Peer Review File

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Reviewer A

Comment 1: The study identified weaknesses of the NNDRS in China and proposes way to address the weaknesses. This is important, however it is unclear how these data can be generalized outside China. In particular, it is unclear if the data can be useful in most of the other countries where there is a less (or no) centralized management of data and less resources to conduct sequencing.

Reply 1: We agree that many countries may not have a centralized data system for hepatitis A, but our approach can be relevant for other notifiable infectious diseases. Actually, China did not have a strong capacity for conducting sequencing-based surveillance either, and our study was among the first efforts in piloting sequencing-based surveillance for selected notifiable infectious diseases. We chose to focus on hepatitis A here for the very reason that the number of hepatitis A cases is relatively small and the sequencing method (300bp and not whole genome) is easy, and thus, a good starting point or low hanging fruit in piloting sequence-based surveillance in sub-national regions before possible larger-scale rollout.

Changes: We revised the title of the manuscript to emphasize this message of piloting and learning. The revised title is: “Implementing sequencing-based surveillance in developing countries: Findings from a pilot rollout for hepatitis A in China”

Comment 2: Also, there are no information on when the program started and how long was the required training for the involved staff.

Reply 2: We provided details in the method section (see page 6, line 502-504, and page 7 line 575-577).

Changes in the text: (1) The sequencing-based enhanced surveillance for hepatitis A was first initiated in Zhejiang province in March 2019, followed by Shandong province in June, and Shaanxi, Hainan, and Hubei provinces in September 2019, and continued through December 2020. (2) Before implementation, key public health professionals from each sentinel province were trained during a one-day training session and were provided with handy problem-solving support by the study team during the first months.

Comment 3: It is unclear who was running the program and what level of specialization and preparation was required to implement the system. In general, the lack of data on the NNDRS itself impairs the usefulness of this study.

Reply 3: We appreciated the reviewer's opinion, we added a brief introduction section of NNDRS in the revised version (see page 6, line 490-494).

Changes in the text:

(1)NNDRS is a national internet-based reporting system for infectious diseases in China. Based on NNDRS, the incidence of notifiable infectious diseases and demographic characteristics of reported cases are monitored at different levels (9). However, the molecular information of pathogens from sequencing is not part of NNDRS. To be better prepared for the next epidemic, researchers from the Chinese CDC and Zhejiang University launched this pilot study.

Reviewer B

Comment 1: In page 14, the authors mentioned that the detection of anti-HAV IgM as screening tool is not appropriate. However, the authors performed the sequence-based surveillance for the patients who were positive for anti-HAV IgM. As you mentioned in page 12, less than 50% of the samples had detectable HAV-RNA. I think that the detection of anti-HAV IgM is more appropriate for screening than the sequencing. Of course, medical examination, interview and elevated serum aminotransferase are also important for screening. I think that the sequencing surveillance is useful not for screening but for the detection of clustered cases and the expectation of further outbreaks.

Reply 1: We totally agree with the review about screening with HAV IgM instead of RNA, and we made the clarification in the revised test (see page 16, line 1657-1659).

Changes in the text: Since performing sequencing for these patients would be a waste of the limited human and financial resources, more accurate and specific criteria for HepA sequencing should be developed.

Comment 2: As mentioned above, the authors should describe further the utility of sequencing-based surveillance compared with that of the detection of anti-HAV IgM. How about the sensitivity and specificity of this system compared with the detection of HAV IgM? Did the authors also calculate the scores for the evaluation of the detection of HAV IgM as described in Table 2?

Reply 2: The simple answer to the question about the utility of sequencing-based surveillance is the virologic characterization of cases and early detection of and faster and more effective response to possible outbreaks. Sequencing-based surveillance is not to replace, but a new add-on to case detection by anti-HAV IgM and the existing case reporting system.

This work is extended and build on the foundation of NNDRS (As shown in figure 1). However, reporting on NNDRS does not require data about the epidemiological details of the cases and the molecular information of pathogens. Meanwhile, reporting on NNDRS has been a daily work for PHPs since 2003, so it's not appropriate to assess its simplicity and acceptability. In terms of data quality and timeliness, case report forms of NNDRS only collect demographic and few clinical information. It is therefore hard for us to calculate the scores for the evaluation of the detection of HAV IgM as described in Table 2. But we do provide information about the utility of sequencing-based surveillance compared with that of the detection of anti-HAV IgM. To make the text more readable, we add figure 6 to illustrated clustered cases that were reported at different times or in different places could be neglected without sequence data. We also rewrite the results section (see page 12, line 981-993, and page 13, line 1155-1160)

Changes in the text: To identify localized epidemic events, local CDC used to base on a case count threshold per month at the catchment area level. An outbreak investigation would be initiated if two cases were occurring in the same area within one month. However, the link between those clustered cases would be unknown without sequencing data. The new information from sequencing-based surveillance may help the early detection and management of an outbreak. For instance, as shown in Figure 6, 40% of cases from Cluster 1 were dispersed over time and geographic locations, and sequencing-based surveillance revealed the virologic links among the sporadic cases, and this information could advance the detection of this outbreak by two months if the virologic data had been shared promptly. According to the abovementioned threshold, The first two cases of outbreak concerns (SD61 and SD60) were triggered in February 2020. But, the first two clustered cases (SD002 and SD086) actually occurred in Dec 2019. Sequencing-based surveillance could improve the efficiency of traceback investigation since patients with identical sequence patterns and similar epidemiologic characteristics usually suggest a common-source exposure. According to laboratory data, only 56% of cases that were reported after Feb 2020 were genetically identical with the outbreak strains. The combination of epidemiological and virologic information among clustered cases implicated raw oysters as the potential vehicle of the outbreak.

Comment 3: I have another question. In page 10 line 164-165, the authors described that the region of the VP1-P2A junction region of the HAV genome was amplified in this sequencing study. How about the primer design? Was that method reported in previous reports? If so, you must cite those as references.

Reply : We added one sentience about the primers, as shown below at lines 160-161 in the final version of the manuscript.

Changes in the text:

The RNA of positive samples was then amplified by nested reverse transcription-polymerase chain reaction (RT-PCR), using the primers that were previously described (14,15).

Reviewer C

Comment 1: Of the 11 genomic clusters found (7 clusters being potentially related to a foodborne outbreak by oyster, what was the possible number of comparisons?

Reply 1: Thank you for these precious comments and suggestions. We revised table 3 to provide that information.

Changes in the text: please see Table 3 in the revised version.

Comment 2: The provinces were selected by social-economics, HepA morbidity, and allocation of healthcare resources. It would be helpful to provide a table summarized each province by these selection factors.

Reply 2: We add the essential data in the revised manuscript (see page 6, line 506-511 and page 7, line 556-557).

Changes in the text: Public health infrastructure, geographic representation, and financial resources needed were considered in the selection and duration of the pilot project. These five provinces (Zhejiang, Shandong, Shaanxi, Hainan, and Hubei) represented all three social-economical tiers of China with different HepA morbidity and healthcare resource levels (9). The incidence of HAV infection varied and was highest in Hubei at1.47/100,000, and lowest in Shandong at 0.53/100,000 in 2015 (10). Government funding for healthcare also varied across the three regions, with the eastern region (Zhejiang, Shandong, and Hainan) having the highest budget (6194

thousand Yuan), the western region(Shaanxi) having the lowest budget (2643 thousand Yuan) (11).

Comment 3: An analysis of covariance would be helpful to better understand the results.

Reply 3: We understand analysis of covariance may be helpful to better understand the results, however, in the present study, the analysis is purely descriptive. And we think the univariate regression models may not be optimal but should be sufficient to draw our conclusion in this descriptive analysis.