centralis*) to humans was provided in 2003. On this occasion, nucleotide sequencing of the virus amplified from the remaining meat matched to that from the patient and a mini-cluster of infection was attributed to genotype 3 [22]. These facts suggest that wild deer can be one of the sources of HEV infection for humans in Japan.

Wild deer known as 'Yezo-deer' (*C. nippon yesoensis*) have inhabited Hokkaido, where hepatitis E is most prevalent in Japan. People have the opportunity to eat deer meat and liver raw (sashimi), more so in Hokkaido than other parts of Japan. In this study, serological surveillance for HEV infection among Yezo-deer was carried out in order to investigate whether the animals harbour HEV, which can potentially be transmitted to human beings.

MATERIALS AND METHODS

Serum samples

A total of 520 serum samples were collected from Yezo-deer hunted in the Hidaka district of Hokkaido from February 2006 to June 2007 and stored at −20 °C until use. Out of the 520 animals, 185 (35.7%) were male and 334 (64.4%) were female. Their age and sex ratio (male/female) were as follows: less than 1 year, 35 animals (21/14); 1 year, 40 (23/17); 2 years, 123 (70/53); 3 years 134 (20/114); over 4 years, 188 (51/137). The age was estimated by the hunters. Animals older than 4 years old were dealt with collectively, as the age-estimation is difficult for this group.

Enzyme-linked immunosorbent assay

Indirect enzyme-linked immunosorbent assay (ELISA) was carried out as described previously with slight modification

Abbreviations: BSA, bovine serum albumin; ELISA, enzyme-linked immunosorbent assay; HEV, hepatitis E virus; HRP, horseradish peroxidase; PBS, phosphate-buffered saline; SD, standard deviation; VLPs, virus-like particles.

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[12]. Briefly, baculovirus-expressed recombinant virus-like particles (VLPs) derived from genotype 3 [10] (50 ng/well) in 50 mM carbonate buffer (pH 9.5) was distributed to a 96-well formatted microplate (FALCON; Becton Dickinson, Franklin Lakes, NJ, USA) and incubated at 4 °C overnight. After blocking with 100 μL of 1% (w/v) bovine serum albumin (BSA, fraction V, Sigma, St Louis, MO, USA) dissolved in phosphate-buffered saline (PBS), pH 7.2, x50 μL of serum sample, diluted 1:200 with PBS containing 0.05% Tween-20 (PBS-T) and 0.5% (w/v) BSA, was added to each well. After incubation at 37 °C for 1 h, the plate was washed four times with PBS-T. Horseradish peroxidase (HRP)-conjugated rabbit anti-deer IgG (H+L) (KPL, Guildford, UK) in 0.5% BSA/PBS-T (1:500 dilution) was added to the wells and incubated at room temperature for 1 h. After further washing, 100 μL/well of substrate (FAST™ OPD; Sigma), was added and incubated for 20 min in the dark. The reaction was stopped by addition of 50 μL of 2.5 M H_2SO_4 and the optical density was measured at 490 nm ($OD_{490}Ag^+$). The optical density ($OD_{490}Ag^-$) was simultaneously measured using an antigen-free coated plate. Specific binding of antibodies to the antigen was expressed by $\Delta OD_{490} = (OD_{490}Ag^+) - (OD_{490}Ag^-)$.

Western blot analysis

Fifteen micrograms of VLPs was boiled in 125 μL of Laemmli sample buffer [9] for 5 min and loaded onto a 1 mm thick × 70 mm width 10% (w/v) polyacrylamide gel. After electrophoresis, VLPs were electroblotted onto a polyvinylidene difluoride membrane (Immobilon®; Millipore, Bedford, MA, USA) [24]. After blocking with 3% BSA overnight at 4 °C, the membrane was washed once with Tris-buffered saline (10 mM Tris–HCl, pH 7.4, 140 mM NaCl) containing 0.05% Tween-20 (TBS-T), separated into strips parallel with the mobility of bands, and incubated with the serum samples diluted 1:100 in Can Get Signal® (Toyobo, Osaka, Japan) at room temperature for 1 h. After washing four times with TBS-T for 15 min each wash, the membranes were incubated with the HRP-conjugated rabbit anti-deer IgG (H+L) diluted with Can Get Signal® buffer at room temperature for 1 h. The enzymatic activity on the membrane was detected using western lightning chemiluminescence reagent plus (Perkin Elmer Life Sciences, Boston, MA, USA) as substrate. The luminescence of bands was detected by a LAS-1000 UV mini® instrument (Fujifilm, Tokyo, Japan).

RESULTS

Detection of anti-HEV by ELISA

In order to investigate the HEV infection status of Yezo-deer, 520 serum samples were collected from these animals between February 2006 and June 2007 in the Hidaka district of Hokkaido, Japan and examined by ELISA using VLPs

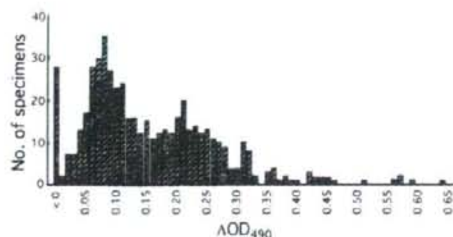

Fig. 1 Distribution of ΔOD_{490} values obtained following testing of serum samples by ELISA.

as antigen. Figure 1 shows the distribution of ΔOD_{490} values obtained by ELISA. The ΔOD_{490} values obtained for the 520 serum samples ranged from −0.141 to 0.639. The frequency of the ΔOD_{490} values showed a bimodal distribution with peaks at 0.079 and 0.207.

Confirmation of anti-HEV antibodies by Western blot analysis

In order to confirm the specificity of the reaction, 60 of the serum samples showing ΔOD_{490} value higher than 0.168 by ELISA, which was the lowest value between the two peaks, were examined by Western blot analysis. As shown in Fig. 2, hyperimmune serum against HEV [12] gave a clear band at the position of 53 kDa. It was found that serum samples #50365, #50678, #50738, #50742 and #50744 gave a

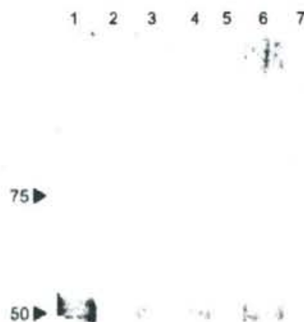

Fig. 2 Detection of anti-HEV antibodies by Western blot analysis. Specificity of the serum samples, of which ΔOD_{490} values were higher than 0.168 in ELISA, was examined by Western blot analysis. Specific bands by anti-VLPs hyperimmune serum (lane 1) and by the serum sample 50365 (lane 2), #50678 (lane 3), #50738 (lane 4) and #50744 (lane 5) are shown. Serum sample #50362, of which the ΔOD_{490} value was 0.049, was used as negative control (lane 6). The positions of the molecular weight markers $\times 10^{-3}$ are shown in the left-hand margin.

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pronounced band at the same position. No band was found with sample #50362, the ΔOD_{490} value of which was 0.049. Serum sample #50546 showed no band, although its ΔOD_{490} value was the highest among the samples (data not shown). These findings indicate that at least five serum samples contained antibodies specific for HEV.

Prevalence of anti-HEV antibodies among Yezo-deer in Hidaka district

The ΔOD_{490} values by ELISA of #50365, #50678, #50738, #50742 and #50744 were 0.307, 0.290, 0.585, 0.206 and 0.458, respectively. In order to define the prevalence of anti-HEV antibodies, the cut-off value for the ELISA was calculated based on the lowest ΔOD_{490} value giving a specific band by Western blot. The mean value for those less than 0.206 was calculated to be 0.051 with a standard deviation (SD) of 0.066. Therefore, the cut-off value, being the mean value plus 2 × SD, was set up at 0.183. Using this criterion, 181 of the 520 serum samples (34.8%) were found to be positive for anti-HEV antibodies by ELISA. Out of the 185 serum samples from male animals, 58 (31.4%) and 123 (36.7%) of the 335 samples from female ones were positive for anti-HEV antibodies, showing no significant difference in the positive rates between males and females. The seroprevalence at each shooting point marked on the centre of a grid on the map for wild life preservation and game is shown in Fig. 3.

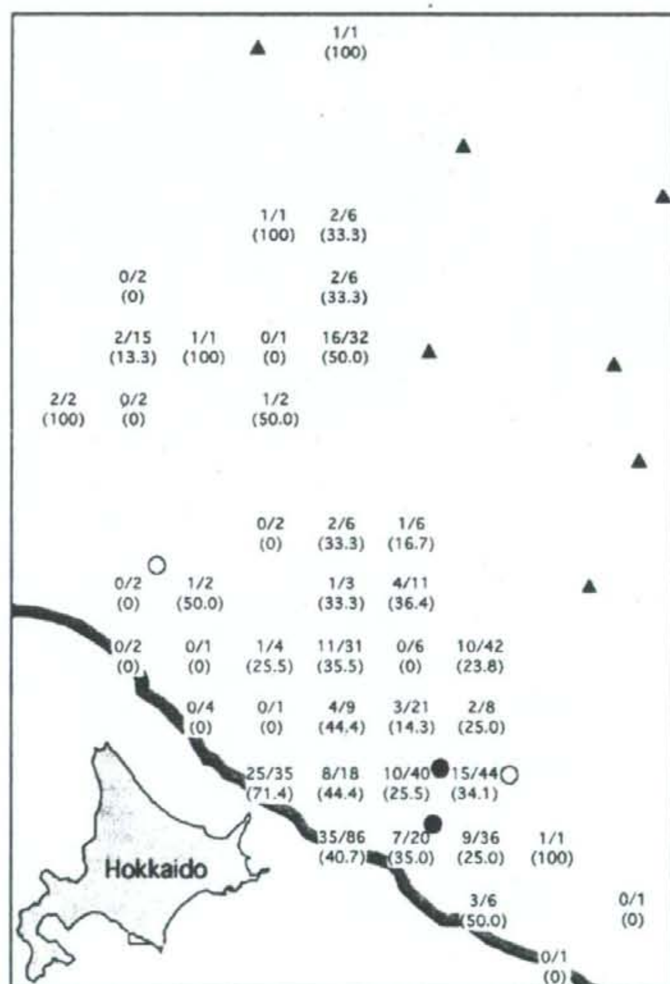

Fig. 3 Prevalence of anti-HEV antibodies among deer hunted in the surveillance area. No. of positive sample/no. of samples tested (%) is indicated on the map for wild life preservation and game. Triangles indicate the summit of a mountain of more than 1000 m above sea level. The circle indicates a pig farm. At least two of the four farms raised pigs on grazing and are indicated by solid circles.

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Although no difference in positive rates was observed between upland and lowland areas, many deer were found to be positive for anti-HEV in lowlands, where pig farms are located.

Cumulative prevalence of anti-HEV antibodies with ageing

In order to study the relation between age and seroprevalence, positive rates for anti-HEV antibodies were compared among age groups. As shown in Fig. 4, nine of the 35 samples from the less than 1-year-old animals (25.7%), five of the 40 one-year-old ones (12.5%), 43 of the 123 two-year-old ones (35.0%), 58 of the 134 three-year-old ones (42.3%) and 66 of the 188 animals more than 4 years old (35.1%) were antibody-positive. A statistically significant difference was observed among 1- to 3-year-old animals and an age-dependent increase in the positive rate was found among the deer.

DISCUSSION

In this study, serological surveillance for HEV antibodies among Yezo-deer in the Hidaka district of Hokkaido, Japan was carried out to investigate whether the animals were infected by the virus. The distribution of ΔOD_{490} values by ELISA using VLPs as antigen showed a bimodal pattern, which was also observed in the main reservoir of HEV such as pigs and wild boars [2,13–15,20,29]. At least five serum samples from the ones investigated reacted with a specific band of 53 kDa in Western blots. Based on the lowest ΔOD_{490} value of a Western blot-positive sample, the cut-off value of the ELISA was determined and the prevalence of anti-HEV antibodies was examined. Comparison of the antibody-positive rate among each age of the deer demonstrated an age-dependent increase of the seroprevalence against HEV. These findings suggest that Yezo-deer in the surveillance area were infected with HEV. It is, therefore, strongly recommended that raw meat or entrails from Yezo-deer should not be ingested to avoid a risk of infection with HEV.

Out of the 520 deer tested, 181 animals (34.8%) were found to possess anti-HEV antibodies by ELISA. The seroprevalence for HEV among deer in this study is lower than that among pigs in Japan (58.0%) [23]. Surprisingly, the positive rate was higher than that among wild boars in Japan (9–25.5%) [14,20] and much higher than that among Yezo-deer reported in a recent study, where the animals inhabiting mainly the Shiretoko district and Nakajima island in Lake Toya in Hokkaido were examined (Dr Y. Matsuura, personal communication) [12]. As shown in Fig. 3, two pig farms raised their animals by grazing in the surveillance area. It has been reported that nearly 90% of the farms in Japan raised pigs infected with HEV [23] and that the infected pigs shed the virus in their feces in large amounts for 3–4 weeks [6]. Interspecies transmission between wild boar and Honsyu-deer (*C. nippon centralis*) was also suggested in Honsyu, Japan, where wild boars and deer inhabit the same areas [20]. As neither wild boar nor grazing pigs inhabit the Shiretoko district and Nakajima island, a lower prevalence of the antibody against HEV has been demonstrated by Matsuura *et al.* [12]. It seems that the deer in the Hidaka district were infected with the virus through eating grass contaminated with feces from the virus-infected pigs.

Only five of the 60 serum samples tested, which were positive for anti-HEV antibodies by ELISA, were found to react with the antigen in Western blots. As the maximum titre of the positive samples reactive in Western blots was 1:800 (as determined by ELISA), the antibody titres in samples other than the five may be too weak to produce specific bands by Western blot. Alternatively, the deer may recognize a conformational epitope rather than a linear one, yielding no bands in Western blots.

The seroprevalence of the deer against HEV was increased in an age-dependent manner. In order to clarify the infection route of these animals, virus detection in the feces of the deer as well as of those of the pigs grazing in the pasture should be undertaken. Animals less than 1-year-old showed a higher prevalence of antibodies than 2-year-old ones. This may reflect maternal antibodies against HEV in the serum of the fawns.

We report here for the first time that Yezo-deer may be infected with HEV. The antibody-positive rate among the deer in the Hidaka district was more than 30%. As females deer were hunted more and had a higher seroprevalence rate than males, much attention should be paid to avoid the risk of acquiring HEV infection from Yezo-deer meat.

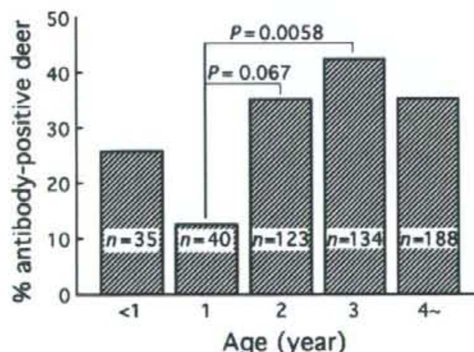

Fig. 4 Age-dependent increase in prevalence of anti-HEV antibodies. Positive rate of anti-HEV antibodies was compared by age. Statistical analysis was performed using the chi-square test. A P value of <0.05 is considered statistically significant.

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