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Evidence of Pestivirus RNA in Human Virus Vaccines

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We examined live virus vaccines against measles, mumps, and rubella for the presence of pestivirus RNA or of pestiviruses by reverse transcription PCR. Pestivirus RNA was detected in two measles-mumps-rubella combined vaccines and in two monovalent vaccines against mumps and rubella. Nucleotide sequence analysis of the PCR products indicated that a modified live vaccine strain used for immunization of cattle against bovine viral diarrhea is not responsible for the contamination of the vaccines.

The presence of pestiviruses in cell cultures and in fetal bovine serum has long been a recognized problem not only in laboratories but also among vaccine manufacturers (9). Therefore, it is quite possible for an adventitious pestivirus to be present in live virus vaccines prepared from pestivirus-contaminated master seed virus stocks and/or cell cultures. Procedures currently in use to detect the adventitious pestivirus in biological products are culture methods and immunological assays. Detection of pestivirus contamination in viral vaccines has been hampered because most (99%) of the pestivirus strains are noncytopathic in cell cultures. Immunological assays are sometimes incapable of direct detection of low-titer contamination of pestiviruses in vaccines. Therefore, the culture methods and immunological assays are not perfect for detection of pestivirus contamination in viral vaccines. To our knowledge, there is no publication available concerning pestivirus contamination in viral vaccines for human use. This report represents the first evidence of contamination with pestivirus RNA or pestiviruses in live human virus vaccines from licensed manufacturers.

We examined five commercial monovalent or combined live vaccines against measles, mumps, and rubella for pestivirus contamination by reverse transcription PCR with nested primer pairs to amplify the 5′ noncoding region conserved among viral genomes of the genus *Pestivirus*. The PCR primers used for the first- and second-round amplifications were selected from published sequences (1, 2, 7, 13) and have no significant nucleotide homology to the genomes of other virus genera reported so far. A single dose of each lyophilized vaccine was dissolved in 200 μl of reconstituent supplied with the vaccine and subjected to RNA extraction with an RNAzol B extraction kit (Biotecx Laboratories, Inc., Houston, Tex.). The RNA was subsequently used for cDNA synthesis and nested PCR as described previously (4). The first-round primers F1 [5′-ATGCCC(A/T)(C/T)AGTAGGACTAGC-3′] and R1 (5′-ACTCCATGTGCCATGTACAG-3′) amplified an approximately 285-bp product, and the second-round primers F2 [5′-AGTCGTCAGT(A/G)GTTCGA C-3′] and R2 (5′-CTCT GCAGCACCCTATCA-3′) amplified an approximately 155-bp product, from the pestivirus cDNA. PCR experiments were repeated two times for each vaccine in separate laboratories. Nucleotide sequences of the second-round PCR products were determined by the method of Sanger et al. (14).

The presence of specific pestivirus RNA was apparent on the gels after the second-round PCR of the monovalent vaccines against mumps and rubella and of the two measles-mumps-rubella combined vaccines (Fig. 1). The source of contamination is currently unknown. The measles and mumps vaccines were prepared in primary chicken embryo cell cultures and the rubella vaccine was prepared in primary rabbit kidney cell cultures. All the cell cultures used for vaccine production were supplemented with fetal bovine serum. Among animals, pigs and ruminants are natural hosts for pestiviruses, which include the causative agents of hog cholera in pigs, border disease in sheep and goats, and mucosal disease or bovine viral diarrhea in cattle. Bovine viral diarrhea virus (BVDV) or BVDV RNA in the fetal bovine serum that is used to grow the host cells used for the preparation of the vaccines might be a source of contamination, because the mumps vaccine prepared in primary chicken embryo cells was positive for the pestivirus RNA. The nucleotide sequences of the PCR products indicated that the contaminants in the viral vaccines are distinct from the BVDV vaccine strain 12, which has been

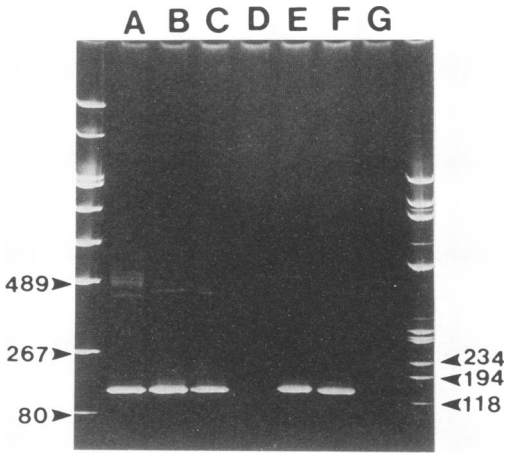

FIG. 1. Second-round PCR products of pestivirus cDNA resolved by 4 to 20% gradient polyacrylamide gel electrophoresis. Lane A, BVDV strain 12 used as a positive control; lanes B and C, measles-mumps-rubella combined vaccines produced by different manufacturers; lane D (negative), a measles vaccine; lane E, a rubella vaccine; lane F, a mumps vaccine; lane G, a live canine parvovirus vaccine used as a negative control. The outer lanes on both sides contain molecular size markers with sizes indicated in base pairs. The significance of weak extra bands appearing in the positive lanes is unknown.

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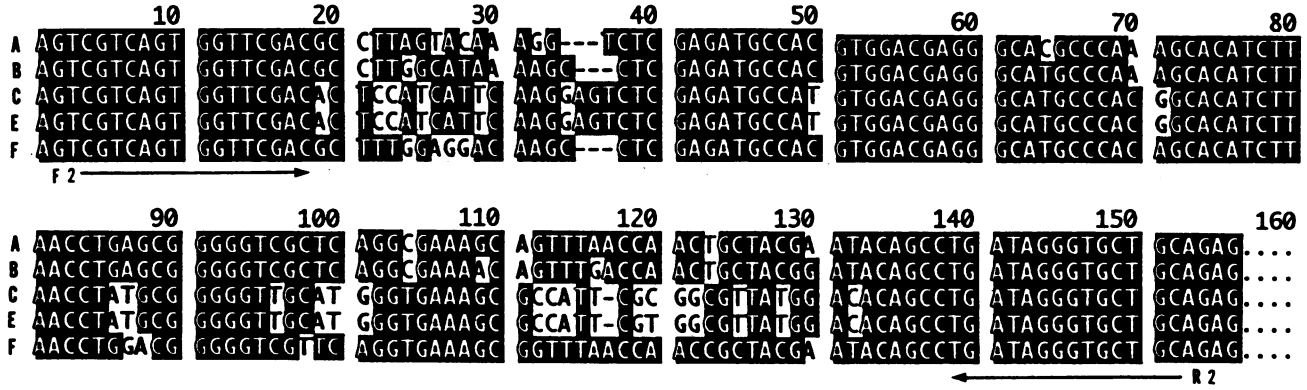

FIG. 2. Nucleotide sequence alignment of the second-round PCR products. A, B, C, E, and F are the sequences of the products shown in the corresponding lanes in Fig. 1. Homologous sequences are shown in white on black. The nucleotide sequence numbers are given from a consensus alignment. Primer sequences for the second-round PCR are indicated by arrows. Dashes represent spaces between adjacent nucleotides introduced for maximum alignment.

used in Japan (Fig. 2). Thus, cattle vaccinated with a modified live BVDV strain do not seem to be a source of the BVDV contamination of fetal bovine serum.

Although it has not been established that BVDV infections cause specific symptoms in humans, infantile gastroenteritis associated with excretion of pestivirus antigens (19) and microcephaly in infants who were born to mothers seropositive for pestiviruses (10) have been reported. Serum antibodies against BVDV have been detected in humans who had no contact with potentially infected animals (3, 18). Since noncytopathic biotypes of BVDV are capable of incorporating the host cellular RNA into their genomes (8, 11), pestivirus contamination would raise another issue with regard to the safety of live virus vaccines produced in continuous cell lines which are potentially oncogenic. Use of continuous cell lines as cell substrates for the production of human biologicals has been approved by the WHO Study Group (17). There is no evidence presented in this paper to substantiate contamination of the human virus vaccines with infectious pestiviruses, but iatrogenic infections have been reported for veterinary virus vaccines contaminated with infectious pestiviruses (5, 6, 15, 16). It is important to avoid the risk of contamination of human viral vaccines. In conclusion, we suggest that human viral vaccines be examined for the presence of adventitious pestiviruses by PCR, which will provide an additional assurance of safety because of its sensitivity. PCR will be particularly useful when it complements other tests (12) such as culture methods or immunological assays.

Nucleotide sequence accession numbers. The nucleotide sequence data first presented in this paper will appear in the DDBJ, EMBL, GSDB, and NCBI nucleotide sequence data bases with the following accession numbers: D26051, D26050, D26052, D26048, and D26049 for sequences A, B, C, E, and F, respectively, in Fig. 2.

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