



Scientific Advisory Board (SAB)

Opinion on the Plague Outbreak in Madagascar

- Conclusions of the SAB teleconference held on 20/10/2017 -

Participants:

GL PID-R		SAB Teleconference _ 20-10-2017 (Plague Outbreak Madagascar)			Confirmed	Attended
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I. Context reminder

- Upon request from the GloPID-R Chairs, the Scientific Advisory Board convened on October 20 of 2017 to provide an opinion on research needs in relation to the Plague outbreak in Madagascar.
- Cases of bubonic and pneumonic plague have been detected in several towns in Madagascar since August 2017. The outbreak of plague in Madagascar was reported to the World Health Organization (WHO) on 13 September 2017 after being detected on 11 September following the death of a 47-year-old woman from Fort Duchene in Soavinandriana Hospital from respiratory complications. After confirming pneumonic plague, the Directorate of Health and Epidemiological Surveillance (DVSSE) immediately carried out investigations. Case detections have increased markedly since late September.
- Although plague is endemic in Madagascar, this current outbreak is concentrated in densely populated urban centers (with almost 80% of the cases in the two largest cities of the country) and other non-endemic areas, where there is little experience in responding to plague. The beginning of the plague season is one month ahead of normal. In addition, a high number of reported cases are pneumonic. Pneumonic plague is highly transmissible from one person to another and, without appropriate treatment, can be rapidly fatal.
- Measures are being put in place to strengthen infection prevention and control within health care settings. But given the importance of the outbreak, its location in densely populated centers, its apparent specificities (reported ratio of pneumonic plague) and the high reliance on antibiotics to combat the outbreak when antimicrobial resistance has previously been reported in plague, an opinion was requested on research needs.

II. Preamble and caveats

- To assess research needs, the Scientific Advisory Board identified research gaps using the standard GloPID-R assessment framework, reaching out to colleagues or experts from their personal network (see **attached detailed assessment**).
- The SAB wishes however to outline that most of its members have little expertise in this specific area. In preparation of the meeting, its members reached out to several bacteriological experts to build a more secured opinion. But this limited expertise should be taken into account when reading the following opinion.

III. Opinion from the SAB

1. As a preamble, SAB members note that – based on the information currently available to them - there appear to be serious capacity problems regarding infection prevention, epidemiological investigation, and diagnostics.
2. In relation to these capacity problems, there is a high degree of uncertainty and significant unanswered questions regarding diagnosis and epidemiology, which require urgent attention. Specifically, is the high prevalence of pneumonic plague due to the insufficient quality of diagnostics, the prioritization in performed diagnostics (given an important backlog of untreated samples), initiation of a chain of human-to-human transmission, or has it arisen from some critical mutation? SAB members agree that this should be the first topic to clarify.
3. In the event that the high incidence of the pneumonic form and the relatively low incidence of mortality are confirmed, research of possible pathogen changes using genomic techniques is a priority. Otherwise, it is still necessary, but less urgent.
4. Therefore, the international community needs to be prepared to initiate research on pathogen strains and disease profile: if the current outbreak does not (only) reflect capacity problems, why is it different? Is there a bacterial factor to consider? Ecological changes that impact on the spread and/or evolution of *Yersinia pestis*? Collection and appropriate storage of samples and data should be ensured as soon as possible. This is all the more important as spread could favor the development of antimicrobial resistance.

5. Protocols for control procedures should be developed and tested in relation to specific transmission modes (e.g. re-burial and other local community practices).
6. The possibility for a phase 3 trial of a candidate vaccine should be considered.
7. More specifically, SAB members have listed and classified the following areas for research or on-site capture of information:

Key research gaps		
	Topic	Priority
1. Pathogen traits	<p>A – Explanations for high proportion of cases that present with the pneumonic form, transmissibility of pneumonic form and apparently reduced mortality from pneumonic plague (NOTE: there are currently significant unanswered questions regarding diagnosis and epidemiology which need urgent attention. In the event that the high incidence of the pneumonic form and the relatively low incidence of mortality are confirmed, research of possible pathogen changes using genomic techniques is a priority; otherwise it is still necessary, just less urgent).</p> <p>A – Ensure collection and appropriate storage of well-pedigreed isolates (ie linked to appropriate meta-data)</p> <p>A – Need for continued screening studies to investigate (evolution of) antibiotic resistance among human and animal hosts/reservoirs</p>	A
2. Disease profile	<p>A – Need to identify if something has fundamentally changed with this outbreak, re Case fatality rate (lower) and pneumonic presentation (higher). Are case definitions applied uniformly?</p> <p>C – Detection of primary sources of infection (also for pneumonic plague)</p>	A
3. Epidemiology	<p>A – Need to identify the drivers for the current outbreak: is there any evidence of changes in the disease ecology that could explain the size of the outbreak and the unusually high percentage of pneumonic plague?</p> <ul style="list-style-type: none"> - Host/vector screening for the presence of <i>Y. pestis</i> to enable more effective host/vector control - Estimation of attack rates in different groups of the population - Although potentially difficult to accomplish in ‘real time’, isolates should be carefully collected with links to full clinical & demographic data, in order to: <ol style="list-style-type: none"> -1. Implement molecular epidemiological studies to detect transmission chains and importance of different sources of infection - 2. Do Comparative genome analysis against historic strains - 3. Assess possible Adaptive microevolution 	A
4. Pathogenesis/host response	<p><i>Depending on investigation:</i></p> <p>A – if high prevalence of pneumonic form confirmed and unrelated to diagnostics backlog and bias</p> <p>C – if not</p> <ul style="list-style-type: none"> - Comparative studies of resistant and non-resistant rat population to identify potential resistance markers - Identification of virulence factors (pathogen and host factors) 	A or C
5. Immune response	<p>B – In order to obtain in depth information on immune responses during natural infection, collection (and optimal storage) of appropriate samples and clinical data should be implemented ASAP (A priority) , even if the research may be done later. Vaccines exist, but are considered to be suboptimal in terms, and such sampling would be needed to study antibody dynamics and (other) correlates of immunity</p>	B
6. Diagnosis and tracking	<p>A – Validation of rapid F1-antigen detection assay</p> <ul style="list-style-type: none"> - Validation of assays for antibody detection 	A

Key research gaps		
	Topic	Priority
	<ul style="list-style-type: none"> - Development of an assay using a second, different target from F1 - Laboratories with diagnostic skills and standardized procedures are needed - Development of a more selective medium for cultivation of <i>Y. pestis</i> 	
7. Treatments	<p><i>Only of interest if resistance proved, which seems unlikely</i></p> <ul style="list-style-type: none"> A – Detection of antibiotic resistance - If resistance, identification of determinants of treatment failure 	A or C
8. Transmission intervention	<ul style="list-style-type: none"> A – Possibility for a phase 3 trial for a candidate vaccine as an intervention? A – Protocols for control measures to be implemented and assessed - Assessment of effectiveness of post exposition prophylaxis (PEP) - Assessment of effectiveness of personal protective equipment (PPE, e.g. surgical masks) B – resistance of fleas to insecticides potentially a major problem but not particularly for this outbreak 	A
9. Prevention	<p>Strategies to reduce the burden of disease structurally, based on knowledge on disease ecology in relation to climate</p> <ul style="list-style-type: none"> A - Host/Vector control - Implementation of timely surveillance C – development of a vaccine 	A
10. Cultural aspects	<ul style="list-style-type: none"> A – unsafe burial practices may have contributed to rapid spread (30+ cases from 1 high-risk burial) B – Investigation of acceptance of contact tracing and isolation methods in the population - Investigation of acceptance of alternative and safe burial practices - Knowledge, attitude, belief and practice studies of health care professionals and general population with respect to plague 	A
11. Behavioral aspects	<i>As above</i>	A
12. Implementation & evaluation of control measures	<ul style="list-style-type: none"> A – Facilities to isolate and antibiotics to treat patients are needed - Evaluation of strategy to identify and find contacts in a timely manner - Protocols for control measures to be implemented and assessed B – Resistance of fleas may make vector control measures much more difficult 	A B
13. Critical infrastructure needs	<ul style="list-style-type: none"> A – Ability to do rapid trials. Sufficiently trained healthcare workers, facilities, PPEs and antibiotic supplies. Sequencing and bioinformatics capacity. Accurate and practicable diagnostics for plague. Appropriate Storage facilities and well-pedigreed clinical isolates (ie linked to metadata (patient and epi data) - Pest control capacity? 	A

Priority A areas of research are areas of research that funders should fund immediately or information of utmost importance that should be captured immediately during an outbreak even if it is only needed for longer-term research.

Priority B areas of research are areas of research relevant to outbreak preparedness, but with a longer-term horizon.

Priority C areas of research relate to other important questions, but that need not be addressed immediately.

Framework for systematic assessment of research gaps

Topic: **Plague outbreak in Madagascar**

Compilation of contributions from the following SAB members: Felicity Burt, Julio Vazquez Moreno (by word of Pedro Anda Fernandez), Marion Koopmans, Jodie McVernon, Oscar Daniel Salomon, Tania Sorrell, Brian Ward, and Lothar Wieler.

Prepared the 22/10/2017

Background, short summary of situation

SitRep for GLOPID-R-Meeting (as of 19.10.2017, 11:00 o'clock):

The current outbreak of plague, primarily pneumonic plague, in Madagascar is very worrisome. In the most recent three WHO External Situation Reports, spanning little more than one week from 9th through 17th October, the total case numbers since August more than doubled from 387 via 684 to 849, including 67 deaths for a for a total case-fatality-ratio of 7.9%. The WHO continues to rate the outbreak as a "grade 2" emergency, on a scale of 1 through 3.

These total case numbers have, however, to be interpreted with caution, because more than half of them are suspected cases without laboratory evidence and based on a rather soft clinical case definition. They may or may not actually be cases of plague. PCR-Laboratory confirmed cases make up less than 10% of the total cases, but their number also doubled during this period; the number of deaths increased by 50%. Thus even if the total size of the outbreak is in doubt, the dynamic increase in recognized confirmed cases, and deaths within just over one week is a fact.

Compared to previous seasonal plague outbreaks in Madagascar, what sets this outbreak apart is

- that case numbers are very high early in the "season", normally between September and April,
- the high percentage of pneumonic as compared to bubonic plague: 67% in most recent report, and
- the large geographic area affected: 18 out of 22 regions in the country have cases, including large cities in the highlands and on the coast, such as the capital Antananarivo and the largest port city Toamasina. Institut Pasteur in France has now discounted a suspected case in a traveller on the Seychelles. Thus, so far no exported case has been confirmed.

The Government of Madagascar, the local Pasteur Institute, WHO and partners through the GOARN-network have recently scaled up their response to the outbreak and have put in place many structures and resources necessary to combat plague transmission on the island and to prevent the export of cases. Just last week, the WHO published a comprehensive plague response plan.

It likely will take some time to bring this outbreak under control, and to prevent future such outbreaks, the island may require help for years to come regarding the natural plague foci in the wild. In terms of research needs, this outbreak is at once reason-for, as well as opportunity-for public-health-oriented research on plague.

The outbreak reportedly does not seriously affect other sectors such as transport, energy, banking, telecommunications, security, etc. However, as these sectors are important to support the health response, a coordinated multi-sectorial response has been activated.

Note: current medicines stocks are only sufficient to treat 5,000 cases (UN sit rep Oct 10, 2017).

Specific questions that GLOPID-R members raised are:

- Are there any obvious genomic differences between this outbreak strains and endemic strains? Are the same strains causing bubonic and pneumonic manifestations, and how do they relate to strains being carried by vectors? – ie to what extent might a ‘new’ strain become endemic in the reservoir?
- Are there identifiable links between the urban areas where the outbreak is most intense and the more remote regions where cases have been identified (i.e. transport routes, types of workers etc.) that might inform movement restrictions within the country?
- What is the effectiveness of PPE in reducing person-person, nosocomial transmission?
- What impact does antibiotic treatment have on infectiousness of cases?
- What is the main mechanism of vaccine action i.e. on acquisition, severity, and transmission?
- Who might be targeted for immunization given these mechanisms?

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Check which aspects apply

Problem	Hosts					Transmission routes									
	Human	Livestock	Wildlife	vectors	Ecology	Person-to-person					Food/water/sewage	Foodborne zoonotic	Environment	Vector-borne	
						contact	airborne	Fecal oral	sexual	bloodborne					
Factors to consider	X		X	X	X	X	X Pneu- monic					x	x	x	X

Caption

Scale of priorities	
Priority A	A - Immediate/urgent research Or - Information of utmost importance to be captured immediately
Priority B	B - Research relevant to outbreak preparedness, but with a longer-term horizon
Priority C	C - Other important questions, but that need not be addressed immediately

Summary of key research gaps		
	Topic	Priority
CAPACITY BUILDING	As a preamble, SAB members note that – based on the information currently available to them - there appear to be serious capacity problems regarding infection prevention, epidemiological investigation, and diagnostics. When recommending urgent outbreak research, research activities should not further compromise the immediate response actions. However, there is a clear need (and opportunity) for some essential research provided these challenges can be solved.	
1. Pathogen traits	A – Explanations for high proportion of cases that present with the pneumonic form, transmissibility of pneumonic form and apparently reduced mortality from pneumonic plague (NOTE: there are currently significant unanswered questions regarding diagnosis and epidemiology which need urgent attention. In the event that the high incidence of the pneumonic form and the relatively low incidence of mortality are confirmed, research of possible pathogen changes using genomic techniques is a priority; otherwise it is still necessary, just less urgent).	A

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	<p>A – Ensure collection and appropriate storage of well-pedigreed isolates (ie linked to appropriate meta-data)</p> <p>A – Need for continued screening studies to investigate (evolution of) antibiotic resistance among human and animal hosts/reservoirs.</p>	
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9. Prevention	<p>Strategies to reduce the burden of disease structurally, based on knowledge on disease ecology in relation to climate</p> <ul style="list-style-type: none"> A - Host/Vector control - Implementation of timely surveillance C – development of a vaccine 	A
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Summary of key research gaps		
Topic		Priority
	B – Resistance of fleas may make vector control measures much more difficult	
13. Critical infrastructure needs	<p>A – Ability to do rapid trials. Sufficiently trained healthcare workers, facilities, PPEs and antibiotic supplies. Sequencing and bioinformatics capacity.</p> <p>Accurate and practicable diagnostics for plague.</p> <p>Appropriate Storage facilities and well-pedigreed clinical isolates (ie linked to metadata (patient and epi data)</p> <p>- Pest control capacity?</p>	A

Check which aspects have been reviewed

Problem	Hosts						Transmission routes									
	Human	Livestock	Wildlife	Vectors	Ecology	Unkno wn	Person-to-person					Food/water /sewage	Foodborne zoonotic	Environment	Vector borne	
							Contact	airborne	Fecal oral	sexual	bloodborne					
Factors to consider	X			X				pneumo nic								X
Pathogen traits	X		X	X			X	X								X
Disease profile	X						X	X								
Epidemiology	X		X	X	X		X	X								X
Pathogenesis / host response	X		X													
Immune response	X															
Diagnosis and tracking	X															
Treatments	X							X								
Transmission intervention	X						X	X								X
Prevention	X		X	X	X											
Cultural aspects/barriers	X						X									
Behavioral aspects/barriers	X						X	X								

Problem	Hosts						Transmission routes								
	Human	Livestock	Wildlife	Vectors	Ecology	Unkno wn	Person-to-person					Food/water /sewage	Foodborne zoonotic	Environment	Vector borne
							Contact	airborne	Fecal oral	sexual	bloodborne				
Implementation /evaluation of control measures	X		X	X											X
Critical infrastructure needs	X		X	X											X

Summary of findings**1. Pathogen traits**Essential knowledge

Plague is caused by the gram negative, rod-shaped bacterium *Yersinia pestis*. It is able to infect humans as well as a wide range of mammals, mostly rodents but also domestic animals like cats and dogs. The developed disease is rapidly progressing and often fatal. Plague bacteria are characterized by their ability to survive for several months in cool and moist conditions and to persist in rodent population without causing excessive deaths.

In theory, *Yersinia pestis* is a well-known organism unless it has acquired new antibiotic resistance pattern (unlikely), a novel tropism or changed its virulence.

Existing work has looked at key virulence determinants (one example referenced below), there is a framework within which to assess pathogen traits and their mapping to the genome.

A less virulent organism might be self-cured in the bubonic form but still recognized in pneumonic form. This might also explain the lower mortality rate for what appears to be pneumonic disease (35% seems low by historical standards).

Relatively small numbers of *Y. pestis* genomes have been sequenced but, in general, reveal small changes in SNPs (microevolution).

Key knowledge gaps

What seems remarkable is the high prevalence of pneumonic plague, compared to what would be expected. That raises questions about possible explanations. Is this the classical "high density urban" form of a zoonotic disease, related more to the demographic changes as we saw for Ebola for instance, or is there a bacterial factor to consider? Is the present predominance of pneumonic plague due to initiation of a chain of human-to-human transmission, or has it arisen from some critical mutation or as an artifact of diagnostic backlog and bias (i.e. testing more severe cases first)? The early onset of the season raises additional concerns about the latter. Examination of the 2015 outbreak suggests that there were multiple endemic circulating strains and that those responsible for human disease varied from year to year.

There is a need for comparative analysis of properties of traits that are relevant for transmissibility in in vitro- and animal models.

Genomic relatedness of current outbreak strain to:

- Historical isolates;
- Concurrent strains causing bubonic manifestations;
- Concurrent strains circulating in rodents/vectors – widespread geographic distribution and concurrent bubonic/pneumonic cases suggest wide environmental spread

Is there any evidence of antimicrobial resistance that would render standard therapies ineffective? Knowledge about molecular dynamics (changes at the level of genetic information) of *Y. pestis* during outbreaks is missing. It has been shown that the mutation rate increases during

1. Pathogen traits

outbreaks/epidemics (Achtman/Cui et al) but effects of these changes with respect to changes of virulence are unknown.

Enforcement of strain isolation from plague patients, particularly during outbreaks followed by subsequent comparative genome analysis could shed light on adaptive microevolution of *Y. pestis*.

In this respect the potential of *Y. pestis* to develop antibiotic resistance also is of utmost importance. In 1995, high-level resistance to antimicrobial agents, including to all of the drugs recommended for plague prophylaxis and therapy, was observed in a clinical isolate of *Y. pestis* from Madagascar (Galimand 2006). This strain exhibited high-level resistance to eight antimicrobial agents, including not only those recommended for therapy (streptomycin, chloramphenicol, and tetracycline) and prophylaxis (sulfonamide and tetracycline) of plague but also drugs used as alternatives to classical therapy, such as ampicillin, kanamycin, and spectinomycin.

More recently, another plasmid-mediated doxycycline resistant *Yersinia pestis* strain isolated from a rat from the capital was analyzed (Cabanel et al; 2009) together with a further streptomycin resistant strain. The fact that the three antibiotic-resistant Malagasy *Y. pestis* strains were isolated from different hosts, at different times, from distant locations, and carried unrelated plasmids indicates independent horizontal acquisition of genetic material and further demonstrates the capacity of *Y. pestis* to acquire antibiotic resistance plasmids under natural conditions. Since these resistance plasmids can frequently carry or easily trap antibiotic resistance cassettes, the emergence of new MDR *Y. pestis* strains may be expected and would represent a major health threat. As the rise of resistance at least at vector level seems associated with horizontal transference from other pathogens, the mapping of resistant strains of main resistant pathogens that could co-circulate by overlapping in time and space could focus the search and assess the likelihood of a resistance event.

The real prevalence of resistant *Y. pestis* in the natural host (rodents) and vectors (fleas) is unknown and need to be investigated. We also do not know whether the level of antibiotic resistance is stable or dynamically increasing. Therefore host/vector screening studies are necessary.

Detection of unusual antibiotic resistances (currently no evidence)

Molecular surveillance of outbreak strain (probably done by the Pasteur Inst)

Phylogenetic characterization (probably done by the Pasteur Inst)

F1 capsule antigen negative strains (unlikely).

Although it is not known if this outbreak is caused by genetic mutation(s) in the bacterium this is not the highest priority for research though it is clearly of interest.

References

WHO SitRep #3, associated fact sheet

Ramasindrazana et al Pneumonic plague transmission in Moramanga, Madagascar, 2015. EID 2017 Senior NJ et al, An integrated computational-experimental approach reveals *Yersinia pestis* genes essential across a narrow or broad range of environmental conditions; BMC Microbiology 2017; 17:163

Cabanel et al. Plague Outbreak in Libya, 2009, Unrelated to Plague in Algeria; Emerg Infect Dis.

<https://www.avma.org/KB/Resources/FAQs/Pages/Plague>

1. Pathogen traits

<https://www.cdc.gov/plague>

Cabanel N, Bouchier C, Rajerison M, Carniel E. Plasmid-mediated doxycycline resistance in a *Yersinia pestis* strain isolated from a rat. *Int J Antimicrob Agents*. 2017. doi: 10.1016/j.ijantimicag.2017.09.015.

Welch TJ, Fricke WF, McDermott PF, White DG, Rosso ML, Rasko DA, Mammel MK, Eppinger M, Rosovitz MJ, Wagner D, Rahalison L, Leclerc JE, Hinshaw JM, Lindler LE, Cebula TA, Carniel E, Ravel J. Multiple antimicrobial resistance in plague: an emerging public health risk. *PLoS One*. 2007;2: e309

Drancourt, M. *Clin Microbiol Infect* 2012; 18: 224-230

2. Disease profile (check with WHO/ECDC RA)

Essential knowledge (for each host)

Why does it appear that so many of the cases appear to be the pneumonic form?

Further to the above, are we confident that this outbreak is predominantly pneumonic in manifestation because of the route of acquisition, or does it represent a hyper-invasive organism with more rapid progression to the pneumonic form? Clarification of this issue is important to understand the dominant transmission mechanism to be interrupted, and also elements of pathogenesis. Such data needs to be combined with genomic/other epidemiologic studies

Humans:

Incubation periods range from 24 hours to 7 days and first symptoms are high fever, headache and general weakness followed by symptoms dependent on the clinical form of plague:

- Pneumonic plague: shortness of breath, coughing, bloody or watery sputum
- Bubonic plague: painfully swollen lymph nodes
- Septicemia: coagulopathies and potentially multi-organ failure. Gangrene may occur in untreated septicemic plague patients.

Animals:

Symptoms often unspecific and might include can include lethargy, signs of depression, anorexia, vomiting, diarrhea, dehydration, fever, enlarged or abscessed lymph nodes.

Key knowledge gaps

Historically the bubonic: pneumonic ratio is at least 6 but appears to be much lower lower in the current outbreak. The case fatality rate for pneumonic plague also seems lower than expected.

Are these results due to under-ascertainment of cases in previous outbreaks or specific for this outbreak? Are the current observations due to some type of bias (ie higher reporting of pneumonic cases) missed cases of bubonic form or something systematic (e.g. burial practices)?

Is the assumption that pneumonic plague has a shorter incubation correct in this instance, as this parameter is critical to determining the ability to interrupt transmission?

Any abnormalities in the clinical course or treatment response? Are deaths within the expected range given challenges in health sector access and resourcing?

2. Disease profile (check with WHO/ECDC RA)
<p>Detection of primary sources of infection (also for pneumonic plague)</p> <p>Investigation of different courses of disease</p> <p>Red flags for plague symptoms (e.g., indicator symptoms of severe courses and/or treatment failure)</p> <p>Further clarification of route of acquisition (where known) and clinical course of cases.</p>
<p><u>References</u></p> <p>WHO SitRep #3, associated fact sheet</p> <p>EMERGE recommendations</p> <p>https://www.cdc.gov/plague</p>

3. Epidemiology (check with WHO/ECDC assessments)
<p><u>Essential knowledge</u></p> <p>From 1 August to 12 October, 684 cases of plague - mainly pneumonic (474), and 57 deaths (case fatality rate 8.3 per cent) were reported in 35/114 health districts across the country. Note that bubonic and pneumonic cases are identified in each of the regions but most of these “cases” have not yet been confirmed microbiologically. The beginning of the plague season 2017-2018 was 1 month ahead of the normal start.</p> <p>With caveats about diagnosis, a high percentage of plague cases in this outbreak appears to be pneumonic plague.</p> <p>There is a need to ensure that the organism hasn’t significantly changed (antimicrobial resistance, novel tropism, new vector, new reservoir).</p> <p>The main host of <i>Y. pestis</i> in MDG is the house rat (<i>Rattus rattus</i>) with their fleas as vectors for transmission of <i>Y. pestis</i>. However, since rats are becoming more and more become resistant to <i>Y. pestis</i>-infection, there may be a novel natural reservoir. It is common knowledge that other rodents and animals can also be reservoirs for <i>Y. pestis</i> or transmit the disease to humans.</p>
<p><u>Key knowledge gaps</u></p> <p>Are drivers for the current outbreak known? Is there any evidence of changes in the disease ecology that could explain the apparent size of the outbreak?</p> <ul style="list-style-type: none"> • It would be of interest to have any further information about the spatiotemporal distribution of cases, to see whether any predominant modes of spread can be identified. These data may inform in-country travel restrictions (e.g. as in 2015 outbreak – admittedly much smaller, see ref); • Are the age groups of cases being affected typical? Is there any sex distribution of cases that may assist in inferring modes of transmission (note M>>F in 2015 although case numbers small)? • Estimation of epidemic parameters using mathematical models of plague infection transmission, for comparison with estimates from past outbreaks. Is this pathogen intrinsically more contagious, or has time to detection/social connectedness been the key to spread (as for the recent EBV outbreak?) • Review of epidemiologic modeling approaches to incorporate super spreader events associated with unsafe burials, as for EBV (see section 9)

3. Epidemiology (check with WHO/ECDC assessments)

Considering emergence in areas where no activity has been documented in recent years and, as in current outbreak, emergence in new area, are there ongoing surveillance programs, what are the risk factors for emergence, what is the capacity in low resource areas for surveillance?

Knowledge regarding reservoirs and activity between outbreaks?

Tools available for molecular epidemiology and genotyping. Understanding of kinetics of plague.

What are the determinants for high percentage of pneumonic plague in this (and other) outbreaks?

It is currently unclear which animals beside the house rat are involved in the transmission of the disease. Therefore potential hosts and vectors should be screened for the presence of *Y. pestis*. Once this knowledge is available, a more effective host/vector control will be possible.

Besides the climate, flea and potential rodent reservoirs, changes in the immune status of the population and socio-cultural aspects should be recorded to understand the factors that contribute to the evolution of the current outbreak, such as migration and transit patterns, urbanization-housing quality, crowding, location on new comers, changes in land use and rodent management in rural and urban areas.

References

Ramasindrazana et al Pneumonic plague transmission in Moramanga, Madagascar, 2015. EID 2017
Gani and Leach, Epidemiologic determinants for modelling pneumonic plague outbreaks. EID 2004

Vogler AJ, Chan F, Wagner DM, et al. Phylogeography and Molecular Epidemiology of *Yersinia pestis* in Madagascar. Picardeau M, ed. PLoS Neglected Tropical Diseases. 2011;5(9):e1319. doi:10.1371/journal.pntd.0001319.

Richard V, Riehm JM, Herindrainy P, et al. Pneumonic Plague Outbreak, Northern Madagascar, 2011. Emerging Infectious Diseases. 2015; 21(1):8-15. doi:10.3201/eid2101.131828.

Grácio AIDS, Grácio MAA. Plague: A Millenary Infectious Disease Reemerging in the XXI Century. Biomed Res Int. 2017; 2017:5696542. doi: 10.1155/2017/5696542

4. Pathogenesis/host response

Essential knowledge

Bubonic plague is usually the most common form of plague, caused by the bite of an infected flea

Bubonic plague can advance to the more severe septicaemic/pneumonic forms

Pneumonic plague can also be acquired by droplet spread from a pneumonic case

Incubation phase 1-7 days (shorter for pneumonic)

A less virulent organism might be self-cured in the bubonic form but still recognized in the pneumonic form. This might also explain the relatively low mortality of ~35%.

4. Pathogenesis/host response

Plague spreads from infected animals to humans primarily by flea because the plague bacteria multiply in the gut of the flea and are injected into the wound during the feeding process. Once inside the body, bacteria spread through the lymphatic vessels and an inflammation of lymph nodes occurs, which causes swelling (Bubonic plague). Bacteria may spread into the blood and endotoxin production leads to massive sepsis and necrosis (Septicemic plague). The lungs of patients may also be affected either by spreading of the bacteria into lung tissue during an infection or by inhalation of particles discharged by infected animals or humans (Pneumonic plague). Without treatment, fatality historically rates range from 50% for the bubonic form to almost 100% for the pneumonic plague, which is also the only form where a transmission from human to human is possible.

Key knowledge gaps

How important are fleabites to the aetiology of the pneumonic cases (i.e. are current cases predominantly human-human or is there also an increased likelihood of bubonic-pneumonic within host), i.e. are we certain that this is amplification via human-human droplet transmission or a more virulent form overall? Epi data tends to indicate the former.

Need clinical case definition and diagnostics to identify both symptomatic and asymptomatic/mild cases.

Rats as host/natural reservoir: Normally rats infected by *Y. pestis* will sicken and die. However, there is an increasing proportion of rats resistant to *Y. pestis* infection leading to an increased survival of infected rats and higher risk of transmission to humans. These animals represent novel natural reservoirs of *Y. pestis*, and are the source for human infections. The mechanism of resistance in rats is currently unknown. However, we know that rat populations with repeated contact to the pathogen develop resistance. Therefore comparative studies of resistant and non-resistant rat population are necessary to identify potential resistance markers. Genome sequencing and comparative genomics of rat populations could be used as an initial approach.

References

Tollenaere et al; Susceptibility to *Yersinia pestis* experimental infection in wild *Rattus rattus*, reservoir of plague in Madagascar. *Ecohealth*. 2010

<https://www.cdc.gov/plague>

<http://plague.emedtv.com/plague>

Andrianaivoarimanana V, Kreppel K, Elissa N, Duplantier JM, Carniel E, Rajerison M, Jambou R. Understanding the persistence of plague foci in Madagascar. *PLoS Negl Trop Dis*. 2013;7(11):e2382. doi: 10.1371/journal.pntd.0002382.

5. Immune response

Essential knowledge

Not applicable within outbreak time frame, but essential to collect consecutive specimens throughout the outbreak for later evaluation of (new) diagnostics and immune responses that inform vaccine development.

Relevant primarily in the context of the F1 vaccine under development.

When *Y. pestis* replicates within macrophages, F1 protein (fraction 1 antigen) is expressed and forms a capsule around the bacterium. This capsule increases bacterial resistance to engulfment by both

5. Immune response

macrophages and neutrophils. *Y. pestis* can cause a global depletion of NK cells and decrease the secretion of IFN- γ , resulting in a reduced production of reactive nitrogen intermediates by macrophages.

Further, it reduces the host adaptive immunity by both influencing the cytokine profile induction and acting directly on the immune cells involved in the adaptive immune responses.

Key knowledge gaps

Vaccine derived immunity – review of current/unpublished evidence, any suitable candidates? Recent low quality review highlights gaps

Is any vaccine ready for a field trial? At least one has undergone a phase 2b trial.

Long term: Are the immune correlates of protection well-defined for development of subunit vaccines.

Analysis of antibody dynamics

Investigation of correlates of immunity

References

Verma et al Plague Vaccine Development: Current Research and Future Trends (Review), *Frontiers in Immunology* 2016

<http://iaj.asm.org/content/76/5/1804.full>

6. Diagnosis and tracking (check with EVDlab)

Essential knowledge

Diagnostic capability – access to rapid tests, accredited lab(s)

Additional resources should be put into case finding.

Is the point-of-care test available in Madagascar? Is it sensitive enough to pick up self-curing (asymptomatic) cases? Is there a reference lab that can establish resistance patterns?

- Choice of clinical specimen for laboratory diagnosis
- Knowledge about antibiotic resistance patterns
- Case definitions (sensitivity and specificity of current definitions)
- Clinical manifestations (depending on the route of infection)
- Differential diagnosis

Preferred clinical specimen depend on the form of plague:

- Pneumonic plague: Blood culture, sputum, bronchial/tracheal lavage
- Septicemic plague: Blood culture
- Bubonic plague: Lymph node aspirate

Diagnostic tools include:

- Rapid antigen detection tests (RDT) targeting the F1-capsule
- Microscopy for bipolar stained gram negative bacteria (bipolar of “safety pin” appearance)

6. Diagnosis and tracking (check with EVDlab)
<ul style="list-style-type: none"> - Cultivation - Polymerase chain reaction - ELISA (Antibody detection)
<p><u>Key knowledge gaps</u></p> <p>With only about 10% of cases confirmed by laboratory tests, it is necessary to speed the confirmation of the rest of cases, especially by informative molecular tools, to increase our knowledge of the epidemiology of this outbreak</p> <p>Only one company in the US provides RDT (F1 capsule-based) for rapid on-site diagnostic of <i>Y. pestis</i>. In addition this test is not currently licensed for human diagnostics. The in-house RDT developed by the IP-MDG is not available to other parties. Since RDT on-site diagnostic is essential, the development and validation of such an assay with clinical material should be encouraged. This requires collection and storage of samples according to a protocol.</p> <p>Can whole genome sequencing be used to track secondary transmissions? Is there any evidence of adaptation to <i>Y. pestis</i> to respiratory spread in humans</p> <p>An assay using a second, different YP-specific target from F1 would be desirable.</p> <p>Development of a more selective medium for isolation of <i>Y. pestis</i>. The currently used medium (CIN agar) allows growth of many other bacteria.</p>
<p><u>References</u></p> <p>EMERGE recommendations</p>

7. Treatments (check with ISARIC, PREPARE)
<p><u>Essential knowledge</u></p> <p>Plague can be treated with specific antibiotics including aminoglycosides, fluoroquinolones and tetracyclines. The classical therapies are either gentamicin or streptomycin. The duration of treatment is 10 to 14 days, or until 2 days after fever subsides.</p> <ul style="list-style-type: none"> - PK/PD of antimicrobials used - Course of disease (with and without treatment) <p>Resistance profile of current strains, to ensure suitability of available stockpile/donor supplies.</p> <p>Are the anti-microbial resistance patterns what we would expect?</p> <p>Though <i>Y. pestis</i> is sensitive to a number of antibiotics, the rapid progression of the pneumonic form makes effective treatment difficult.</p>
<p><u>Key knowledge gaps</u></p> <p>Further pathogen characterization as in (1).</p> <p>What are the determinants for failure to respond to treatment?</p> <p>Detection of unusual antibiotic resistances (currently no evidence)</p>

7. Treatments (check with ISARIC, PREPARE)

Safety and efficacy of different treatment regimens (e.g., Prospective controlled data on Cipro vs streptomycin and Cipro + Streptomycin vs. streptomycin only)

Long term: vaccine development.

References

Roger D. Pechous et al.: Pneumonic Plague: The Darker Side of Yersinia pestis, In Trends in Microbiology, Volume 24, Issue 3, 2016

<https://www.cdc.gov/plague/healthcare/clinicians.html>

8. Transmission intervention

Essential knowledge

Short-term: Post exposition prophylaxis (PEP)

Long-term: Development of a vaccine

Infection control measures (isolation) for suspected/probable/confirmed cases

Containment of person-person spread is a key objective to reduce pneumonic-pneumonic cases. The CDC recommends that individuals suspected of having pneumonic plague be placed in isolation. Those with confirmed pneumonic plague should remain in isolation under droplet precautions until all laboratory cultures are negative.

WHO/US AID are strengthening infection prevention and control measures in hospitals (note at least 15 HCWs have contracted the infection to date)

Safe burial practices – being implemented as an intervention.

Availability of vector control and rodent control during outbreaks.

To what extent is outbreak driven by vector-reservoir dynamics or human crowding/behaviors?

Key knowledge gaps

Currently no licensed vaccine is available. There are several candidate vaccines at various stages of clinical trials. Are any of these candidates ready for an “emergency release” phase 3 intervention trial, similar to what was done during the last stages of the Ebola outbreak?

Are the pneumonic strains the same as those continuing to cause bubonic plague?

How do these relate to strains in rodents/vectors (i.e. how likely to cause ongoing spread even if human-human containment effective?) Are the fleas implicated in Madagascar’s outbreak rat or mouse origin? Are the fleas becoming resistant to pesticides?

Is the reproduction number on average higher than previously observed (see 3)?

What is the role of super-spreader events associated with unsafe burials in driving this epidemic? (Important to understand to determine how containable it is).

8. Transmission intervention

Protection of HCWs is of utmost importance. How effective is protective equipment (PPE, e.g. surgical masks)? Specifically, effectiveness of surgical masks to prevent small droplet spread? Any other PPE recommended? Here, the pressing need is to assess what is needed for proper implementation of infection control procedures and the possible barriers, including cultural barriers. The emphasis should be on applied research as opposed to theoretical studies.

References

Kumar Verma and Tuteja Plague Vaccine Development: Current Research and Future Trends Front. Immunol., 2016

<http://www.centerforhealthsecurity.org/our-work/publications/plague-fact-sheet>

9. Prevention (check with WHO R&D blueprint)

Essential knowledge

The following measures have proven useful in the prevention of plague:

- Rodent control
- Limit contact to potentially infected animals
- Vector control and use of repellents.

Rodent/vector controls are clearly challenging. To avoid both insect bites and contact with infected individuals, behavioral strategies are key.

Role of vaccine? (Covered in other sections).

Key knowledge gaps

Outbreaks are usually tackled with a fire-fighting approach. Teams move into an infected area to kill fleas with insecticides, treat human cases, and give chemoprophylaxis to exposed people. Many experts have argued that this crisis management approach is insufficient as the outbreak is likely to be on the wane by the time action is taken. Informed, pre-emptive decisions about plague management and prevention before outbreaks occur would certainly be more sustainable and cost-beneficial.

What are the main drivers for the recurring outbreaks in Madagascar, and is it possible to reduce the burden of disease structurally, based on knowledge on disease ecology in relation to climate?

Vaccine development. Currently no licensed vaccine is available.

- See also 8. Transmission intervention
- Host/Vector control
- Evaluation of timely surveillance under low resource conditions
- Measures to eliminate natural plague foci

References

CDC (<https://www.cdc.gov/plague/prevention/index.html>)

Giorgi E, Kreppel K, Diggle PJ, Caminade C, Ratsitorahina M, Rajerison M, Baylis M. Modeling of spatio-temporal variation in plague incidence in Madagascar from 1980 to 2007. *Spat Spatiotemporal Epidemiol.* 2016 Nov;19:125-135. doi: 10.1016/j.sste.2016.10.001. Epub 2016 Oct 18.

Vogler AJ, Andrianaivoarimanana V, Telfer S, Hall CM, Sahl JW, Hepp CM, Centner H, Andersen G, Birdsell DN, Rahalison L, Nottingham R, Keim P, Wagner DM, Rajerison M. Temporal phylogeography

9. Prevention (check with WHO R&D blueprint)

of *Yersinia pestis* in Madagascar: Insights into the long-term maintenance of plague. *PLoS Negl Trop Dis.* 2017 Sep 5;11(9):e0005887. doi: 10.1371/journal.pntd.0005887. eCollection 2017 Sep.

10. Cultural aspects

Essential knowledge

To what extent are cultural practices driving outbreak as well as pattern (eg: pneumonic >> bubonic)? Traditional burial practices appear to promote these outbreaks. Widespread traditional burial practices involving intervention with corpses a known transmission risk in Madagascar. Case description of a 31-year-old male who died on 27 August, 31 secondary cases including 4 deaths so clear evidence of superspreader events contributing to the epidemic’s time course.

Key knowledge gaps

None identified regarding implementation – WHO is focusing on safe and appropriate burial as a component of outbreak response.

Education and awareness.

Acceptance of alternative burial practices

- Investigation of acceptance of contact tracing and isolation methods in the population
- Investigation of acceptance of alternative and safe burial practices
- Knowledge, attitude, belief and practice studies of health care professionals and general population with respect to plague
- Knowledge on rodent management in urban and rural settlements, housing-crowding, and movements along the country.
- Knowledge about cultural practices that impact on spread and how to best elicit behavior change to reduce spread.

References

Ramasindrazana et al Pneumonic plague transmission in Moramanga, Madagascar, 2015. *EID* 2017
 ECDC Rapid risk assessment – Outbreak of plague in Madagascar, 9 October 2017

11. Behavioral aspects

Essential knowledge

See above

Traditional burial practices promote these outbreaks

Key knowledge gaps

Education and awareness.

Acceptance of alternative burial practices

11. Behavioral aspects

Population-based studies of the knowledge, attitudes and practices of health care professionals with respect to plague are clearly required. Such studies would make it possible to identify the sociocultural and technical factors responsible for the persistence of such a high CFR, despite the existence of a long-standing, established control programme.

References

Migliani et al., Epidemiological trends for human plague in Madagascar during the second half of the 20th century: a survey of 20 900 notified cases, Tropical medicine and international health, 2006

12. Implementation and evaluation of control measures

Essential knowledge

Multiple interventions being implemented across sectors, it will be challenging to determine which are most effective.

Outstanding query about the role of vaccines given the widespread distribution of this outbreak

See pest control above – resistance in fleas?

Facilities to isolate patients are needed.

Key knowledge gaps

Vaccines – multiple candidates in development, current live whole cell vaccines reactogenic and of limited efficacy. Any front line candidates appropriate?

Diagnostic and surveillance laboratories in low resource areas.

Unknown if there are sufficient facilities

Facilities to isolate and antibiotics to treat patients are needed

Evaluation of strategy to identify contacts in a timely manner

References

Low quality review but covered the field: Verma et al Plague Vaccine Development: Current Research and Future Trends (Review), Frontiers in Immunology 2016

Stenseth et al. Plague: past, present, and future. PLoS Med. 2008

13. Critical infrastructure needs

Essential knowledge

Limited health sector resources, Red Cross, MSF, other NGOs supporting international response.

Availability of point-of-care diagnostics as well as reference lab capacities.

Capacity to isolate and cryopreserve bacteria throughout the outbreak for later, detailed analysis.

Easily accessible healthcare facilities with sufficient members of staff are needed to diagnose and treat the patients and trace contacts.

Ability to do rapid trials

Sequencing and bioinformatics capacity

Pest control capacity?

Sufficient supplies of antibiotics are needed to treat patients and give PEP to contacts.

13. Critical infrastructure needs

Key knowledge gaps

Are the human resources available in Madagascar sufficient to respond to the outbreak?

Antimicrobial resistance, resistance of fleas, possibly genetic analysis of organisms. The ability to perform pathogen genomics in real time should be developed.

Are sufficient supplies available for appropriate PPE and have local HCW been appropriately trained?

Identification of need in terms of

- Timely lab diagnosis
- adequate clinical treatment
- contact tracing
- information campaigns concerning medical professionals and the general population
- safety of health care workers and other staff

References