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ABSTRACT BOOK

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Isolation of HEV-3 strains from swine fecal samples on human A549 cell line

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Background: Hepatitis E virus is an emerging virus, recently recognized as zoonotic. The disease is considered emerging and, in humans, the infection can be asymptomatic or causes a disease generally self-limiting that can become chronic in immunocompromised. The zoonotic genotypes, named HEV-3 and HEV-4, infect pigs and wild boar that are the main reservoirs. A few data are available on virus replication and mechanism of infection, due to lack of an efficient system of cultivation. This study aimed to cultivate different strains of HEV-3 detected in Italian pigs and to develop a reproducible protocol of isolation of wild-type HEV strains on cell cultures.

Methods: fecal suspensions from HEV-positive pig samples were obtained in Tris-HCl 0,1 M (10% w/v) and used for the cell inoculum. Cell lines A549, ST100 and PK15 were growth in MEM (10% FBS + 2.5 g/mL amphotericin B) at 34.5 °C and 5% CO2 for 3 days before being infected.

Afterwards, cell monolayers were inoculated at MOI 0.1 (starting tire of inocula ≥10^5 genome copies GC/ml).The supernatant was refreshed with MEM every 3-4 days. The infected cell monolayers were split regularly, 1:2 at the same growth conditions. The HEV GCs of supernatant were quantified by quantitative real-time RT-PCR and viral particles were visualised by transmission electron microscopy (TEM).

Results: three wild-type HEV-3 strains derived from fecal samples collected from Italian pigs were isolated on A549 cell monolayers. No growth was observed with HEV-3 strain from positive wild boar liver. Neither ST100 nor PK15 cell lines were permissive for the growth of the same isolated strains. At 60 days post infection, the isolates, namely 2BN5_IT20, 3AC47_IT21 19M2_IT21, reached titres comprises between 10^5-10^6 HEV RNA GC/ml. Infected cells were successfully split as confirmed by virus production in the supernatant for at least 14days post-split. Electron microscopic observations of supernatants showed assembled structured viral particles with size ranging from 28 to 33 nm.

Conclusions: the protocol developed for human HEV virus propagation was also successfully used for swine HEV-3 cultivation from fecal samples. The protocol is troublesome but ensures reproducible cultivation of wild type viruses. Further studies will be performed to ameliorate the protocol and to produce the virus for future study focusing on the replication cycle, immune responses induced by the virus and its resistance to temperature and other treatments.