Author's response to reviews

**Title:** The First Clinical Case due to AP92 like strain of Crimean-Congo Hemorrhagic Fever Virus and A Field Survey

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**Author's response to reviews:** see over
Responses to the comments

Referee 1:
This reviewer pointed out that the attack rate of CCHF virus strain KMAG cannot be calculated in the previous review. Although the authors replied that the attack rate was given for overall CCHF infection, not specific for CCHFV KMAG in the replies to my review, it is still written as “The attack rate, that was defined as the proportion of diseased subjects among the infected ones was two out of 38 (5.2%)” (Page 8, lines 14-15). The authors should clearly mention that the attack rate was given for overall CCHF virus infection, not specific for CCHFV KMAG in this sentence. Without this clarification, it makes the readers misunderstand the context. The authors described that the attack rate was very low compared to the CCHF strains” (Page 8, lines 15-16). It is written that the attack rate was compared to the CCHF strains. Clarify what were the CCHF (virus?) strains, which were the target compared to by the attack rate. Based on the descriptions, this reviewer considers that the authors compared the attack rate of CCHFV KMAG with those of the other strains such as CCHFV isolated in Russia. In the entire text, the terms “CCHF strains”, “CCHF viral strains”, “CCHF virus”, “CCHFV”, etc, appear. Unify the terms throughout the entire text according to the meaning of each term.

Response:
1. The sentence was revised as: “The attack rate for overall CCHFV infection, that was defined as the proportion of diseased subjects among the infected ones was two out of 38 (5.2%)”
2. CCHFV term was preferred and revised accordingly.
3. The sentence was revise as: “The attack rate was very low compared to the CCHFV strains reported from Russia, where the attack rate was described as 20% previously [14].”

Referee 2
Although the information concerning material of the study can be found somewhere in the text, it would be very useful if all Material will be as a section before the Methods, as I suggested previously.

Response:
A material subtitle was inserted under the Methods section.

In Figure 1, the proportions are not given. I had suggested as an example "14/420, 3.33%”. By this way, the reader will validate easily the positivity.

Response:
Figure 1 was revised accordingly.

Title has to include at least the country, better the city, as well. Not "field survey" but "serosurvey"

Response:
The title was revised at the first revision according to the suggestion of the first referee.

Referee 4:
Reviewer's report For the most part, the author's have addressed most of my original criticisms. However,
1. On the use of the term "attack rate" This would imply that a virus "attacks" humans. Viruses do not "attack" anybody, they "infect" so the proper terminology is "infection rate" NOT "attack rate"

We used the term “attack rate” by reference to “Epidemiology: An Introduction, K.J. Rothman, Oxford Univ Press, 2002”. In this reference, the attack rate was described as: “A term for risk or incidence proportion that is sometimes used in connection with infectious outbreaks is attack rate. An attack rate is simply the incidence proportion or risk of becoming afflicted with a condition during an epidemic period. For example we might speak of an influenza epidemic with an attack rate 10%, which means that 10% of the population developed the disease during the epidemic period.” In our study, we described the attack rate as the proportion of the number of diseased individuals among number of infected individuals.

2. On the issue of the PCR assay used in the study. The author's state that it is for "amplification of exclusively AP92 strain" Again, since no validation of this assay was described, how is the reader to know that the assay is specific for the AP92 strain? Was it tested against several other CCHF strains and shown to be negative?

We tested the primers used in this study with the Eastern European strains and one African strain, and all were found to be negative.

3. 2nd paragraph on page 4 "Table X" needs to be corrected.

Revised as Table 3.

4. Also, as stated by one of the other reviewers, it is NOT correct to use AP92 strain as the outgroup in the phylogenetic analysis since the relateness to this sequence is what is being compared. A related, but non-CCHF, virus strain (such as Dugbe virus) should be used as the outgroup.

Figure2 was revised, and Dugbe was added and used as out group.

Reviewer 3:

Reviewer's report:
This important article presents highly relevant facts that may change our understanding of CCHFV ecology and epidemiology. However, because of their importance these data deserve a better methodological foundation (see point 5). If for any reason authors cannot provide extra testing in due time, they might want to report these important data in a more preliminary format.

Major compulsory points:
Authors should be more careful about claims regarding circulation and traits of the AP92-like virus. They have no identification of virus from vectors and reservoir, and they have no exact idea regarding time of exposure and reliability of reporting in people. Definition of infected persons only relies on an IgM result in a single EIA test that has not been in use in many studies.

Response:
Virus was detected from ticks collected in the neighbouring villages. We did not report this data in this manuscript, but we have already entered these sequences to the gene bank (FJ392603, FJ392602, FJ392601). Additionally, in summer months of 2008, a second human case was detected in the same region, which was entered to the gene bank (FJ392604).

Definition of infection does not only rely on IgM result in a single EIA test. All individuals were re-tested four months later for both IgG and IgM, simultaneously with the initial serum samples. All IgM positive individuals but one were found to be seroconverted to IgG four months later. This is an evidence for newly acquired infection. The EIA test used in this study was bought from Russia. This is the sole commercial kit in the market that we can find.

In this light, sentences like "This difference can be explained by different virulences among the circulating strains" (Page 8) sound overstated. If authors want to raise hypotheses, these should be clearly identified as such, and developed a bit further (see below).

Response:
The sentence was revised: “One of the possible explanations for this difference could be the different virulent strains of CCHFV, but further studies are needed for precise elaboration.

2. The Abstract suggests that antibody testing was done specifically against the new AP92-like strain, which is almost certainly not the case. Please identify the test antigen in the EIA test. Authors should also keep in mind that there are no differential CCHFV serotypes, and should avoid any indirect implications into this direction unless they have proof of it.

Response:
Antibody testing was not done specifically against the new AP92-like strain. The antigen used in the EIA kit was not given in the package of the kit, but there is no different serotypes of CCHFV. In order to avoid confusion, a sentence was inserted to the abstract as: “Diagnosis was confirmed by RT-PCR and sequencing.”

3. Has virus isolation from patient blood been attempted? It would be a very high priority to have an isolate of an AP92-like strain (pathogenicity modelling / vaccine template). This should be discussed.

Response:
These sentences were inserted to the page 8: “We couldn’t isolate this strain, because of lack of laboratory facilities with appropriate biosafety level. However, since this strain could be the agent for asymptomatic infection, its isolation and further characterization could be useful for understanding the pathogenetic mechanisms and for vaccine development against CCHFV.”

4. Page 5 "Since the original kit didn’t contain any reagent for hindering false IgM seropositivities due to IgG type antibodies, before IgM detection, IgG was eliminated using a commercial IgG removal kit (Zeus®, USA)." - To my knowledge IgG is removed from plasma before IgM detection in order to prevent shielding of antigens by IgG if both isotypes are co-existing. To exclude the effect of rheumatoid factor, authors should conduct a
rheumatoid factor test in all patients positive for IgM. Positives should not be counted as anti-CCHFV IgM positives.

Response:
This sentence was inserted to the Methods section, page 3: “A commercial variant capture ELISA kit (vectorbest®, Russia) was used for detection of IgM antibodies of CCHFV.” This sentence was missed in the first draft. The sentence “Since the original kit didn’t contain any reagent for hindering false IgM seropositivities due to IgG type antibodies, before IgM detection, IgG was eliminated using a commercial IgG removal kit (Zeus®, USA).” was removed from the methods section.

5. All antibody-positive sera (or ideally, all sera) should be re-tested by a second EIA test, or by IFA.

This is ideal for more robust results, but there is no alternative commercial EIA kits. Under these circumstances, we retested the same individuals twice 4 months apart. And first samples were retested simultaneously with the second samples (Methods, page 3).

6. Figure 2: This seems to be an unrooted cladogram. The phylogeny should be rooted with Dugbe virus, which is a well acceptable outgroup.

The sequence of Dugbe virus was added to the phlogenetic analysis and used as the outgroup. The figure 2 was revised accordingly.

Minor essential points:

7. Page 6, "Thirty four out of 35 (97.4%) IgG positive patients were also IgM positive." – Please state how many patients had IgM positive / IgG negative status in total (I calculate five).

There are four patients, who are IgM positive but IgG negative, as it was indicated in the first paragraph of the “survey in the region” section.

8. Page 6, "All the IgM positivities except one, co Since the original kit didn’t contain any reagent for hindering false IgM seropositivities due to IgG type antibodies, before IgM detection, IgG was eliminated using a commercial IgG removal kit (Zeus®, USA)." inverted to IgG positivity." – Does this mean that all patients who had only IgM / no IgG in the first test seroconverted to IgG positive?

Yes. This is a strong point for the study, which shows a recent infection. And the sentence “Since the original kit didn’t contain any reagent for hindering false IgM seropositivities due to IgG type antibodies, before IgM detection, IgG was eliminated using a commercial IgG removal kit (Zeus®, USA)." was deleted as explained in response to the 4th comment.

9. Page 7: In the population survey, have people been asked for tick morphology and have they been shown Boophilus / Rhipicephalus / Hyalomma ticks as examples? Can any conclusions been made in this direction?
People were asked for the history of tick bite, but not for the types of the ticks. Because practically it is not likely get reliable data about tick morphology by asking to the individuals.