TRSTMH

Emergence or improved detection of Japanese encephalitis virus in the Himalayan highlands?

--Manuscript Draft--

Article Type:	Commentary
Full Title:	Emergence or improved detection of Japanese encephalitis virus in the Himalayan highlands?
Manuscript Number:	TRSTMH-D-15-00327R1
Order of Authors:	Matthew Baylis, BA, DPhil
	Christopher M Barker, Phd
	Cyril Caminade, PhD
	Ganesh R Pant, PhD
	Ajit Rayamajhi, PhD
	Bhoj R Joshi, PhD
	William K Reisen, PhD
	Daniel E Impoinvil, PhD

UNIVERSITY OF LIVERPOOL

Professor Matthew Baylis
Oxenhale Chair of Veterinary Epidemiology
Head, Department of Epidemiology & Population Health,
Institute of Infection & Global Health,
University of Liverpool.

Leahurst Campus
Chester High Road
Neston
Cheshire CH64 7TE

Telephone: 0151 794 6084
Facsimile: 0151 794 6028
Email: matthew.baylis@liv.ac.uk

Transactions of the Royal Society of
Tropical Medicine and Hygiene

30 November 2015

Dear TRSTMH

I am pleased to enclose a manuscript for consideration as a Commentary:
Emergence or improved detection of Japanese encephalitis virus in the Himalayan highlands?
Matthew Baylis, Christopher M. Barker, Cyril Caminade, Bhoj R. Joshi, Ganesh R. Pant, Ajit Rayamajhi, William K. Reisen & Daniel E. Impoinvil

The Commentary was prepared following an email I received from Shalene Singh-Shepherd, who had heard a talk I gave on the subject at the February 2015 ISNTD Bites meeting in London. I will append her email below. The article has never been submitted for publication elsewhere.

Kind regards

With best wishes,

MBaylis

Matthew Baylis

Email from Shalene Singh-Shepherd (11 May 2015):

Dear Dr Baylis,

You gave a very interesting talk at the ISNTD meeting in earlier this year. As your talk was based on a topic of great interest to the Royal Society of Tropical Medicine & Hygiene, I would like to invite you to submit a Commentary based around your presentation Transactions of the Royal Society of Tropical Medicine & Hygiene. We suggest that the Commentary could comment on the lack of coordination between medical and veterinary efforts to control Japenese Encephalitis and what could be done to improve the situation as we think that this topic would be of great interest to our readership.

Transactions of the Royal Society of Tropical Medicine and Hygiene is an official journal of the Royal Society of Tropical Medicine and Hygiene. It publishes authoritative and impactful original, peer-reviewed articles and reviews on all aspects of tropical medicine. The journal offers a respected voice for clinicians, health-related scientists, development organisations and students and our website can be viewed at http://trstmh.oxfordjournals.org/

Our commentaries are quite short, 1000 words in length with a 100 word abstract and 10 references. Benefits to publishing in the RSTMH journals include rapid peer-review and a short time to first decision, a short time from acceptance to publication online and appearance in a print issue of the journal, authors also benefit from an in-house team of expert copyeditors who will ensure the accepted paper is carefully edited and that the proof is properly prepared for publication. Also, authors publishing papers in the RSTMH journals have the choice of publishing their paper for free so that it can be viewed by all RSTMH Fellows, subscribers and pay-per-view customers or after acceptance can opt to publish their paper Open Access, making it freely available to all. This usually carries a fee of £1750–£2000, although some papers are eligible for a reduced fee or fee waiver (assessed on a case-by-case basis).

We encourage the inclusion of relevant and useful tables and figures. Authors should to view our "Instructions for authors" before submitting (http://www.oxfordjournals.org/our_journals/trstmh/for_authors/manuscript_instructions.html

If you do decide to submit a paper our online manuscript submission system can be found at http://www.editorialmanager.com/trstmh/default.asp

We would be grateful for an initial response if you are interested in making a submission to us. We would be also happy to consider your research for publication in Transactions as it will fit well within the scope of our journal.

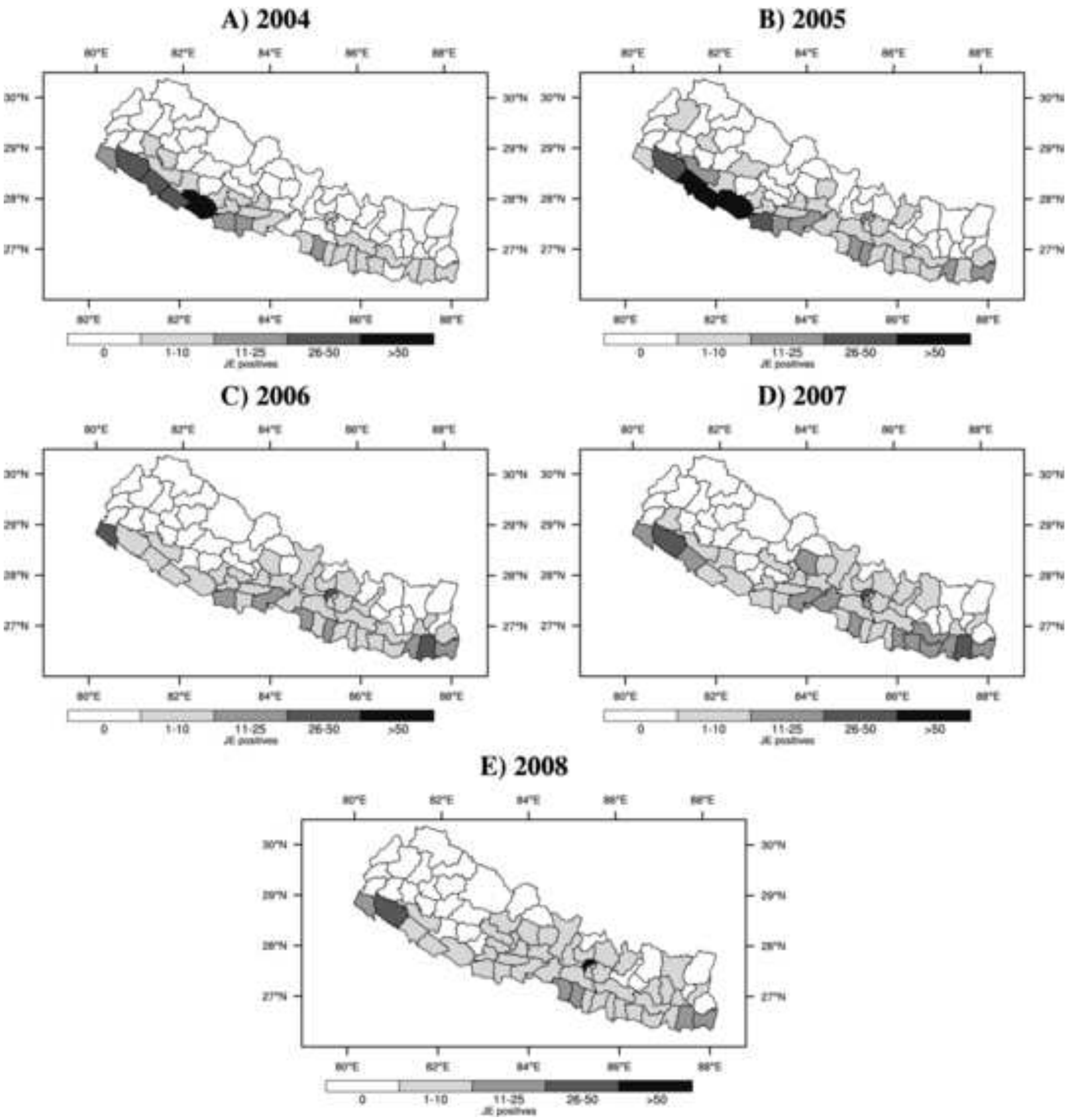
We look forward to hearing from you.

Best wishes
Shalene Singh-Shepherd

Departmental Contact
Janis Paine
j.paine@liv.ac.uk
Tel: +44 (0)151 795 6253

Figure

Click here to download Abstract fig1.jpg



Emergence or improved detection of Japanese encephalitis virus in the Himalayan highlands?

Matthew Baylis[a,f], Christopher M. Barker[b], Cyril Caminade[a], Bhoj R. Joshi[c], Ganesh R. Pant[d], Ajit Rayamajhi[a,e], William K. Reisen[b], Daniel E. Impoinvil[a]

a. Institute of Infection and Global Health, University of Liverpool, Liverpool, UK
b. Department of Pathology, Microbiology, and Immunology and Center for Vectorborne Disease, School of Veterinary Medicine, University of California, Davis, CA 95616, USA
c. Centre for Environmental and Agricultural Policy Research, Extension and Development (CEAPRED), Nayabato, Lalitpur, Nepal
d. Department of Animal Science, Veterinary Science and Fisheries, Agriculture and Forestry University, Chitwan, Nepal
e. Department of Pediatrics, National Academy of Medical Sciences, Kanti Children's Hospital, Maharajgunj, Kathmandu, Nepal
f. Health Protection Research Unit in Emerging and Zoonotic Infections, University of Liverpool, UK.

Abstract

The emergence of Japanese encephalitis virus (JEV) in the Himalayan highlands is of significant veterinary and public health concern and may be related to climate warming and anthropogenic landscape change, or simply improved surveillance. To investigate this phenomenon, a One Health approach focusing on the phylogeography of JEV, the distribution and abundance of the mosquito vectors, and seroprevalence in humans and animal reservoirs would be useful to understand the epidemiology of JE in highland areas.

Commentary

Japanese encephalitis (JE) is a zoonotic neuro-tropic vector-borne disease that remains a major cause of viral encephalitis in Asia [1]. The distribution of JE virus (JEV) transmission risk extends latitudinally from eastern Russia to the northern tip of Queensland, Australia and longitudinally from eastern Pakistan to Papua New Guinea. An important reason for JEV's persistence, despite the availability of an effective human vaccine since 1954,[1] is that the causative virus is sustained in an enzootic cycle and un-inoculated humans (especially children) are at continual risk from infectious mosquito bites. Maintenance of JEV transmission purportedly involves ardeids (herons, egrets) and rice-paddy mosquitoes, with epizootic transmission to pigs that amplify the virus and serve as a source of infection for peridomestic mosquitoes that infect humans. The mosquito *Culex tritaeniorhynchus* is the primary vector of JEV, due to its abundance throughout the JEV geographical range, catholic feeding habit which includes birds, pigs and humans, frequent infection during entomological surveys, and high competency in experimental infection studies.[1] Other mosquito species may be locally important. Infection in sows frequently results in abortion, whereas humans and horses are dead-end hosts, not contributing to transmission, but may suffer severe neurological disease.[1] The spill-over of virus from the pig epizootic cycle to humans and horses indicates the need for One Health approaches to integrate medical and veterinary measures for control.

Complicating measures to focus JE control are recent reports of its spread to higher elevations in the Himalayas. In Nepal and Tibet, China, studies suggest the recent emergence of JEV at elevations from 1,000 to 3,000 meters above sea level (a.s.l.).[2-4] These studies provide some evidence of recent infection in pigs and humans, with no reported history of movement from low elevation JE-endemic regions. *Culex tritaeniorhynchus* is well-established at lower elevations (< 2000 m a.s.l.), but has not been found at higher elevations (>2000 m a.s.l),[3] despite its ability to survive cold winters in diapause.[5] Although these studies document the presence of JEV at higher elevations, it is not clear if this represents the emergence of novel transmission at high elevation due to climate warming or the discovery of pre-existing seasonal transmission through extended surveillance.

Climate change in the Himalayas is a plausible explanation for JEV's spread to higher elevations. Warming trends would allow the upslope expansion of suitable climatic conditions for the vector; and also rice production, providing breeding sites for the vector, and pig production, providing amplifying hosts for JEV.[6, 7] Temperature increases are implicated as drivers of the spread of other vector-borne diseases to areas of higher elevation, such as malaria in Colombia and Ethiopia.[8]

Alternatively, JEV may have been present in highland regions for some time and is simply being detected because of improved disease surveillance, diagnostics and health systems. Without laboratory confirmation, JE is often classified as acute encephalitis syndrome (AES), where the disease aetiology of the patient is not specified. With better health services, JE may be detected more frequently, despite no actual change in disease incidence. For instance, reports of JE in the highlands of Nepal coincided with the introduction of AES surveillance in 2004 through a national sentinel surveillance network, conducted by the government of Nepal, and supported by the World Health Organization (WHO).[9] Similarly, the expansion of China's public health and research infrastructure also correspond to discoveries of JE in Tibet. Vaccination programs may also confound the perceived distribution of transmission. For example, the vaccination strategy of Nepal provided universal vaccination in high risk districts in the lowland ('Terai') regions, but only targeted vaccination in moderate risk, hill regions and no vaccination in the lowest risk, high elevation, mountain regions. The shift in spatial clustering of Nepal's JE cases, from highly endemic districts of Terai to the higher elevation Kathmandu Valley (Figure 1) after the mass vaccination in 2006,[10] could be attributed to the reduction of cases in high risk Terai districts through more intense vaccination relative to the other districts,[11] as well as to the natural temporal cycling of the virus.

Phylogeographic studies of JEV and its vectors collected from highland and lowland areas may be a useful way to identify recent and past pathogen transmission. Tools such as Bayesian Evolutionary Analysis Sampling of Trees (BEAST) are being used to estimate the time since emergence of JEV,[12] and could be applied to determine the origin of mosquito populations as well. Such an approach, if applied to JEV and vectors in highlands, may provide information on whether emergence is recent or recently discovered. Because JE is a zoonotic disease, investigations of pathogen transmission in animals, particularly birds, may be helpful in understanding emerging transmission. As birds are a reservoir for JEV, comparing sero-prevalence of birds from the lowland and highland regions may provide insights into the relative extent of transmission in these two regions. Such studies would also help bridge the gap between medical and veterinary control efforts and wildlife health.

In conclusion, several factors must be considered when addressing the recent emergence of JE at higher elevation in the Himalaya. It is currently not clear whether increased detection of JE in the highlands is due to change in environmental factors leading to its upslope spread, or is a consequence of extended surveillance. New tools such as phylogenetic analytical methods and sero-epidemiology may be useful in integrating medical and veterinary surveillance efforts to better understand the distribution of transmission. By developing a clear understanding of transmission, ministries of health can be better informed and prepared to deliver effective public health interventions.

Acknowledgement

This Commentary arose from discussions held at a 2014 workshop in Nagarkot, Nepal, funded by BBSRC UK-US Partnership Award (BB/K021389/1), *Emergence of Japanese Encephalitis in the highlands of Nepal,* to MB and DI.

References

1. Impoinvil DE, Baylis M, Solomon T; Japanese Encephalitis: On the One Health Agenda. In: Mackenzie JS, Jeggo M, Daszak P, et al.s (eds)*. One Health: The Human-Animal-Environment Interfaces in Emerging Infectious Diseases*. New York: Springer Berlin Heidelberg, 2013; 205-47.
2. Bhattachan A, Amatya S, Sedai TR, et al. Japanese Encephalitis in Hill and Mountain Districts, Nepal. Emerging Infect Dis 2009;15:1691-2.
3. Li Y-X, Li M-H, Fu S-H, et al. Japanese Encephalitis, Tibet, China. Emerging Infect Dis 2011;17:934-6.
4. Thakur K, Pant G, Wang L, et al. Seroprevalence of Japanese encephalitis virus and risk factors associated with seropositivity in pigs in four mountain districts in Nepal. Zoonoses Pub Health 2012;59:393-400.
5. Rosen L Overwintering mechanisms of mosquito-borne arboviruses in temperate climates. Am J Trop Med Hyg 1987;37:69S-76S.
6. Erlanger TE, Weiss S, Keiser J, et al. Past, Present, and Future of Japanese Encephalitis. Emerging Infect Dis 2009;15:1-7.
7. Keiser J, Maltese MF, Erlanger TE, et al. Effect of irrigated rice agriculture on Japanese encephalitis, including challenges and opportunities for integrated vector management. Acta Trop 2005;95:40-57.
8. Siraj AS, Santos-Vega M, Bouma MJ, et al. Altitudinal Changes in Malaria Incidence in Highlands of Ethiopia and Colombia. Science 2014;343:1154-8.
9. Wierzba TF, Ghimire P, Malla S, et al. Laboratory-based Japanese encephalitis surveillance in Nepal and the implications for a national immunization strategy. Am J Trop Med Hyg 2008;78:1002-6.
10. Impoinvil DE, Solomon T, Schluter WW, et al. The spatial heterogeneity between Japanese encephalitis incidence distribution and environmental variables in Nepal. PLoS ONE 2011;6:e22192.
11. Upreti SR, Janusz KB, Schluter WW, et al. Estimation of the impact of a Japanese encephalitis immunization program with live, attenuated SA 14-14-2 vaccine in Nepal. Am J Trop Med Hyg 2013;88:464-8.
12. Schuh AJ, Ward MJ, Brown A, et al. Phylogeography of Japanese encephalitis virus: genotype is associated with climate. PLoS Negl Trop Dis 2013;7:e2411.

Figure captions

Figure 1: Number of lab confirmed cases of Japanese encephalitis at the district level for A) 2004, B) 2005, C) 2006, D) 2007 and E) 2008. See [10] for more information on the clinical data.

TRSTMH-S-15-00382
Response to referees

Editor's comments:
Thank you for submitting your Commentary manuscript to us for consideration. Having read the paper and the reviewers' comments I am pleased to invite you to revise your manuscript in line with the comments from the reviewers. Please ensure that you provide a point-by-point response to each of the points raised by the reviewers. I should also mention that Reviewer 3 has provided comments on the PDF of the manuscript itself. To access the attachment please login and view it via the action links for your paper. Please can you also provide a response to each of the points raised by Reviewer 3 on the PDF of the manuscript and upload it as an attachment when submitting the revised version of your manuscript. Please get in touch if you have any problems accessing the PDF with Reviewer 3's comments and we can send it to you separately.

When revising your paper I would be grateful if you could use the track changes function so that it is easy to see what amendments have been made; not doing so might delay the assessment of the revised paper.

In addition to the reviewers' comments, please could you address the following journal style points in the revised paper:
1) Please can you include the author statements section at the end of the main text, before the reference list (see our instructions for authors for details: http://www.oxfordjournals.org/our_journals/inthealth/for_authors/manuscript_instructions.html). These should include in the following order: Authors' disclaimers (if required); Authors' contributions (including details of a guarantor for the paper); Acknowledgements (if required); Funding (if none state 'None'); Competing interests (if none state 'None declared'); Ethical approval (if not relevant state 'Not required').
RESPONSE: DONE

Reviewers' comments:

Reviewer #1:
I'm not convinced that this commentary enlightens anyone familiar with JEV research. It certainly appears to have missed a trick!
In the paper described by Li et al (2011) (ie ref no. 3) it states:
"653 mosquitoes were trapped in Mainling county (a hilly region) 75% of which were Armigeres obturba and none of which

were Cx. tritaeniorhynchus. However, 22% of the sampled human sera from this area were seropositive for JEV" (although they used a very stringent neutralisation test, ie PRNT90). In other words, Armigeres obturba might very well be a significant contributor to this apparent emergence of JEV in the higher regions.
RESPONSE: Li et al provide strong evidence of JEV transmission (to pigs and people) at high elevation (2900 m) but only weak evidence that Culex tritaeniorhynchus is not present. They surveyed for 10 days only, and provide no information on the sampling effort (number of traps, etc). They made two isolations of virus from mosquitoes (the second is reported in a separate paper in Plos NTD); both were made from Culex tritaeniorhychus, albeit from individuals caught at lower elevation.
In a separate study of JEV vectors in Yunnan province, China, Liu et al (DOI: 10.1089/vbz.2012.1016) reported infection rates of 13.2% in Culex tritaeniorhynchus, but only 0.7% in a species of Armigeres (albeit, not A. obturbans). On balance, it is possible that other vectors are involved in JEV transmission at high elevation, but further research is required to confirm this. We have, therefore, amended the text as follows (new text in italics):
Line 51-52 “Although these studies document the presence of JEV at higher elevations, and the possible involvement of other vector species, …”

I also felt that the authors should consider whether or not the apparent increase in JEV in higher regions is merely due to natural annual fluctuations in temperature, ie not "climate change". Are there any data to support this - if not this idea might be included in the type of studies required in the future.
RESPONSE – we think this question relates to ‘climate variability’, the natural changes in climate about the mean, on scales of weeks to decades (not just annual), versus ‘climate change’, long term change in the mean itself. An extensive analysis of weather station data in Nepal (https://practicalaction.org/file/region_nepal/ClimateChange1976-2005.pdf) found convincing evidence of long-term change in many climate variables over a 30 year period, suggestive of climate change. In the Executive Summary of the above report (page 6) it says “A general increasing trend in temperature has been found over Nepal. The maximum temperature was found to be increasing at a greater rate (0.05° C/year) than the minimum temperature (0.03° C /year).” There are several independent publications which support this, and further show pronounced regional warming over the central northern

districts where JE was reported to have emerged. The best is http://link.springer.com/article/10.1007/s12040-012-0257-8).

While there is fairly convincing evidence of climate change in the region we cannot, however, exclude the possibility of an additional effect of shorter term climate variability. We have therefore modified line 59 to now say "Climate change in the Himalayas or, possibly, the effects of shorter-term climate variability, are plausible explanations for the spread of JEV to higher elevations"

Line 34 change "un-inoculated" to "non-vaccinated"
RESPONSE. Done.

Lines 39 et seq: Poor English "frequent infection during entomological survey" does this phrase refer to the higher presence/detection of JEV in the surveyed (tritaeniorhynchus) mosquitoes when compared with other mosquito species (I suspect this is what the authors mean) or is it implying that the mosquitoes become infected during the entomological survey (presumably the authors do not mean this but the sentence could be interpreted this way) or could it be translated as implying that the scientists carrying out the investigation were frequently infected? I would recommend re-writing this sentence thus "Culex tritaeniorhynchus is considered to be the primary vector of JEV based on its higher abundance throughout the JEV geographical range, together with its catholic feeding habit, higher frequency of infection detected during entomological surveys and high vector competence, demonstrated in experimental infection studies".
RESPONSE. A sentence similar to this suggestion is now provided.

Line 40 I recommend the authors to cite reference number 3 at the end of the sentence "Other mosquito species may be locally important" Ref no. 3 demonstrates clearly that a significant proportion of humans (22%) were seropositive for JEV in Mainling County (Tibet) where no Cx tritaeniorhynchus (0/653 mosquitoes trapped) were captured during a mosquito-trapping session (also see my earlier comment on this).
RESPONSE. Done.

Line 54 change "for JEV's spread…." to "for the dispersion of JEV…."
RESPONSE. Changed to "for the spread of JEV.."

Lines 81 and 82 The authors state "As birds are a reservoir for JEV…….....". The authors should provide a robust reference to support this statement. For example has it been reliably

demonstrated that birds become viraemic in their natural habitats? If the answer is yes, do these birds become sick? I don't recall reading about bird die-off during JEV epidemics (this contrasts with West Nile virus and Usutu virus)! Do the authors know of any published evidence that JEV is carried in the natural environment as a transmissible virus by healthy or sick birds (ie not bats)?
RESPONSE. The classic studies are Buescher et al (1959) and Scherer et al (1959). Both address JEV infection in wild ardeids (egrets, herons) in their natural habitat in Japan. They provide evidence of both antibody responses to JEV, and viraemia. They do not report any clinical impact (disease) – just evidence of infection.
We do not have the space to include further references; however, ardeid infection is discussed in reference 1 and both these classic papers are cited by it, so we have added a citation to reference 1 here.

Line 85 correct me if I am wrong but I thought there was doubt as to whether or not JEV has emerged recently at higher elevation. I suggest the sentence should read "…..when addressing the apparent recent emergence……"
RESPONSE – DONE

Line 86 change "Himalaya" to "Himalayas"
RESPONSE – done.

Line 89 "to better understand" - ugh "to improve our understanding" does not have a split infinitive and scans more nicely.
RESPONSE – to improve clarity, this sentence has been changed to "Phylogenetic analytical methods, sero-epidemiological studies and integrated medical and veterinary surveillance efforts will improve understanding of the distribution of transmission"

Reviewer #2:
The commentary is well written and significant in terms of today's increasing JE case load in highlands.
The present day scenario should also be presented with respective figures, so that it would be easy to compare the distribution of JE in Nepal. The given figures are not clearly visible. Also the maps should depict the altitude of the locations showing the progression of JE from low lands to highlands. Meteorological data if any in these localities also can provide the base for interpretation in regards to the role of climatic change in these areas.
RESPONSE – unfortunately, we do not have ready access to the most recent data on JEV cases in Nepal, and so we are unable

to provide the present day scenario without a significant effort. We will do this if requested by the editor.
It is worth stressing that our paper addresses reports of JEV's emergence in the first decade of this century, and so we are happy to only present data for years in that period.
We have added a sixth panel (f Altitude) to the figure; this makes it easier to see that the progression of JEV in maps A to E was to higher elevation.
References should be reduced to 10.
RESPONSE – we have removed 2 references which were not specific to JEV. One has led to a small change in the text. The sentence "Temperature increases are implicated as drivers of the spread of other vector-borne diseases to areas of higher elevation, such as malaria in Colombia and Ethiopia (REF)" has been changed to "Temperature increases are implicated as drivers of the spread of other vector-borne diseases to areas of higher elevation, such as malaria in highland areas of South America and Africa".

Reviewer #3:
See comments on the manuscript PDF attached.
RESPONSES

COMMENT 1. This has been addressed already (4th response to Reviewer 1)

COMMENT 2. This is in regard to other examples of climate change driving vectors to higher elevation. We have already generalized the sentence somewhat (see 2nd response to reviewer 2) and lack space to add more examples.

COMMENT 3. Agreed, the expansion of rice production will also provide new feeding grounds for ardeid birds. We have amended the text accordingly.

Emergence or improved detection of Japanese encephalitis virus in the Himalayan highlands?

Matthew Baylis[a,f], Christopher M. Barker[b], Cyril Caminade[a, f], Bhoj R. Joshi[c], Ganesh R. Pant[d], Ajit Rayamajhi[a,e], William K. Reisen[b], Daniel E. Impoinvil[a]

a. Institute of Infection and Global Health, University of Liverpool, Liverpool, UK
b. Davis Arbovirus Research and Training, Department of Pathology, Microbiology, and Immunology, School of Veterinary Medicine, University of California, Davis, CA 95616, USA
c. Centre for Environmental and Agricultural Policy Research, Extension and Development (CEAPRED), Nayabato, Lalitpur, Nepal
d. Department of Animal Science, Veterinary Science and Fisheries, Agriculture and Forestry University, Chitwan, Nepal
e. Department of Pediatrics, National Academy of Medical Sciences, Kanti Children's Hospital, Maharajgunj, Kathmandu, Nepal
f. Health Protection Research Unit in Emerging and Zoonotic Infections, University of Liverpool, UK.

Abstract

The emergence of Japanese encephalitis virus (JEV) in the Himalayan highlands is of significant veterinary and public health concern and may be related to climate warming and anthropogenic landscape change, or simply improved surveillance. To investigate this phenomenon, a One Health approach focusing on the phylogeography of JEV, the distribution and abundance of the mosquito vectors, and seroprevalence in humans and animal reservoirs would be useful to understand the epidemiology of JE in highland areas.

Commentary

Japanese encephalitis (JE) is a zoonotic neuro-tropic vector-borne disease that remains a major cause of viral encephalitis in Asia~~[1]~~.[1] The distribution of JE virus (JEV) transmission risk extends latitudinally from eastern Russia to the northern tip of Queensland, Australia and longitudinally from eastern Pakistan to Papua New Guinea. An important reason for JEV's persistence, despite the availability of an effective human vaccine since 1954,[1] is that the causative virus is sustained in an enzootic cycle and ~~un-inoculated~~non-vaccinated humans (especially children) are at continual risk from infectious mosquito bites. Maintenance of JEV transmission purportedly involves ardeids (herons, egrets) and rice-paddy mosquitoes, with epizootic transmission ~~to~~ among pigs that amplify the virus and serve as a source of infection for peridomestic mosquitoes that infect humans. The mosquito *Culex tritaeniorhynchus* is considered to be the primary vector of JEV, due to its abundance throughout the JEV geographical range, catholic feeding habit which includes birds, pigs and humans, high frequency of~~t~~ infection ~~during~~ detected by entomological surveys, and high competency for virus transmission in experimental infection studies.[1] Other mosquito species may be locally important.[2] Infection in sows frequently results in abortion, whereas humans and horses are dead-end hosts, not contributing to transmission, but may suffer severe neurological disease.[1] The spill-over of virus from the pig epizootic cycle to humans and horses indicates the need for One Health approaches to integrate medical and veterinary measures for control.

Complicating measures to focus JE control are recent reports of its spread to higher elevations in the Himalayas. In Nepal and Tibet, China, studies suggest the recent emergence of JEV at elevations from 1,000 to 3,000 meters above sea level (a.s.l.).[2-4] These studies provide ~~some~~ evidence of recent infection in pigs and humans, with no reported history of movement from low elevation JE-endemic regions. *Culex tritaeniorhynchus* is well-established at lower elevations (< 2000 m a.s.l.), but has not been found at higher elevations (>2000 m a.s.l),[2] despite its ability to survive cold winters in diapause. [1]~~{Rosen, 1987 #3452}~~ Although these studies document the presence of JEV at higher elevations, and the possible involvement of other vector species, [2] it is not clear if this represents the emergence of novel transmission at high elevation due to climate warming or the discovery of pre-existing seasonal transmission through extended surveillance.

Climate change in the Himalayas~~s~~ or, possibly, the effects of short term climate variability, are~~is a~~ plausible explanations for the spread of JEV~~'s spread~~ to high~~er~~ elevations. Warming trends would allow the upslope expansion of suitable climatic conditions for the vector and virus replication within it; and also rice production, providing breeding sites for the vector and feeding grounds for ardeids;~~,~~ and pig production, providing amplifying hosts for JEV.[5,6] Temperature increases are implicated as drivers of the spread of other vector-borne diseases to areas of higher elevation, such as malaria in highland areas of South America and Africa.~~Colombia and Ethiopia.{Siraj, 2014 #3300}~~

Alternatively, JEV may have been present in highland regions for some time and is simply being detected because of improved disease surveillance, diagnostics and health systems. Without laboratory confirmation, JE is often ~~classified a~~grouped within~~s~~ acute encephalitis syndrome (AES) cases, where the disease aetiology of the patient is not specified. With better health services, JE may be detected more frequently, despite no actual change in disease incidence. For instance, reports of JE in the highlands of Nepal coincided with the introduction of AES surveillance in 2004 through a national sentinel surveillance network, conducted by the government of Nepal, and supported by the World Health Organization (WHO).[7] Similarly, the expansion of China's public health and research infrastructure ~~also~~ corresponded to discoveries of JE in Tibet. Vaccination programs may also confound the perceived distribution of transmission. For example, the vaccination strategy of Nepal provided universal vaccination in high risk districts in the lowland ('Terai') regions, but only targeted vaccination in moderate risk, hill regions and no vaccination in the lowest risk, high elevation, mountain regions. The shift in spatial clustering of Nepal's JE cases, from highly endemic districts of Terai to the higher elevation Kathmandu Valley (Figure 1) after the mass vaccination in 2006,[8] could be attributed to the reduction of cases in high risk Terai districts through more intense vaccination relative to the other districts,[9] as well as to the natural temporal cycling of JEV and its vectors~~the virus~~.

Phylogeographic studies of JEV and its vectors collected from highland and lowland areas may be a useful way to identify recent and past pathogen transmission and spread. Tools such as Bayesian Evolutionary Analysis Sampling of Trees (BEAST) are being used to estimate the time since emergence of JEV,[10] and could be applied to determine the origin of mosquito populations as well. Such an approach, if applied to JEV and vectors in highlands, may provide information on whether emergence is recent or recently discovered. ~~Because~~ As JE is ~~a~~ zoonotic ~~disease~~, investigations of pathogen transmission in animals, particularly birds and pigs, may be helpful in understanding emerging transmission. As birds are a reservoir for JEV,[1] comparing sero-prevalence of birds from the lowland and highland regions may provide insights into the relative extent of transmission in these two regions. Such studies would also help bridge the gap between medical and veterinary control efforts and wildlife health.

In conclusion, several factors must be considered when addressing the apparent recent emergence of JE at high~~er~~ elevation in the Himalayas. It is currently not clear whether increased detection of JE in the highlands is due to change in environmental factors leading to its upslope spread, or is a consequence of extended surveillance. P~~New tools such as p~~hylogenetic analytical methods, ~~and~~

sero-epidemiological studies and ~~y may be useful in~~ integrated~~ing~~ medical and veterinary surveillance efforts will improve understanding of ~~to better understand~~ the distribution of transmission. By developing a clear understanding of transmission, ministries of health can be better informed and prepared to deliver effective public health interventions.

Authors' contributions

MB conceived the study; DI drafted the manuscript with help from MB and WK; CC prepared the figure; all other authors critically revised the draft. All authors read and approved the final manuscript. MB and DI are guarantors of the paper.

~~Acknowledgement~~Funding

This Commentary arose from discussions held at a 2014 workshop in Nagarkot, Nepal, funded by BBSRC UK-US Partnership Award (BB/K021389/1), *Emergence of Japanese Encephalitis in the highlands of Nepal,* to MB and DI. CC acknowledges support by The Farr Institute for Health Informatics Research (MRC grant: MR/M0501633/1).

Formatted: Font: (Default) +Body (Calibri), 12 pt, English (United Kingdom)

Competing interests

Formatted: Font: Bold

None declared

Ethical approval

Not required

References

1. Impoinvil DE, Baylis M, Solomon T; Japanese Encephalitis: On the One Health Agenda. In: Mackenzie JS, Jeggo M, Daszak P, et al.s (eds). *One Health: The Human-Animal-Environment Interfaces in Emerging Infectious Diseases*. New York: Springer Berlin Heidelberg, 2013; 205-47.
2. Li Y-X, Li M-H, Fu S-H, et al. Japanese Encephalitis, Tibet, China. Emerging Infect Dis 2011;17:934-6.
3. Bhattachan A, Amatya S, Sedai TR, et al. Japanese Encephalitis in Hill and Mountain Districts, Nepal. Emerging Infect Dis 2009;15:1691-2.
4. Thakur K, Pant G, Wang L, et al. Seroprevalence of Japanese encephalitis virus and risk factors associated with seropositivity in pigs in four mountain districts in Nepal. Zoonoses Pub Health 2012;59:393-400.
5. Erlanger TE, Weiss S, Keiser J, et al. Past, Present, and Future of Japanese Encephalitis. Emerging Infect Dis 2009;15:1-7.
6. Keiser J, Maltese MF, Erlanger TE, et al. Effect of irrigated rice agriculture on Japanese encephalitis, including challenges and opportunities for integrated vector management. Acta Trop 2005;95:40-57.
7. Wierzba TF, Ghimire P, Malla S, et al. Laboratory-based Japanese encephalitis surveillance in Nepal and the implications for a national immunization strategy. Am J Trop Med Hyg 2008;78:1002-6.
8. Impoinvil DE, Solomon T, Schluter WW, et al. The spatial heterogeneity between Japanese encephalitis incidence distribution and environmental variables in Nepal. PLoS ONE 2011;6:e22192.
9. Upreti SR, Janusz KB, Schluter WW, et al. Estimation of the impact of a Japanese encephalitis immunization program with live, attenuated SA 14-14-2 vaccine in Nepal. Am J Trop Med Hyg 2013;88:464-8.
10. Schuh AJ, Ward MJ, Brown A, et al. Phylogeography of Japanese encephalitis virus: genotype is associated with climate. PLoS Negl Trop Dis 2013;7:e2411.

Figure captions

Figure 1: Number of lab confirmed cases of Japanese encephalitis at the district level for A) 2004, B) 2005, C) 2006, D) 2007 and E) 2008. See [10] for more information on the clinical data. F) Altitude (metres).

Formatted: Font: Bold

Formatted: Space After: 0 pt

REVISED FIG 1

Click here to download Manuscript fig1 REVISED.jpg