

24 one time point, when patients demanded medical assistance. Ten preimmune urine
25 samples were also included in the study presented.

26 RNA was extracted from 1 ml of freshly thawed urine by using the inRICHMENT
27 Virus Reagent (Analytik Jena AG, Jena, Germany), followed by QIAmp Viral RNA Mini
28 Kit (Qiagen, Ca, USA) according to the manufacturer's instructions. Specific YF-17D
29 genome was detected by quantitative real time RT-PCR performed as described
30 previously (2).

31 In total, 18 out of 129 samples yielded positive amplification of YF-17D genome,
32 while all preimmune sera were negative. Among the healthy YF-17D vaccinees, four
33 exhibited the presence of YFV RNA in their urine (28.6%), including 3 first-time
34 vaccinees and the re-vaccinated one. The YF-17D genome was detected in the urine of
35 these individuals in an intermittent mode, with more than one consecutive day yielding
36 positive amplification. From our results, it seems that a first excretion of YF-17D occurs
37 in the first days after vaccination, and a second viral shedding (days 4-7) might happen,
38 probably reflecting the viral replication in the vaccinees (Figure 1).

39 Among suspected YFVAE patients, YF-17D genome was detected in eight out of 18
40 patients (44.4%) at different time points. Paired sera from these patients did not yield a
41 positive amplification of YFV-17D genome. Remarkably, we found the presence of viral
42 genome 20, 24, and 25 days after vaccination in the suspected YFVAE patients (Figure
43 1). We can only hypothesize whether the presence of viral genome in urine at this time
44 was a response to the prolonged replication of the virus in the patients affected or to a
45 persistent viral shedding of the vaccine virus which may occur in some individuals
46 without further pathological significance.

47 The average viral load detected in positive samples was 8.8×10^2 genome
48 equivalents (GE)/ml, ranging between 30 and 70 GE/ml (n=4 samples) to 10^4 GE/ml
49 (n=1 sample), without any significant difference regarding the day of sample collection.

50 A more comprehensive study to determine the features of YFV-17D shedding in
51 urine is ongoing. It would be highly desirable to explore the presence of YFV genome in
52 wild-type cases, as we would then be able to anticipate that YF genome could be
53 present in the urine of the patients during the course of the disease, providing a feature
54 that could be extremely useful for diagnosis and identification of clinical cases.

55 This work is the first report of YF-17D genome detection in urine of vaccinees. The
56 finding could be related to the fact that YFV replicates in the kidney and provides the
57 opportunity for further research regarding YF and YF-17D pathogenesis and organ
58 tropism. Moreover, the data reported have relevance for diagnostic purposes since urine
59 samples are very easy to collect, even from cases with hemorrhagic alterations or from
60 newborns without the need of invasive methods or trained personnel. Moreover, its use
61 would be of great interest under field conditions such as suspected outbreaks or mass-
62 vaccination campaigns.

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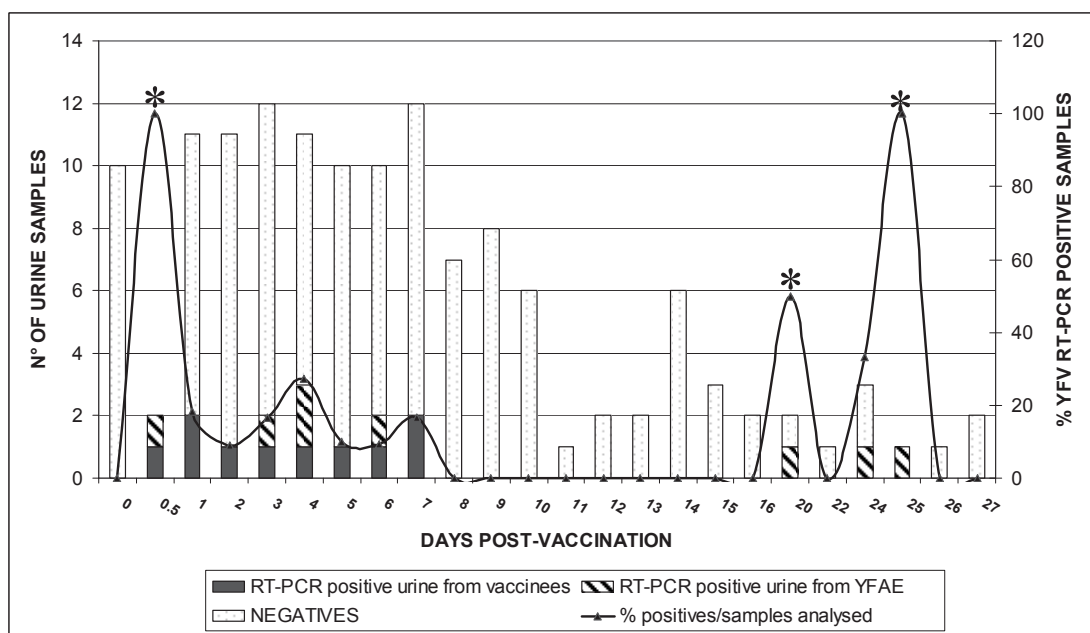
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Figure 1: Detection of YF-17D genome in urine of vaccinees. Black bars represent positive samples from healthy vaccinees; stripped bars represent positive samples from suspected YFAE. Dotted bars represent negative samples (both healthy and suspected YFAE vaccinees). The black line indicates the percentage of positive samples from the total of samples assayed at this time point.



* Percentages could be biased by the small number of samples