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Originally published as:

Domingo, C., Yactayo, S., Agbenu, E., Demanou, M., Schulz, A.R., Daskalow, K., Niedrig, M.
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(2011) Journal of Clinical Microbiology, 49 (2), pp. 760-762.

DOI: 10.1128/JCM.01775-10

This is an author manuscript.
The definitive version is available at: http://jcm.asm.org/

Detection of yellow fever 17D genome in urine

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Yellow fever (YF) remains an important public health problem in endemic regions, with a dramatic upsurge in the number of cases in recent years. So far, extensive YF epizooties occurred in South America in 2008, and during the past year YF outbreaks arose in Cameroon, Democratic Republic of Congo, Guinea, Côte d´Ivoire, Central African Republic and Liberia (http://www.who.int/). Highly effective, live attenuated YF vaccines against the disease have been available for decades and have had a major impact on the incidence of the disease (17).

The clinical diagnosis of isolated cases of YF or identification of vaccine-associated adverse events (YFVAE) is particularly difficult because the symptoms are quite similar to those of many other diseases (20). Laboratory confirmation is therefore essential and relies on the detection of YF-specific IgM or a fourfold or greater rise in serum IgG levels (in the absence of recent YF vaccination), isolation of yellow fever virus (YFV), positive post-mortem liver histopathology, detection of YF antigen in tissues by immunohistochemistry, or detection of YFV RNA by PCR which provides the earliest diagnosis possible. Samples recommended for diagnostics are blood, serum, CSF, peritoneal or pleural fluid and liver biopsies (1, 20). However, biopsies and invasive techniques must be avoided or practiced with extreme caution due to the risk of bleeding complications (5).

To explore the suitability of non invasive samples for the diagnosis of acute YF infections or YFVAE, we have collected urine samples (n=129) from YF-17D vaccinees (day 0 to day 28), comprising sequential samples from 13 healthy primary vaccinees, one revaccinated individual, and 18 suspected YFVAE detected during mass24 vaccination campaigns in Liberia and Cameroon. Urine samples from suspected YFVAE were collected at only one time point, when patients demanded 25 medical assistance. Ten preimmune urine samples were also included in the study presented.

RNA was extracted from 1 ml of freshly thawed urine by using the inRICHMENT Virus Reagent (Analitik Jena AG, Jena, Germany), followed by QIAmp Viral RNA Mini Kit (Qiagen, Ca, USA) according to the manufacturer's instructions. Specific YF-17D genome was detected by quantitative real time RT-PCR performed as described previously (3).

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

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

The average viral load detected in positive samples 48 was 8.8+E02 genome equivalents (GE)/ml, ranging between 30 and 70 GE/ml (n=4 samples) to 104 49 GE/ml (n=1 sample), without any significant difference regarding the day of sample collection.

It has been reported that a low transient viremia is detectable on YF-17D vaccinees 4 to 6 days after vaccination and do not exceed 2.3 log10 PFU/ml or 2.23 log10 copies/ml (12, 16, 19). However, higher levels have been reported in wild type infections (2, 14) and severe YFVAE (4, 7, 13). The results presented are in agreement with these observations and deserve being thoroughly studied. A large scale study to determine the features of YFV-17D shedding in urine, and the correlation with viremia levels is ongoing. It would be highly desirable to explore the presence of YFV genome in wild58 type cases, as we would then be able to anticipate that YF genome could be present in the urine of the patients during the course of the disease, providing a feature that could be extremely useful for diagnosis and identification of clinical cases.

The presence of viral RNA or antigens in urine has been demonstrated for other relevant flavivirus causing human infections like West Nile virus (18), dengue (10, 15), Japanese encephalitis (9), or St. Louis Encephalitis virus (8). This work is the first report of YF-17D genome detection in urine of vaccinees. The finding could be related to YFV replication which may occur in the kidney (6, 11) and provides the opportunity for further research regarding YF and YF-17D pathogenesis and organ tropism. Moreover, the data reported have relevance for diagnostic purposes since urine samples are very easy to collect, even from cases with hemorrhagic alterations or from newborns without the need of invasive methods or trained personnel. Moreover, its use would be of great interest under field conditions such as suspected outbreaks or mass-vaccination campaigns.

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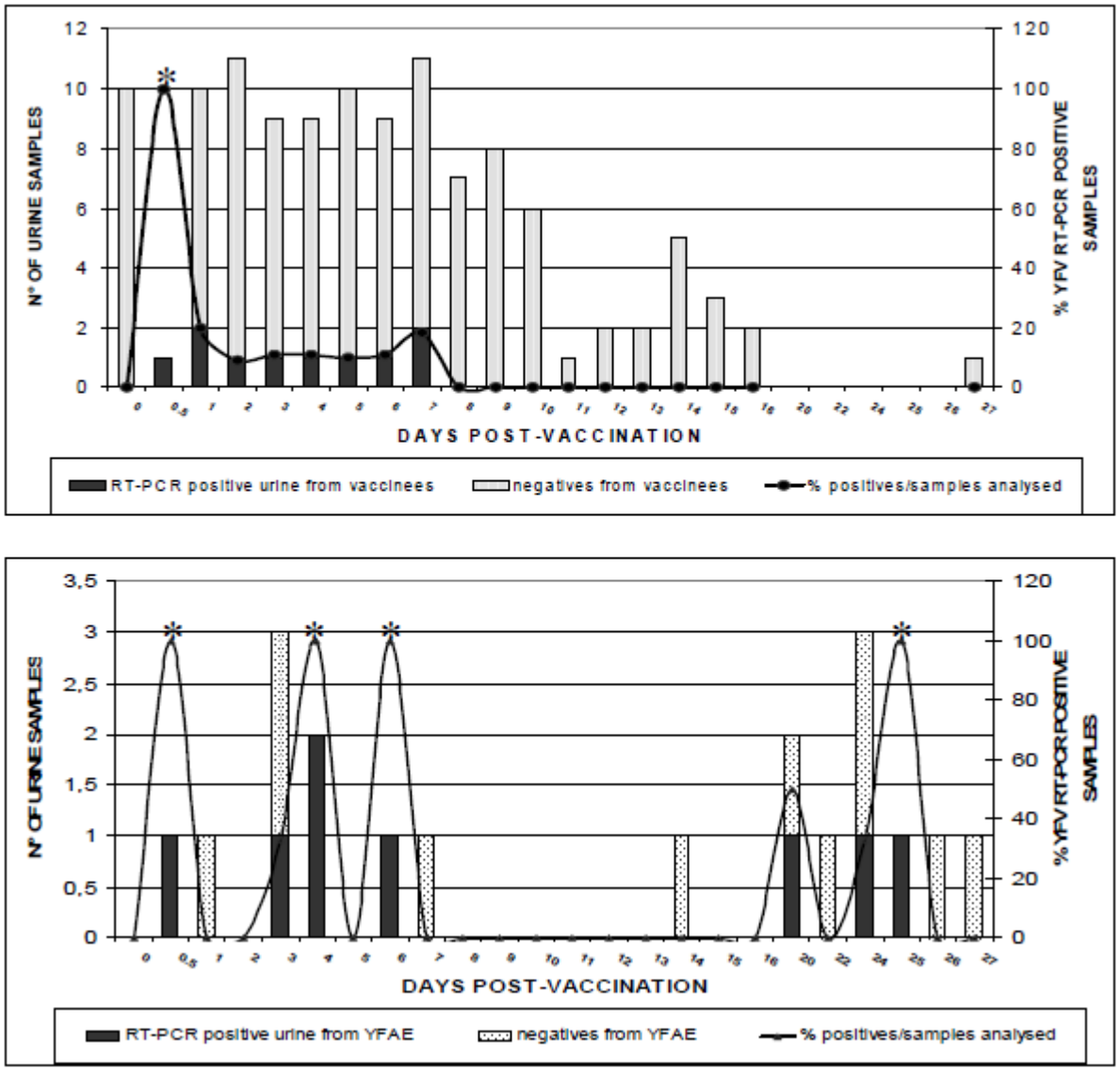
Tables and Figures

Figure 1: Detection of YF-17D genome in urine of healthy vaccinees (upper graph) or vaccinees with suspected adverse events (lower graph). Black bars represent positive samples; dotted bars represent negative samples. 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