表3　北海道内の市販ブタレバーから検出されたHEVのウイルス量，遺伝子型およびE型肝炎患者由来HEV株との塩基配列の比較

ブタレバー No.	HEV RNA (copies/g)	HEV 遺伝子型	HEV株	もっとも近縁のヒト由来HEV株	
				株名	一致率
82	10^5	3	swJL 82	HE-JA 6	94.4%
97	10^7	3	swJL 97	HE-JA 9	92.9%
98	10^7	3	swJL 98	HE-JA 9	92.9%
131	10^2	3	swJL 131	HE-JA 6	91.0%
145	10^3	4	swJL 145	HE-JA 18*	100%
234	10^5	3	swJL 234	HE-JA 4*	98.5～100%
325	10^3	3	swJL 325	HE-JA 4*	98.5～100%

*：北海道在住のE型肝炎患者由来のHEV株

〔Yazaki, Y., et al.：J. Gen. Virol. 84；2351-2357, 2003[18]により作成〕

HEV株と遺伝子配列が最大100%一致するものもあることが明らかになった(表3)[18]．これらは市販のブタレバーの一部がHEVを含んでおり，非加熱または不十分な加熱調理の状態で摂食することによりHEVに感染する危険性があること示唆するものである．

ブタは一般に6カ月齢で加工され食肉用として出荷されるが，前述のように6カ月齢のブタの末梢血中にはHEVは存在しない．しかしながら，HEVの増殖部位である肝臓には，市販ブタレバーの一部からHEV RNAが検出されたように，ウイルスが残存していることがあると考えられる．市販ブタホルモンでのHEV RNAの検出結果は公表されていないが，ブタへのHEVの感染実験において，肝臓よりも長く腸管でHEVの増殖が続いていたというデータが報告されていることから[20]，ブタレバーと同様，これらの部位を食するときには，感染予防のために十分に加熱調理することが重要である．E型肝炎と診断された患者に対しては危険食材の摂食の有無，時期，調理の状況(同じ包丁やまな板で生肉とほかの食材を扱ったか，加熱は十分であったか)，焼き肉の場合には生肉に触れた箸を口にしていないかなどについて詳細に聞き取り調査を行い，感染源および感染経路の特定に努めることが，感染予防の観点からも重要であると考えられる．

おわりに

従前，わが国ではE型肝炎は輸入感染症と考えられ，流行地域への渡航歴がない場合，E型肝炎診断のための検査が行われることはなかった．しかしながら，先進諸国にもブタなどの動物をリザーバーとする固有のHEV株(遺伝子型3型または4型)による人獣共通感染症としてのE型肝炎が存在していることが明らかになった．そして平成15年の感染症法の改正により，E型肝炎は診断後ただちに届け出ることが義務付けられた四類感染症に分類された．A型，B型およびC型が否定された急性肝炎患者には積極的にE型の検査を実施することとなり，今後，臨床現場においてE型肝炎患者に遭遇する機会は増えるものと思われる．

HEVはブタのほかにもイノシシやシカの生肉および内臓の摂食により感染することが明らかにされている一方で，感染源を特定できない

症例も少なからず存在している．また，きわめてまれではあるが輸血によるHEV感染もある．しかし，基本的にHEVは経口感染するウイルスであり，新たな感染様式を解明し，E型肝炎の予防策を講じるためにも，患者の食餌の内容や調理法，食習慣などに関する詳細，かつ具体的なアナムネーゼの聴取はますます重要であると考えられる．

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Summary

Hepatitis E as a Zoonotic Disease : Hepatitis E Virus Infection among Pigs

Masaharu Takahashi* and Hiroaki Okamoto*

Infection with the hepatitis E virus(HEV) is highly prevalent among domestic pigs in many countries around the world including Japan, where genotype 3 and 4 HEVs circulate. Although there has been no direct evidence indicating HEV transmission from pigs to humans, experimental infection with HEV revealed that HEV from swine is transmissible to a chimpanzee. Furthermore, swine HEV strains with a nucleotide sequence identity of 100% to human HEV strains have been recognized ; the majority of hepatitis E patients in Hokkaido reported a history of ingesting uncooked or undercooked pig liver 0.5-2 months before the onset of the disease ; and HEV RNA was detected in 1.9%(7/363) packages of raw pig liver sold in grocery stores as food in Hokkaido. Therefore, it is beyond doubt that hepatitis E is transmitted by ingestion of uncooked or undercooked meat or viscera from pigs infected with HEV.

Key words : hepatitis E, zoonosis, pig liver

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特集 E型肝炎—最新の知見

4 人獣共通感染症としてのE型肝炎
(2) イノシシにおけるE型肝炎ウイルス感染

道堯浩二郎* 堀池 典生**

Key words : E型肝炎ウイルス，野生イノシシ，人獣共通感染症

要 旨

E型肝炎ウイルス(HEV)は人獣共通感染症で，ブタ，イノシシ，シカに感染が確認されている．本邦の海外渡航歴のないE型肝炎例のうち，イノシシ肉や内臓を食した後に発症したE型肝炎例が散見され，イノシシ肉(内臓)からの経口感染はHEVの重要な感染経路と考えられる．発症例は中高年の男に多い．本州，九州，四国でHEV-RNA陽性のイノシシが証明され，どの地方でもイノシシ肉や内臓の生食はHEV感染の危険があると考えられる．また，急性肝炎診察時には野生動物の肉，内臓の摂取歴を聞く習慣をつけることが重要である．

はじめに

E型肝炎ウイルス(HEV)は，おもにアジア，アフリカに存在する水系感染(waterborne transmission)するウイルスとして認識されていた．近年，ヨーロッパ，アメリカや日本においても散発的に海外渡航歴のないE型肝炎例が発生していることが判明し，その後の検討によりこれらの国にも土着と思われるHEVが存在することが明らかになった[1]．これらの国での感染源並びに感染ルートの一つとして，動物からの感染が報告されている．本稿では，イノシシからヒトへのHEV感染と，イノシシにおけるHEV感染状況に関して概説する．

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Ⅰ．人獣共通感染症としてのHEV

この項のポイント
- HEVは人獣共通感染症で，ブタ，イノシシ，シカに感染が確認されている．

HEVは人獣共通感染症であることが知られている[2]．HEVに対する抗体が検出されたおもな動物種として，表1に示すものがあげられる[3),4)]．これらのうち，すべての動物種で感染

表1 E型肝炎ウイルスとの関連が報告されている動物

ブタ
イノシシ
シカ
ウシ
サル
ネコ
イヌ
ラット，マウス，ドブネズミ
ニワトリ

0911-601X/06/JCLS

が確認されたわけではないが，ブタ，イノシシ，シカではHEV-RNAが証明されており，少なくともこれら3種では感染が確認されている．ニワトリに関しては，ウイルスRNAが証明されているものの，ヒトのHEVとは塩基配列が大きく異なり，ヒトへの感染の可能性は低い．一方，ブタ，イノシシから分離同定されたHEVとヒトから分離されたHEVの塩基配列が複数の論文で検討され[5]，分子系統樹解析で宿主動物種特異的なクラスターは形成されないことから，ブタ，イノシシに感染するHEVはヒトにも感染しうることが分子系統樹からも推察される．ブタ，野生イノシシやシカに関しては，肉や内臓を食したヒトにおける感染が報告されており，これらの動物からヒトへの経口感染は感染経路として重要である．

Ⅱ．本邦におけるイノシシの生態

日本のイノシシには，ニホンイノシシ(*Sus scrofa leucomystax*)とリュウキュウイノシシ(*Sus scrofa riukiuanus*)の2種の亜種が棲息する．低山帯から平地のおもに森林に棲む．イノシシは雑食性で，植物の根茎，葉，ドングリ，またカエル，ヘビなどを食べる．北海道では野生イノシシはほとんど棲息せず，本州以南に多く棲息する．日本に野生イノシシは約15万頭程度棲息し，増加傾向といわれている．イノシシ捕獲数は九州，中国，近畿，四国で多く，東北，北海道で少ない．1960～2000年までのイノシシ通算捕獲数の集計によれば，都道府県別では多い順に鹿児島，宮崎，大分，兵庫，山口，三重，熊本，島根，広島でこれらの県では10万頭を超えており，一方，少ない順に山形，秋田，新潟，岩手，富山，北海道，青森となっており，これらの道県では100頭以下である．イノシシの猟期は通常11～2月頃であるが，畑や集落近くに出没するイノシシの捕獲(駆除)は通年で行われている．近年，獣害による農業被害が増大してきており，里山近くの田畑の農作物を食い荒らす頭数が増えてきているため，イノシシ捕獲数に占める農業被害防止の目的での集落や里山での捕獲数(駆除)の比率が上昇している．

Ⅲ．イノシシからの感染が推察されるE型肝炎例

この項のポイント
- イノシシ肉や内臓を食した後に発症したE型肝炎例が散見される．
- 発症例は中高年の男に多い．

イノシシの肉や内臓を食した後にE型肝炎を発症し，イノシシからの感染が推察されるE型肝炎例の報告を**表2**に示す[6)~9)]．肝を生食した後発症した報告が2件と，一緒にバーベキューで食した後の複数例でのE型肝炎発症の報告が2件みられる．発症例は全例50歳以上の男で，潜伏期は1～3カ月であった．HEVのgenotypeは長崎で3，鳥取と熊本で4であった．重症度は，genotype 4の感染例で通常の急性肝炎，急性肝炎重症型，劇症肝炎が各1例ずつ，genotype 3では，重症肝炎例はなかった．

筆者らも最近，イノシシ肉を調理して食した後に発症した女性例を経験しており，この例のHEVはgenotype 3で，重症化せずに治癒した．本邦でのE型肝炎の重症化例はgenotype 3よりgenotype 4感染例で多く，イノシシからの感染例でも同様の傾向であった．なお，Masudaらの報告で，イノシシ肉摂取後IgM型HEV抗体は陽性であったが肝機能は正常であった例が示されており，不顕性感染例も少なからず存在する可能性が推察される[9)]．

表2　イノシシを食した後に発症したE型肝炎例の報告

報告者	地　区	内　容	肝炎の重症度	潜伏期	genotype
肱岡ら[6]	熊本	シカ，イノシシ肉と内臓を頻回に生食した猟師にE型肝炎(59歳，男)	通常の急性肝炎	2カ月未満	4
Matsudaら[7]	鳥取	イノシシ肝を一緒に生食した2例にE型肝炎(53歳，男，70歳，男)	1例劇症肝炎，1例急性肝炎重症型	1～2カ月	4
Tamadaら[8]	長崎	イノシシ肉をバーベキューで一緒に食した12例中7例にIgM-HEV陽性，5例で肝炎(69～81歳，全例男)	5例とも通常の急性肝炎	1～2カ月	3
Masudaら[9]	長崎	イノシシ肉をバーベキューで一緒に食した3例中2例にIgM-HEV陽性，うち肝炎発症1例にRNA陽性(71歳，男)	通常の急性肝炎 1例は肝機能異常なし	59日	3

表3　イノシシのHEVに関するおもな報告

報告者	地　区	内　容	genotype
北嶋ら[10]	兵庫	野生イノシシ7頭中4頭に抗体陽性，3頭(推定年齢1歳未満)にRNA陽性	3
三好ら[11]	和歌山	9頭中1頭に抗体とRNA陽性	3
Sonodaら[12]	全国	35頭中3頭に抗体陽性，佐賀からの1頭(体重60 kg)にRNA陽性	3
中村ら[13]	沖縄(西表島)	15頭中2頭にRNA陽性	4

Ⅳ．イノシシにおけるHEVマーカー陽性率

この項のポイント
- 本州，九州，四国でHEV-RNA陽性のイノシシが証明され，どの地方でもイノシシ肉や内臓の生食はHEV感染の危険があると考えられる．

イノシシを食べた後にE型肝炎が発症した例があることから，イノシシにおけるHEV感染の頻度，とくにHEV-RNA保有率が検討された．**表3**にイノシシにおけるHEVマーカー陽性率について記載された報告を示す[10)~13)]．兵庫，和歌山，佐賀，沖縄からHEV-RNA陽性のイノシシの存在が報告されている．沖縄からはgenotype 4，他の3県からはgenotype 3が同定されている．四つの報告をまとめると，HEV-RNA陽性イノシシは66頭中7頭である．

筆者らが集めた愛媛県の野生イノシシ269頭のHEVマーカーを東芝病院の三代俊治先生に測定していただいたところ，RNAは8頭に陽性で，すべてgenotype 3であった[14)]．なお，HEV抗体は269頭中50頭(18.6%)に陽性であった．一方，飼育イノシシでは14頭中HEV抗体は10頭と有意に高く検出され，飼育イノシシではイノシシ間で感染しやすいことが推察された．北嶋らの報告ではHEV-RNAが検出されたのは3頭とも1歳未満のイノシシであったが[10)]，筆者らが調べたイノシシでは若いイノシシだけではなく，成獣にもHEV-

RNAが検出された．飼育ブタでは6カ月以上の月齢ではHEV-RNAは検出されないため[15]，食しても安全なブタの年齢(月例)が判明しているが，イノシシの年齢(体重)からはHEVの感染している確率の低いイノシシを推定することは困難で，食しても安全と判断できる年齢(体重)はなさそうである．

おわりに

捕獲されたイノシシの肉はハンターやその家族，友人らにより食されることが多い．最近，ジビエ料理(狩猟鳥獣肉の料理)がブームになりつつあり，通信販売などで野生動物の肉が入手できるようになってきている．野生動物の肉や内臓を食べる際は，十分加熱することを徹底するとともに，血液などが付着する調理器具の洗浄にも注意を払うべきであろう．また，急性肝炎例の病歴聴取の際には，野生動物の肉や内臓を食べた既往を聞く習慣をつけることも重要である．

本邦のイノシシの生態に関するデータは，ウェブサイトhttp://www.chugoku-np.co.jp/kikaku/ihen/data1.htlmより引用．

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Summary

Hepatitis E as a Zoonotic Disease : Hepatitis E Virus Infection among Wild Boars

Kojiro Michitaka* and Norio Horiike**

Zoonotic transmission of the hepatitis E virus(HEV) has been reported. The significance of wild boars as a source of HEV infection was reviewed. The number of Japanese patients with acute hepatitis E, including fulminant hepatitis who had a history of eating wild boar meat, including liver, has totaled four. Several investigators have studied the prevalence of anti-HEV and HEV-RNA in wild boars, and found that HEV-RNA was present in several % of wild boars. Boars positive for HEV-RNA were found in the Kyushu, Shikoku and Honshu areas in Japan. From these reports, wild boars have been identified as an important source of zoonotic HEV transmission.

Key words : hepatitis E virus, wild boar, zoonotic transmission

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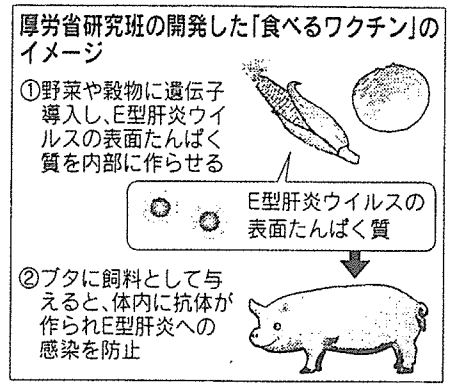
動物の感染拡大防ぐ技術

厚労省研究班 飼料をワクチンに

E型肝炎ウイルス

ブタの生肉などを介して人に感染するE型肝炎ウイルスの、動物での感染拡大を防ぐ基礎技術を、厚生労働省のE型肝炎研究班が開発した。植物の遺伝子を組み換えてE型肝炎ウイルス特有のたんぱく質を合成させる。飼料としてブタに食べさせるだけで、ウイルスに対する抗体が作られて感染しにくくなる「食べるワクチン」の実現につながる可能性があるという。



中央農業総合研究センターの津田新哉室長と大西純リサーチ・レジデントらが、モデル植物のタバコを使って開発した。

E型肝炎ウイルスにはカプセルのような外皮があるが、この外皮の成分である「キャプシド」というたんぱく質を作る遺伝子をアグロバクテリウムという土壌細菌に組み込み、タバコに感染させて導入する。

ウイルスと植物では、たんぱく質を合成する仕組みが異なるため、遺伝子を植物に導入するだけではたんぱく質が合成されない。そこで、遺伝子の一部を植物向けに書き換えてから導入する工夫をした。タバコの葉一㌘当たり、数㌦（㌦は百万分の一）㌘のたんぱく質が合成されたという。

この技術で穀物や野菜に遺伝子導入し、ブタに食べさせれば、キャプシドたんぱく質がブタの腸で吸収される。たんぱく質自体はE型肝炎ウイルスの遺伝情報を含まないため感染しないが、ブタの体内に抗体が作られ、E型肝炎ウイルスに感染しにくくできる可能性があるという。

E型肝炎ウイルスはブタなどの生肉や内臓を食べることで人に感染し、肝炎を起こして死ぬこともある。国内で飼育されるブタの多くに広がっているが、感染を防ぐ有効な手段が見つかっていない。

V. 参考

何故かなかなか公表されないでいた
GSK-sponsored hepatitis E vaccine field trial in Nepal
の結果が漸くパブリッシュされた

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Safety and Efficacy of a Recombinant Hepatitis E Vaccine

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ABSTRACT

BACKGROUND

Hepatitis E virus (HEV) is an important cause of viral hepatitis. We evaluated the safety and efficacy of an HEV recombinant protein (rHEV) vaccine in a phase 2, randomized, double-blind, placebo-controlled trial.

METHODS

In Nepal, we studied 2000 healthy adults susceptible to HEV infection who were randomly assigned to receive three doses of either the rHEV vaccine or placebo at months 0, 1, and 6. Active (including hospital) surveillance was used to identify acute hepatitis and adverse events. The primary end point was the development of hepatitis E after three vaccine doses.

RESULTS

A total of 1794 subjects (898 in the vaccine group and 896 in the placebo group) received three vaccine doses; the total vaccinated cohort was followed for a median of 804 days. After three vaccine doses, hepatitis E developed in 69 subjects, of whom 66 were in the placebo group. The vaccine efficacy was 95.5% (95% confidence interval [CI], 85.6 to 98.6). In an intention-to-treat analysis that included all 87 subjects in whom hepatitis E developed after the first vaccine dose, 9 subjects were in the vaccine group, with a vaccine efficacy of 88.5% (95% CI, 77.1 to 94.2). Among subjects in a subgroup randomly selected for analysis of injection-site findings and general symptoms (reactogenicity subgroup) during the 8-day period after the administration of any dose, the proportion of subjects with adverse events was similar in the two study groups, except that injection-site pain was increased in the vaccine group (P=0.03).

CONCLUSIONS

In a high-risk population, the rHEV vaccine was effective in the prevention of hepatitis E. (ClinicalTrials.gov number, NCT00287469.)

From the Walter Reed–Armed Forces Research Institute of Medical Sciences Research Unit Nepal (M.P.S., R.M.S., S.K.S.) and the Nepalese Army (D.M.J., G.B.T., N.T.) — both in Kathmandu, Nepal; the Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand (M.P.M., K.S.A.M., T.P.E.); GlaxoSmithKline Biologicals, Rixensart, Belgium (M.F., M.P.D., A.S.) and King of Prussia, PA (B.L.I.); and the Walter Reed Army Institute of Research, Silver Spring (R.A.K., J.S., D.W.V., T.P.E.), and the Military Infectious Diseases Research Program, Army Medical Research and Materiel Command, Fort Detrick (D.W.V.) — both in Maryland. Address reprint requests to Dr. Innis at GlaxoSmithKline, 2301 Renaissance Blvd., King of Prussia, PA 19406-2772, or at bruce.2.innis@gsk.com.

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HEPATITIS E VIRUS (HEV) INFECTION IS a major public health problem in many developing countries.[1] Hepatitis E occurs sporadically and in epidemics, causing substantial rates of death and complications, especially in pregnant women.[2] On the basis of seroprevalence, an estimated one third of the world's population has been infected with HEV.[3] In India, the lifetime infection risk is more than 60%, which translates to hundreds of thousands of illnesses annually.[4] Hepatitis E is usually self-limited and typically occurs in locations where laboratory diagnosis is unavailable.[5] Consequently, the true burden of hepatitis E is unknown.

Hepatitis E is clinically indistinguishable from other types of acute viral hepatitis.[5] In outbreaks of infection, the average incubation period is approximately 40 days; the highest attack rates are among persons between the ages of 15 and 40 years.[6] The severity of illness increases with age; the overall case fatality ratio is estimated to be 1 to 3%.[7,8] Pregnant women have the highest risk of associated acute hepatic failure. Among these women, the case fatality ratio is 5 to 25%, and survivors have high rates of spontaneous abortion and stillbirth.[5]

HEV, a nonenveloped, single-strand, positive-sense RNA virus of the genus hepevirus, has a genome comprising three overlapping open reading frames (ORFs); ORF-2 encodes the principal capsid protein.[9] There are four HEV genotypes: genotype 1 causes most human disease, genotype 2 is rare, and genotypes 3 and 4 (although prevalent in domestic animals such as swine) may have reduced pathogenicity for humans.[6] Nevertheless, all HEVs can be considered to belong to one serotype.[4] Therefore, a vaccine that is shown to be efficacious in one country should provide protection against hepatitis E elsewhere.

A genotype 1 HEV recombinant protein (rHEV) vaccine, which provided protection in nonhuman primates,[10] was found to be immunogenic in humans.[11] These results prompted a clinical trial of the vaccine's efficacy in volunteers from the Nepalese Army, a population at high risk for hepatitis E.[12,13]

METHODS

STUDY DESIGN

We conducted the study in accordance with good clinical practice guidelines, the provisions of the Declaration of Helsinki, and regulations of both the United States and Nepal. The institutional review boards of the Nepal Health Research Council and the U.S. Army approved the study protocol. The U.S. Army Medical Materiel Development Activity office monitored the conduct of the trial and the veracity of the data. An independent data and safety monitoring board monitored adverse events and confirmed end points before investigators were made aware of study-group assignments. Each subject provided written informed consent before participation.

ROLE OF THE SPONSORS

The study was designed by the U.S. Army with GlaxoSmithKline. Investigators in Nepal and Thailand collected the data; statisticians at GlaxoSmithKline analyzed the data according to a prespecified sponsor-approved plan. All the authors had complete and unfettered access to the data, wrote the manuscript, and vouch for the accuracy and completeness of the article.

STUDY SUBJECTS

A total of 5323 healthy men and nonpregnant women were recruited from 61 Nepalese Army units in Kathmandu. Serologic assessment was performed to assess eligibility.[14] Of these subjects, 66.3% had levels of anti-rHEV immunoglobulin of less than 20 Walter Reed antibody units (WR U) per milliliter. Of these subjects, 1885 who had anti-rHEV immunoglobulin levels of less than 10 WR U per milliliter were initially randomly assigned to study groups; subsequently, 115 who had anti-rHEV immunoglobulin levels of 10 or more WR U per milliliter but less than 20 WR U per milliliter were randomly assigned to study groups, so the entire cohort included 2000 subjects from 45 Nepalese Army units.

GlaxoSmithKline Biologicals prepared a permuted-block, 1:1 randomization list (with 20 subjects to a block) with the use of an algorithm of pseudorandom numbers provided by RS/1 data-analysis software (Bolt Beranek and Newman). Randomization of all subjects was performed at one site without stratification. During the double-blind trial, all investigators and subjects were unaware of study-group assignments.

VACCINE AND PLACEBO

The vaccine was a purified polypeptide produced in *Spodoptera frugiperda* cells infected with a recom-

binant baculovirus containing a truncated HEV genomic sequence encoding the capsid antigen.[15] Vaccine doses contained 20 μg of rHEV antigen in 0.5 ml of buffered saline adsorbed to 0.5 mg of aluminum hydroxide. Placebo doses, which looked identical to the vaccine doses, contained 0.5 mg of aluminum hydroxide in 0.5 ml of saline. Three doses of vaccine or placebo were administered intramuscularly, at months 0, 1, and 6. In addition, all subjects in both study groups were offered hepatitis B vaccine (Engerix-B), beginning 3 months after study entry. A total of 84% of the subjects received all three doses of hepatitis B vaccine.

CASE DEFINITION AND EVALUATION OF EFFICACY

Subjects with hepatitis E were identified through active surveillance every other week at military units and through daily hospital surveillance. Definite hepatitis E was defined as jaundice or illness that lasted for at least 3 days, with at least three of the following symptoms: fatigue, loss of appetite, abdominal discomfort, abdominal pain in the right upper quadrant, nausea, or vomiting.[16] Liver injury had to be confirmed by a serum alanine aminotransferase level of more than 2.5 times the upper limit of the normal range or a serum total bilirubin level of more than 2 mg per deciliter (34 μmol per liter). The presence of HEV RNA had to be detected in serum or stool by reverse-transcriptase–polymerase-chain-reaction (RT-PCR) assay.[17] HEV infection had to be confirmed by detection of either anti-rHEV IgM of at least 100 WR U per milliliter[18] or anti-rHEV immunoglobulin of at least 2500 WR U per milliliter. The immune response at months 0, 2, 6, 7, and 24 was determined by anti-rHEV immunoglobulin immunoassay with the use of the vaccine antigen.[14]

ADVERSE EVENTS

Investigators asked subjects about any adverse events at all study visits. In addition, investigators reviewed all clinic and hospital admission records daily to identify trial subjects. Subjects in a randomly selected subgroup were interviewed on days 1, 3, 5, and 7 after each vaccination to record injection-site findings and general symptoms (reactogenicity subgroup). Serious adverse events (which were defined as medically significant events, including those resulting in hospitalization, disability, or death) were recorded throughout the study. Adverse events were coded with the use of the *Medical Dictionary for Regulatory Activities*.[19] To analyze safety for this report, adverse events that were coded as HEV infection or hepatitis E were censored.

STUDY END POINTS

The primary efficacy end point was the prevention of definite hepatitis E occurring at least 14 days after the administration of the third dose of vaccine. A secondary efficacy end point was the prevention of definite hepatitis E occurring at least 14 days after the administration of the second dose but before the administration of the third dose.

STATISTICAL ANALYSIS

We estimated that the incidence rate of hepatitis E would be 1.6% during a 1-year period.[12,13] Assuming a vaccine efficacy of 80%, a two-group continuity-corrected chi-square test with a one-sided significance level of 0.05 would have a power of 80% to detect a difference in the incidence of hepatitis E with 866 subjects per group, as calculated by nQuery Advisor, version 5.0 (Statistical Solutions). To compensate for dropouts, 1000 subjects per group were needed.

The vaccine-efficacy cohort included all subjects who received three doses for the primary analysis and all subjects who received two doses for a secondary analysis. A two-sided Fisher's exact test was used to compare the percentages of subjects with hepatitis E in the two study groups. A two-sided 95% confidence interval (CI) for vaccine efficacy (1 minus the relative risk) was computed with the use of the Mantel–Haenszel confidence interval for relative risk.

For robustness, efficacy also was computed in the total vaccinated cohort (all subjects who received at least one vaccine dose) on the basis of the relative risk and by the Cox regression model. The cumulative incidence, expressed as hazard-ratio curves, including the group effect as regressor, was generated to analyze time to occurrence of hepatitis E. The log-rank test was used to compare the groups.

Among the 200 subjects in the reactogenicity subgroup, the proportions of subjects who reported symptoms when questioned by investigators during the 8-day period after vaccination were compared between groups. Among the 1800 subjects in the total vaccinated cohort minus the reactogenicity group and in the reactogenicity subgroup, the proportions of subjects who spontaneously reported adverse events at a follow-up

visit during the 31-day period after receiving any dose were compared between groups. In the total vaccinated cohort, the occurrence of severe adverse events was compared between groups. All comparisons used a two-sided Fisher's exact test.

In a randomly selected immunogenicity subgroup of subjects who complied with all protocol requirements regarding vaccination and blood sampling (including 80 subjects in the vaccine group and 160 subjects in the placebo group), the proportion of subjects with anti-rHEV immunoglobulin levels of at least 20 WR U per milliliter and the geometric mean concentrations of anti-rHEV immunoglobulin were analyzed. Data analysis was performed with the use of SAS software (version 8.2) and ProcStatXact 5 with Windows NT 4.0. All reported P values are two-sided.

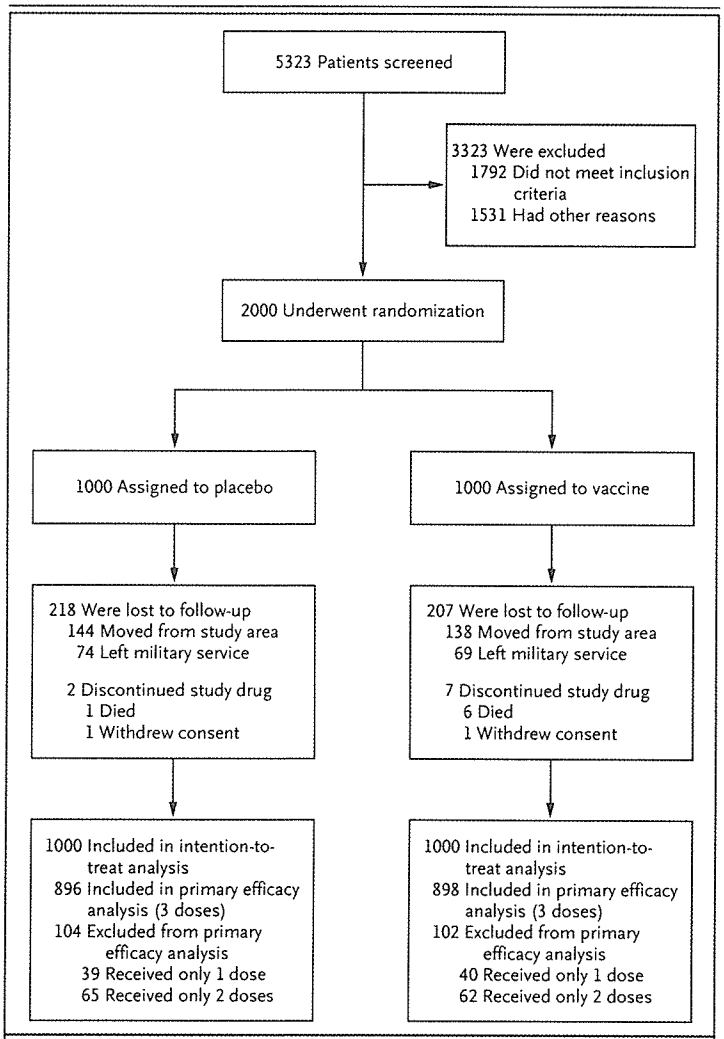


Figure 1. Enrollment and Outcomes.

RESULTS

STUDY POPULATION

From July to August 2001, 2000 healthy subjects (99.6% of whom were men), with a mean (±SD) age of 25.2±6.25 years (range, 18 to 62) were randomly assigned to receive either rHEV vaccine or placebo (Fig. 1). Follow-up ended in January 2004. The study groups (with 1000 subjects in each) were similar with respect to mean age, sex, and rates of withdrawal from the study. A total of 1566 subjects were followed for a median of 804 days.

VACCINE EFFICACY

The data and safety monitoring board reviewed 111 episodes of acute hepatitis and certified 87 definite cases of hepatitis E (see the Supplementary Appendix, available with the full text of this article at www.nejm.org, for details regarding the 24 subjects who were deemed not to have hepatitis E). Of the 87 subjects with definite hepatitis E, 84 were icteric and 3 were anicteric (all in the placebo group). The median duration of illness for the 87 subjects was 29 days (interquartile range, 23 to 39); the median maximum serum alanine aminotransferase level was 1248 U per liter (interquartile range, 756 to 1995), and the median maximum total bilirubin level was 9.0 mg per deciliter (154 μmol per liter) (interquartile range, 6.6 to 13.1 mg per deciliter [113 to 224 μmol per liter]).

The primary objective was to evaluate the efficacy of a three-dose vaccination course. During the period from 14 days after the administration of the third dose until the end of the study, hepatitis E developed in 69 subjects: 3 in the vaccine group (0.3%) and 66 in the placebo group (7.4%) ($P<0.001$ by Fisher's exact test). The efficacy of the vaccine was 95.5% (95% CI, 85.6 to 98.6) (Table 1). By logistic regression, neither age (<25 years in 1117 subjects and ≥25 years in 677 subjects) nor the level of prevaccination antibody to rHEV (≤10 WR U per milliliter in 1692 subjects and >10 WR U per milliliter in 102 subjects) had an effect on vaccine efficacy (see the Supplementary Appendix).

A secondary objective was to evaluate the efficacy of a two-dose vaccination course. During the period from 14 days after the administration of the second dose until the time of administration of the third dose, hepatitis E developed in eight subjects: one in the vaccine group

(0.1%) and seven in the placebo group (0.7%) (P=0.07 by Fisher's exact test). Among these subjects, the vaccine efficacy was 85.7% (95% CI, −16.0 to 98.2) (Table 1).

An intention-to-treat analysis was performed to estimate the vaccine's efficacy when administered during ongoing disease transmission. From randomization, HEV infection developed in 87 subjects: 9 in the vaccine group (0.9%) and 78 in the placebo group (7.8%) (P<0.001 by Fisher's exact test). Among these subjects, the efficacy of the vaccine, on the basis of the relative risk, was 88.5% (95% CI, 77.1 to 94.2) (Table 1). The cumulative incidence of HEV infection as a hazard-ratio curve, plotted for the vaccine group and the placebo group to analyze the time until infection, differed between groups; efficacy as calculated by the Cox-regression model was 89.9% (95% CI, 77.9 to 94.5) (P<0.001 by the log-rank test) (Fig. 2).

In nine subjects in the vaccine group, hepatitis E developed after the following intervals after the administration of the first vaccine dose: 1, 13, 13, 30, 194, 665, 694, 706, and 767 days. Hepatitis E developed in the first four subjects before they received the second dose; all had an acute-illness antibody pattern that was consistent with a primary response (ratio of anti-rHEV IgM to anti-rHEV immunoglobulin, >0.1), suggesting that HEV infection occurred before they received the first dose of vaccine. Infection developed in the remaining five subjects months after they had been vaccinated with the first dose; all had an acute-illness antibody pattern that was consistent with an anamnestic response (ratio of anti-rHEV IgM to anti-rHEV immunoglobulin, <0.1, with a markedly elevated level of anti-rHEV immunoglobulin), suggesting that infection occurred despite vaccination. The subject with an illness onset on day 194 had received one vaccine dose; the subject with an illness onset on day 706 had received two vaccine doses 223 days apart, and the subjects with an illness onset on days 665, 694, and 767 had received a third vaccine dose 187, 180, and 182 days, respectively, after the first dose.

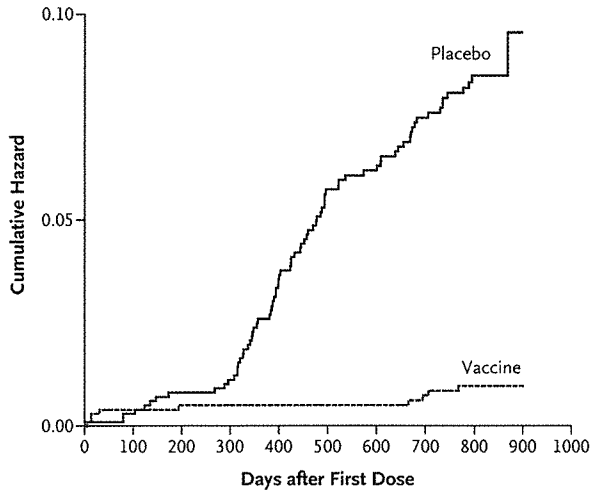
VACCINE SAFETY

The two study groups had a similar rate of loss to follow-up (21.8% in the vaccine group and 20.7% in the placebo group), implying similar overall tolerability of the study treatment. The rates of reporting of symptoms in the reactogenicity subgroup when subjects were questioned by investigators were similar between groups, except for subjects who had pain at the injection site (Table 2). The proportions of subjects spontaneously reporting any adverse event were similar in the two study groups (in the reactogenicity subgroup, 28.0% in the vaccine group and 27.0% in the placebo group; in the total vaccinated cohort minus the reactogenicity subgroup, 25.2% in the vaccine group and 24.9% in the placebo group). Likewise, the proportions of subjects who spontaneously reported any adverse event that prevented them from engaging in normal activities were similar in the two groups (in the total vaccinated cohort minus

Table 1. Efficacy of the rHEV Vaccine against HEV.

Period of Observation	Subjects with HEV Infection		Vaccine Efficacy*
	Vaccine	Placebo	
	no./total no.		*% (95% CI)*
From 14 days after dose 3 until end of study (a priori primary end point)	3/898	66/896	95.5 (85.6 to 98.6)
From 14 days after dose 2 until dose 3 (a priori secondary end point)	1/960	7/961	85.7 (−16.0 to 98.2)
From 14 days after dose 2 until 14 days after dose 3 (a posteriori secondary end point)	1/960	8/961†	87.5 (0.1 to 98.4)
From dose 1 until end of study (exploratory end point)	9/1000	78/1000	88.5 (77.1 to 94.2)

* Efficacy was estimated as 1 minus the relative risk, with the 95% CI based on the Mantel–Haenszel CI for the relative risk.
† One additional case occurred 6 days after the administration of dose 3, before the surveillance period for the a priori primary end point.



No. at Risk										
Placebo	1000	985	970	944	901	851	807	775	539	0
Vaccine	1000	986	972	948	924	905	864	851	577	2

Figure 2. Cumulative Hazard of Hepatitis E.

The cumulative hazard of a first hepatitis E episode (i.e., the incidence rate) was estimated as a minus-log transformation (log data to nonlog data) of the Kaplan–Meier survival curve during the period after the administration of the first dose of study drug until the end of the study among all subjects who received at least one dose of either vaccine or placebo. The numbers below the graph are the numbers of patients in each group remaining under surveillance at 100-day intervals. The difference between subjects who received placebo and those who received vaccine was significant ($P<0.001$ by the log-rank test). Vaccine efficacy from the first dose, calculated with the Cox regression model, was 89.9% (95% CI, 77.9 to 94.5%).

the reactogenicity subgroup, 3.3% in the vaccine group and 3.0% in the placebo group).

The proportions of subjects reporting any serious adverse event, excluding acute hepatitis E, were similar in the two groups: 13.5% in the vaccine group and 13.7% in the placebo group. Subjects in the placebo group had 5.7% more serious adverse events owing to acute hepatitis E than did those in the vaccine group. The most common category of adverse events was infections (excluding hepatitis E), which accounted for 73 of 135 events in the vaccine group and 73 of 137 events in the placebo group. The most frequent diagnosis, excluding hepatitis E, was enteric fever (in 2.0% of subjects in the vaccine group and 2.4% in the placebo group). Among all serious adverse events, which were stratified according to body system and diagnosis, rates of events were similar in the two groups except for leptospirosis (0.2% in the vaccine group and 1.2% in the placebo group). However, the difference probably resulted from differential testing, since only subjects with a clinical diagnosis of hepatitis were tested for leptospirosis (see the Supplementary Appendix). Seven subjects died during the study, six in the vaccine group (four in combat, one from cholangiocarcinoma, and one from an undetermined cause 130 days after a second vaccination) and one in the placebo group (after a vehicle accident). The data and safety monitoring board did not consider any of the deaths to be related to vaccination.

ANTIBODY RESPONSE

Among subjects in the immunogenicity subgroup who received vaccine, 81.3% had a level of anti-rHEV immunoglobulin of at least 20 WR U per milliliter 1 month after the second vaccine dose, and 100% had this level 1 month after the third vaccine dose; by the end of the study, the proportion had declined to 56.3%. In contrast, the proportion of such subjects in the placebo group rose to 10.6%, reflecting the rate of HEV infection (Fig. 3A). Vaccination elicited antibody responses that differed with respect to the geometric mean concentration between the groups from 1 month after the administration of the second dose until the end of the study (Fig. 3B).

DISCUSSION

The rHEV vaccine was protective against hepatitis E during a median of 804 days. According to the primary analysis, the estimated efficacy of three doses of vaccine was 95.5%. The intention-to-treat analysis supported this finding, with an estimate that the efficacy of the rHEV vaccine after the administration of the first dose was 88.5 to 89.9%. Vaccination was conducted during active HEV transmission, affording an opportunity to evaluate the onset of protection. Before the administration of a second dose, hepatitis E developed in four subjects in the vaccine group (on days 1, 13, 13, and 30), as compared with one subject in the placebo group (on day 5). The other four subjects with hepatitis E among those who received one dose (one subject in the vaccine group and three in the placebo group) had an illness onset 104 to 288 days after vaccination. Therefore, we conclude that the vaccine has not been shown to provide any protection after one dose. Vaccination with two doses may afford protection, but the study did not establish this with certainty, since the 95%

CI included zero. Two vaccine doses may control outbreaks of hepatitis E, but this hypothesis requires confirmation.

Several lines of evidence establish that antibody to rHEV is a correlate of protection against hepatitis E. Nonhuman primates that received convalescent serum were protected from disease when challenged with HEV.[10] The convalescent serum bound to rHEV vaccine antigen, which also binds a monoclonal antibody that can neutralize infectious HEV.[20] The development of anti-rHEV immunoglobulin levels of at least 20 WR U per milliliter in 81.3% of subjects in the vaccine group 1 month after the administration of the second dose was related temporally to the apparent onset of protection, although the minimum protective level of antibody is unknown. The antibody level increased after the second dose and then declined until the administration of the third dose, but the protection persisted (Fig. 2), suggesting that a declining level of anti-rHEV immunoglobulin after the second dose does not indicate a loss of immunity. Moreover, the increase by a factor of 10 in anti-rHEV immunoglobulin levels 1 month after the administration of the third dose is evidence that the first two vaccine doses elicited immunologic memory that when boosted by a third dose provided protection against hepatitis E, even after the serum antibody level had waned. This finding is true for hepatitis B vaccine, another recombinant subunit vaccine, which confers immunity despite waning levels of antibody.[21]

The profile of adverse events associated with the administration of rHEV vaccine was similar to that of placebo, although our experience is limited with respect to the number of people at risk and the duration of observation. The symptom profile compiled in response to investigators' queries was similar to that of placebo, except that injection-site pain occurred more frequently among vaccine recipients. There were no significant differences between the groups with regard to spontaneously reported adverse events or serious adverse events. The number of deaths in the vaccine group was larger than that in the placebo group, but none of the deaths were considered to have been related to vaccination. The number of subjects was too small to exclude the possibility of rare vaccine-related adverse events.

By enrolling subjects without antibody evidence of previous HEV infection,[14] we evaluated vaccine in persons who were at greatest risk for infection. The total vaccinated cohort may have included some subjects who were immunologically primed by previous exposure but who did not have detectable antibody to rHEV. Although randomization should have distributed primed subjects equally between the groups, any priming may have offered protection against hepatitis E and an enhanced response to vaccination. Two types of evidence support the exclusion of most primed subjects. Among 80 subjects in the immunogenicity subgroup of the vaccine group, the maximum levels of anti-rHEV immunoglobulin 1 and 5 months after the administration of the second dose were 537.7 and 430.3 WR U per milliliter, respectively — levels that are inconsistent with an anamnestic response. Moreover, among 78 subjects with hepatitis E in the placebo group, 75 had ratios of anti-rHEV IgM to immunoglobulin that were consistent with a primary antibody response.[14] There-

Table 2. Rates of Symptoms Reported to Investigators (Reactogenicity Subgroup) during the 8-Day Period after the Administration of Any of Three Doses of Study Drug.

Symptom	Intensity*	Subjects Reporting Event		P Value†
		Vaccine (N=100)	Placebo (N=100)	
		% (95% CI)		
At injection site				
Pain	Any	82 (73.1–89.0)	68 (57.9–77.0)	0.03‡
	Grade 3	1 (0–5.4)	0 (0–3.6)	1.00
Redness	Any	24 (16.0–33.6)	19 (11.8–28.1)	0.49
	Grade 3	0 (0–3.6)	0 (0–3.6)	—
Swelling	Any	20 (12.7–29.2)	17 (10.2–25.8)	0.72
	Grade 3	0 (0–3.6)	0 (0–3.6)	—
Systemic				
Fatigue	Any	43 (33.1–53.3)	47 (36.9–57.2)	0.67
	Grade 3	0 (0–3.6)	0 (0–3.6)	—
Headache	Any	46 (36.0–56.3)	46 (36.0–56.3)	1.00
	Grade 3	0 (0–3.6)	0 (0–3.6)	—
Fever	Any	30 (21.2–40.0)	36 (26.6–46.2)	0.45
	Grade 3	1 (0–5.4)	1 (0–5.4)	1.00

* Grade 3 pain, headache, and fatigue were defined as preventing normal activities; grade 3 redness or swelling was defined as having a diameter of more than 50 mm; and grade 3 fever was defined as a temperature of more than 39.0°C.
† P values are two-sided and were calculated by Fisher's exact test. Dashes indicate that P values could not be calculated.
‡ The absolute rate difference between the vaccine group and the placebo group was 14.0% (95% CI, 2.0 to 25.8).

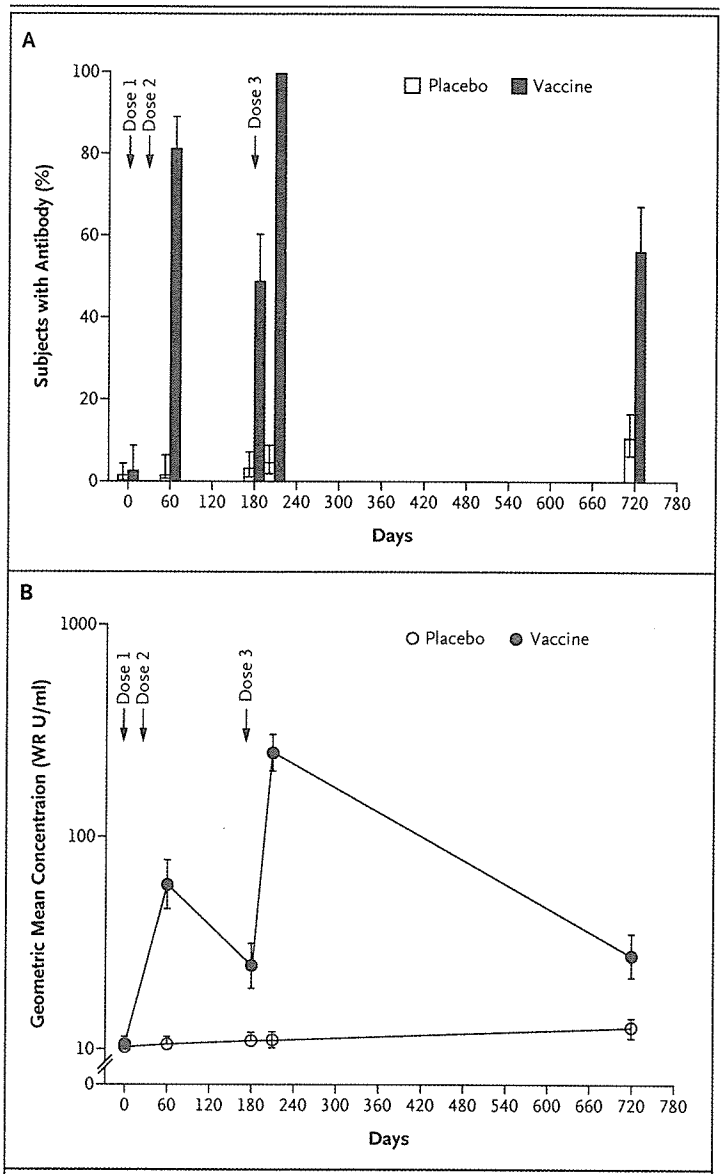


Figure 3. Antibody Response to rHEV Vaccine.

Panel A shows the proportion of subjects who had a level of antibody to rHEV of at least 20 WR U per milliliter in each study group. Panel B shows the geometric mean concentration of antibody to rHEV. In the calculation of the geometric mean concentration, values below the assay cutoff of 20 WR U per milliliter were coded as 10 WR U per milliliter. In both panels, I bars indicate 95% CIs.

fore, our results should apply to persons without previous exposure to HEV.

The contribution of HEV to overall morbidity among the subjects in our trial was substantial and supports the assertion that the burden of hepatitis E is grossly underestimated. Hepatitis E was the most common medically significant illness (including illness resulting in hospitalization, disability, or death) in the placebo group. The potential effect of rHEV vaccine to improve well-being may be substantial in adult populations with similar disease exposures.

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CLINICAL TRIAL REGISTRATION

The *Journal* requires investigators to register their clinical trials in a public trials registry. The members of the International Committee of Medical Journal Editors (ICMJE) will consider most clinical trials for publication only if they have been registered (see N Engl J Med 2004;351:1250-1). Current information on requirements and appropriate registries is available at www.icmje.org/faq.pdf.

EDITORIALS

Hepatitis E Vaccine — Ready for Prime Time?

Krzysztof Krawczynski, M.D., Ph.D.

Large outbreaks of hepatitis E virus (HEV) infection were first recognized as a distinct clinical entity in the early 1980s.[1,2] Outbreaks continue and involve thousands of patients in certain geographic regions of the world, including the Indian subcontinent, northwest China, and the Central Asian Republics (the former Soviet Union) — areas where HEV infection is endemic.[3] HEV is transmitted predominantly through the fecal–oral route, and most reported outbreaks of infection have been related to the consumption of fecally contaminated drinking water; person-to-person transmission of the disease appears to be uncommon. There is a high attack rate in adults and a lower rate in children under the age of 14 years.

In many areas where outbreaks of hepatitis E have been reported, the disease accounts for a substantial proportion of sporadic acute hepatitis in both children and adults. The presence of anti-HEV antibody, evidence of previous HEV infection, has been detected in up to 5% of children under the age of 10 years in countries where the disease is endemic. In countries where HEV infection is not endemic, the disease has been associated with travel to endemic regions and accounts for less than 1% of reported cases of acute viral hepatitis. In recent years, cases of acute hepatitis E have been reported outside the regions where the disease is endemic and without the typical risk factors — in sporadic cases in Japan, it was related to the consumption of undercooked boar or deer meat.[3] Seroprevalence studies among blood donors in some countries where the disease is not endemic have found a prevalence of anti-HEV antibody of 1 to 5%, which is relatively high in comparison with the low rate of clinically evident disease. Possible reasons for these findings include subclinical or anicteric HEV infection, serologic cross-reactivity with other agents, and false positive test results. The higher prevalence rates of anti-HEV antibody among swine handlers in regions where HEV infection is not endemic may reflect true antibody positivity to HEV found in swine.[4]

Identified only in 1983, HEV is a small (32 to 34 nm in diameter), nonenveloped RNA virus of the genus hepevirus in the Hepeviridae family. A single-stranded, positive-sense, and polyadenylated RNA genome of HEV is composed of three open reading frames. Various geographically distinct isolates of HEV have been classified into at least four genotypes, but all genotypes share at least one major serologically cross-reactive epitope despite substantial genomic variability, an important finding in the search for a protective vaccine.[5]

Hepatitis E is typically a self-limited, acute viral hepatitis lasting 1 to 4 weeks; it does not progress to chronic disease. In rare cases, some patients may have severe disease, which progresses to fulminant liver failure; the overall case fatality rate for the general population in disease-endemic countries ranges from 0.1 to 4%. Case fatality rates are much higher (up to 25%) among pregnant women infected with HEV during the third trimester. In such cases, rapidly progressive disease may develop, with a short preencephalopathy period, cerebral edema, and disseminated intravascular coagulation.[3,6] The substantial morbidity associated with large epidemics of hepatitis E, the lack of effective therapy for the disease, and strikingly high mortality among HEV-infected pregnant women have inspired several groups to search for an efficacious vaccine to prevent both the infection and the disease.[7-9]

In this issue of the *Journal*, Shrestha et al. re-