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SHORT COMMUNICATION

Zika virus isolation in semen

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SUMMARY

Zika virus (ZIKV) can be sexually transmitted and replicative particles were first detected in a semen sample from a patient during the 2013-14 French Polynesia outbreak. Here we describe the virus isolation from semen of a patient returning to Italy from Brazil.

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Zika virus (ZIKV), a neglected tropical disease before 2015 when it was first identified in Brazil, is a mosquito-borne *Flavivirus* closely related to dengue (DENV), West Nile, Japanese encephalitis and yellow fever. This emerging virus usually causes asymptomatic infections or mild disease in humans, but has recently been associated with adverse fetal outcomes (Panchaud *et al.*, 2016) and Guillain Barré syndrome (Cao-Lormeau *et al.*, 2016). ZIKV is primarily transmitted by *Aedes* mosquitoes, but there is also evidence of sexual transmission (McCarthy, 2016; Hills *et al*., 2016). Although ZIKV can be detected in plasma, whole blood, urine, saliva and semen by PCR (Fourcade *et al.*, 2016; Atkinson *et al*., 2016), its isolation from biological specimens is difficult. In particular, only three isolates from semen have been reported (D'Ortenzio *et al*., 2016; Mansuy *et al*., 2016; Jang *et al*., 2016).

Here, we describe the case of a male subject with arthralgia, rash, asthenia, myalgia and conjunctivitis returning to Italy from Brazil in April 2016. Three days after his return, plasma, urine and saliva were collected and resulted positive for ZIKV by PCR (Lanciotti *et al*., 2008). Virus isolation from these materials was attempted with the VERO E6 cell line. Three days later (day 6 after symptom onset), semen was collected, tested by RT-PCR and inoculated on VERO E6 for virus isolation. The viral load in semen was $1.1x10^5$ ZIKV RNA copies/ml with no observed significant differences in ZIKV RNA load between semen supernatant ($1x10^5$ copies/ml) and the cell pellet ($5x10^4$ copies/ml). Semen was diluted 1:10 in Earle medium (EMEM) plus antibiotics and kept at room temperature for 30 min before inoculation. Saliva ($1.1x10^2$ copies/ml), collected with *Copan's* universal transport medium (UTM) (Copan, Brescia, Italy), and urine ($8.1x10^3$ copies/ml) were treated for 30 min with antibiotics for decontamination, plasma (90 copies/ml) was inoculated undiluted. 100 µl of each samples were inoculated into a VERO E6 confluent 24-well microplate and centrifuged for 30 min 1800 rpm. After one hour incubation at 37°C 5% CO_2, the inoculum was discarded and 1 ml 2% FBS EMEM was added to each well and incubated at 37°C 5% CO_2. Each well was observed by light microscope every day for cytopathic effect (CPE) appearance. A CPE, characterised by round cells rapidly detaching from the monolayer, was observed on day four only in semen. The presence of the virus was confirmed by RT-PCR. ZIKV virus was isolated only from semen, whereas the other samples were negative also after a second passage. The isolate was then propagated in two cell lines: VERO E6 and ARPE; but only in VERO E6 was a significant increase in RNA load detected from the first to the second passage (*Table 1*). In ARPE, the CPE was weaker and, despite having been passaged in new culture, after seven days the titre remained lower compared with VERO E6 (*Table 1*). A $1x10^{10}$ $TCID_{50}$/ml titre was obtained at the second passage in VERO E6 at day four (*Figure 1*). At the time of virus isolation, only IgM antibodies were present in the patient's serum and no antibodies were detected for dengue or chikungunya virus. Twenty-two days after the first semen collection another sample was taken but tested negative by RT-PCR. Few ZIKV semen isolates from the ongoing epidemic in South America (D'Ortenzio *et al*., 2016; Mansuy *et al*., 2016; Jang *et al*., 2016) or from other biological samples are available due to the difficulties in isolating this virus. It might be speculated that in residents of tropical countries the presence of previous Dengue antibodies cross-reacting with ZIKV (Stettler *et al*., 2016) may block the virus and prevent ZIKV isolation. In patients without previous flavivirus antibodies, the presence of specific ZIKV antibodies associated with a low viral load in plasma, urine and saliva, compared to semen (Reusken *et al*., 2016) can impair virus isolation. A similar event has

Table 1 - *Semen ZIKV isolation in cell culture.*

Cell line	*Passage 1 RT-PCR*	*Passage 2 RT-PCR*	*Passage 1 Virus titration*	*Passage 2 Virus titration*
VERO E6	$1.3x10^4$ copies/ml	$4.6x10^8$ copies/ml	$4x10^6$ TCD_{50}/ml	$1x10^{10}$ TCD_{50}/ml
ARPE	$7x10^2$ copies/ml	$5.6x10^5$ copies/ml	$2x10^3$ TCD_{50}/ml	$1x10^6$ TCD_{50}/ml

Key words:
Zika virus, Semen, Viral isolation.

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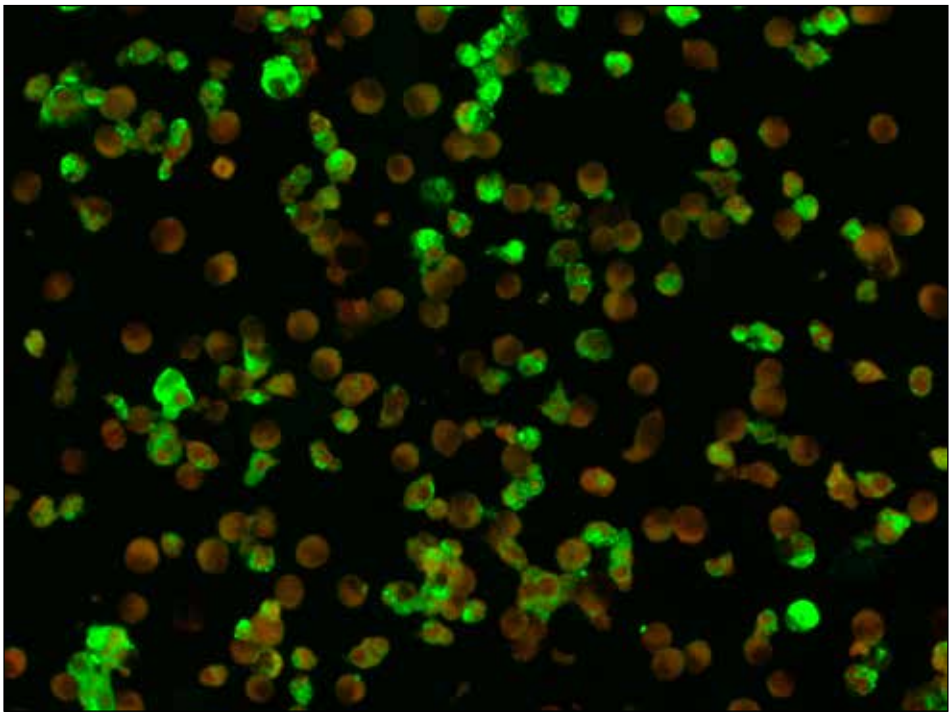

Figure 1 - *ZIKV isolation in the VERO E6 cell line stained with patient serum.*

been proposed in parvovirus B19 infection where the appearance of antibodies hampers virus isolation (Wolfisberg *et al*., 2013). Moreover, the isolation of ZIKV from semen in our patients further confirms that sexual transmission of ZIKV might be more common than reported, considering that the virus can be detected in semen some months after disease onset (Barzon *et al*., 2016). CDC guidance suggests that men with ZIKV disease should wait at least six months after symptom onset to attempt conception (Petersen *et al*., 2016). Semen should be routinely checked with suspected ZIKV infection and patients should be monitored until semen is negative to avoid sexual transmission and gamete storage.

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