**#15790**

**Answers to reviewers’ comments**

**Reviewer B**

* In INTRODUCTION the reviewer suggests the following amendments:
  + Line 5: “(…) Small rodents and lagomorphs are the main hosts of F.tularensis, but a wide range of wild and domestic animals may be infected. A number of different arthropods can also act as vectors (…)”

Answer: Thank you very much, changed.

* + Line 6-8 - transmission to humans could be better detailed. A suggestion could be “Transmission to humans occurs directly from the animal reservoir (handling tissues or fluids, ingestion of undercooked meat or animal bite), through arthropod bites, following exposure to contaminated environmental sources (water, soil, dust), or during sample manipulation in the laboratory.”

Answer: Thank you very much, suggestion accepted.

* + Line 15 – “(…) POSSIBILITY OF aerosolization (…)”

Answer: “possibility of” added to the sentence.

* + Consider (if there are no constraints in the length of the document) adding a word on diagnosis and prognosis.

Answer: The authors considered the referral to diagnosis and prognosis of the disease in the introduction in the initial version of the manuscript, but due to word constraints, they aren’t able to include it.

* In CASE REPORT and DISCUSSION
  + Although not mandatory, stating that the rest of the physical exam at admission was unremarkable could be considered.

Answer: Thank you very much, suggestion accepted.

* + Type of agglutination test used (TAT or MAT; commercial or in-house; etc) should be specified; Titer of 407 means 1:163840000000?? – in the reviewer’s opinion the difference of magnitude between the result and the cut-off used (1:20) should be made clearer for the reader.

Answer: As suggested, the authors have included the type of the test (TAT) in case report section. The titre detected was 40 is the double of the cut-off.

* + Table with laboratory results: the reference values are missing.

Answer: Corrected.

* + **I**n DISCUSSION, lines 12-13 about treatment, the following amendment is suggested: ”(…) except for meningitis and endocarditis, where combination therapy is recommended”.

Answer: Thank you, suggestion accepted.

* + It should be clarified what was the rationale behind a 21-day empirical treatment with doxycycline. First (in CASE REPORT section) the idea is conveyed that the suspected diagnosis was leptospirosis (which does not justify a 3-week course) and consideration of the diagnosis of tularemia was only made retrospectively when the case was reviewed and after the treatment course. Later (in DISCUSSION section) it’s not that clear – was the diagnosis of tularemia considered during the treatment course after all?

Answer: The patient completed the antibiotic treatment in outpatient setting. He was discharged in less than a week and several serological tests’ results were not available at the time of discharge. The medical team started reviewing the case and considering less frequent diagnosis when they received all the negative results from the previous serological tests. It was during this period, after considering tularemia as a possible diagnosis that it was decided to extend the antibiotic treatment up to 21 days.

* + The organization of the DISCUSSION section could be improved. The first paragraph covers well the under-recognition of tularemia in Portugal and mentions one of the facts that supports its existence in our country (previous detection in ticks and lagomorphs). However, another important fact is left to be mentioned in the third paragraph (seroprevalence study following outbreak in Spain and the subsequent study with PCR). The reviewer suggests that the information in these 2 paragraphs be merged, leaving another paragraph for diagnosis and treatment discussion and the 2 final ones (public health and final remark) as they are.

Answer: Thank you, suggestion accepted.

* + Why was not an acute phase sample sent for tularemia when the diagnosis was considered (at the same time the convalescence sample was sent)? Had the initial samples been eliminated already?

Answer: The hospital laboratory wasn’t able to store serum samples at the time due to technical issues. The initial samples had already been eliminated by the time the medical team suspected of tularemia.

* + Why was not the convalescent sample sent also for Brucella, Coxiella, Leptospira and Rickettsia, to rule out seroconversion?

Answer: Convalescent samples were also tested for those agents in the laboratory hospital and the results were negative. A reference to these results was added to the manuscript. It was not included initially due to word constraints.

* + Following the previous points, diagnosis limitations (recent versus past infection; cross-reactions; etc) should be discussed for this case, given that a single serological test does not allow a confirmed diagnosis – in particular, lines 17-20 in the DISCUSSION section should be reviewed.

Answer: Cross-reactivity was considered at the time of the diagnosis and all serological tests performed during the hospitalization were repeated.

* + In none of the literature provided it is stated that a negative follow-up serological test “confirms resolution of the acute infection”.
  + Most of the references provided (and the additional one suggested by the reviewer) state that MOST OFTEN the antibody titers decline slowly over time and persist for years after infection (and only rarely may be transient) – in that line of thought, a justification should be sought for their early disappearance in this case

Answer (to both points above): In this case, the test performed at INSA is a TAT, which according to the literature (including the one suggested by the reviewer), detects mainly IgM-type antibodies. Considering that the second test, performed six months after the infection (the previous date was incorrect, the sample admission’s date in INSA was confirmed during this revision), was negative, it suggests the resolution of the acute infection.

* + Why were not the animals fed on the contaminated food also tested/screened? Wouldn’t these be the most at risk to be contaminated?

Answer: The contaminated feed bags were destroyed (identified for lack of integrity), so they were not given to any animal.

* About REFERENCES:
  + In DISCUSSION, lines 21-22 – please check REFERENCE number 3. It does not seem (nor references 12 and 13) to say that the area with highest number of tularemia in Europe is NW Spain.

Answer: The references were reviewed and the mistranslation was corrected.

* + An important and updated reference on the serological diagnosis should have been included in the author’s research. Please find it attached at the website.

Answer: Thank you for the update, it was added as a reference in the text.