

# WHO experts consultation on Ebola Reston pathogenicity in humans

Geneva, Switzerland  
1 April 2009



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Printed by the WHO Document Production Services, Geneva, Switzerland

## I. Introduction

The discovery of *Reston ebolavirus* in pigs in the Philippines in November 2008 and subsequent human infections (WHO, 2009), prompted WHO to organize an informal meeting of international experts to provide guidance to WHO in responding to specific questions related to the pathogenicity of *Reston ebolavirus* in humans.

A panel of 18 experts was convened by teleconference on 1 April 2009 (see Annex List of participants) bringing together expertise on *Reston ebolavirus* and emerging infectious diseases in the fields of virology, pathogenicity, immunology, epidemiology, laboratory techniques and ecology. Dr Thomas Ksiazek, from the University of Texas, Medical Branch, United States, kindly agreed to chair the discussion.

The meeting focused on the pathogenicity of *Reston ebolavirus* in humans and addressed a specific set of questions. A background paper on *Reston ebolavirus*, prepared by Drs T.G. Ksiazek and P.E. Rollin, was distributed to participants in advance of the meeting, together with the specific questions.

The following report presents a summary of the discussion together with the experts' response to each of the specific questions.

Note: The Reston ebolavirus taxonomically belongs to the *Filoviridae* family, *Ebolavirus* genus, *Reston ebolavirus* species.

## II. Questions, experts' discussions and answers

### Question 1. *Can humans be infected with Reston ebolavirus? What is the existing evidence: serology, virus isolation, etc*

Yes there is evidence that people can be infected with *Reston ebolavirus*. The strongest evidence comes from the United States, from where 4 documented infections and seroconversions were reported in 1989–90, including the case of a worker at the primate facility in Reston, Virginia who cut himself while performing a necropsy on an infected monkey. Daily samples were taken following the incident. He was shown to be viraemic on days 9, 10 and 11 post inoculation. The individual was shown to have seroconverted on day 14 following the incident. Additional evidence comes from serological studies on animal handlers at monkey facilities and people with occupational exposure to pigs in the Philippines (see table 1).

Table 1. Summary of *Reston ebolavirus* serological studies on animal handlers from monkey facilities and people with occupational exposure to pigs in USA, Italy and the Philippines, 1989–2009\*.

Year of Report	Place	IgG positive	No. of tested	%	Exposure
1989–1990	USA, Reston	4	149	3%	Monkeys
1989–1990	USA, Alice, Philadelphia	0	29	0%	Monkeys
1989–1990	The Philippines, Calamba	3	18	17%	Monkeys
	The Philippines, other sites	0	168	0%	Monkeys
1993	The Philippines, Calamba	2	21	10%	Monkeys
1992	Italy, Sienna	0	16	0%	Monkeys
1996	USA, Alice	0	10	0%	Monkeys
1996	The Philippines, Calamba	1**	21	0%	Monkeys
1996	The Philippines, other sites	0	230	0%	Monkeys
2008–2009	The Philippines	6	102	6%	Pigs
	Total	15	764	2%	

\*Sources: (CDC, 1990a; b; c; d; e), (Miranda et al., 1992); (Miranda et al., 1999); (Rollin et al., 1999); (WHO, 1990); (WHO, 1992); (WHO, 2009)

\*\* same person already IgG pos in 1993

**Answer 1:** Humans can be infected with *Reston ebolavirus* and a resultant viraemia has been documented in one case.

**Question 2. *What clinical manifestations have been observed in humans infected with Reston ebolavirus? Is there any evidence surrounding the pathophysiological process or specific target organs?***

To date only 15 persons have been shown to be positive for *Reston ebolavirus* antibodies (9 adult males from studies held between 1989 and 1996, and 6 adult males from 2008-09 studies). None of these people recalls any significant illness that could be related to infection with *Reston ebolavirus*.

Seroconversion was demonstrated in only the 4 cases reported in USA. In the other 11 people, seropositivity, not seroconversion, was demonstrated. As no baseline blood samples were available for these people, changes in antibody status could not be shown and hence time of infection was difficult to estimate. For some of these 11 people, potential exposure to *Reston ebolavirus* may have pre-dated the documented seropositivity by a number of years. As a result, there may be inaccuracies in their recall of any clinical symptoms that could be associated with *Reston ebolavirus* infection.

All but one of the 15 individuals with antibodies against *Reston ebolavirus* were, at the time of testing, reported to be healthy and to not have any underlying medical conditions.

In the US, one person - the animal handler who developed a *Reston ebolavirus* viraemia and in whom seroconversion had occurred, after accidental inoculation while performing a necropsy on an infected monkey - did have some underlying medical conditions. He was reported to have been suffering from diabetes, complicated by retinopathy and peripheral vascular disease at the time of the percutaneous exposure. No illness was observed during the period he was under medical observation following the percutaneous exposure, including the time when he was viraemic and underwent seroconversion. He did, however, undergo surgical intervention for removal of a gangrenous toe during this period, with no complications.

**Answer 2: It was concluded that there is insufficient evidence to draw any conclusions about the clinical outcomes or the absence thereof associated with *Reston ebolavirus* infection in humans.**

**The level of evidence was thought to be insufficient for the following reasons:**

- the very small number of people with proven infections (n = 15)
- all people with proven infections were adult males
- the timing of infection was unknown for all but four of the individuals and exposure may have predated the study period by several years, hence recall of clinical symptoms may lack accuracy.

**Question 3. *If it is considered that there is no or insufficient evidence to determine the pathophysiological process of Reston ebolavirus infection in humans, can this be extrapolated from experience and evidence with other Filovirus infections?***

It was thought by the expert group to be difficult to extrapolate this information directly from either:

- experience with other Filovirus infections in humans; or
- experience with *Reston ebolavirus* in non-human species

However, it was clear that:

a) Within the same Filovirus species a wide range of clinical outcomes have been demonstrated in humans. Examples of the range in the case fatality ratio (CFR) between outbreaks due to same species of Ebola and Marburg viruses include:

- *Zaire ebolavirus* (CFR varies from 60% in Minkébé, Gabon 1994 to 90% in Kéllé Congo 2003),
- *Sudan ebolavirus* (CFR varies from 41% in Yambio, Sudan 2004 to 65 % in Nzara, Sudan 1976),
- *Côte d'Ivoire ebolavirus* (only one case, who survived),
- *Bundibugyo ebolavirus* (CFR was 25%),
- *Marburg virus* (CFR varies from 24% in Germany 1967 to 90% in Uige, Angola 2005).

b) Relatively minor genetic differences between strains within the same Filovirus species have resulted in significant differences in clinical outcomes in both humans and non-human primates.

- During the *Marburg virus* outbreak in Uige, Angola 2005, the CFR was 90% (252 cases including 227 deaths, CFR 90%). The CFR associated with the *Marburg virus* outbreak in Germany and Yugoslavia in 1967 was only 24% (31 cases including 7 deaths).
- These findings were also corroborated by experimental inoculations of monkeys. The Uige, 2005 *Marburg virus* strain was more pathogenic in monkeys than the initial 1967 *Marburg virus* strain (Geisbert T, personal communication).
- Partial *Marburg virus* RNA sequence analysis revealed that the Angolan strain was only 7% different from the main group of East African *Marburg virus*, called POP lineage (Towner et al., 2006).
- Difference in pathogenicity in monkeys has been reported between the Boniface and Gulu strains of *Sudan ebolavirus*.

c) Relatively minor genetic differences between strains within the *Reston ebolavirus* species have been shown to result in significant difference in clinical outcomes in non-human primates.

- *Reston ebolavirus* showed different pathogenicity in different monkey species in an experiment conducted by the Centers for Disease Control and Prevention, Atlanta, US (Fisher-Hoch et al., 1992; Jahrling et al., 1996). African green monkeys (*Chlorocebus aethiops*) showed significantly higher survival rates than cynomolgus monkeys (*Macaca fascicularis cynomolgus*), and some were asymptomatic.
- Two different isolates of *Reston ebolavirus* showed different pathogenicity when inoculated to cynomolgus monkeys. The F4A strain, isolated during the *Reston ebolavirus* monkey outbreak in 1990 in Philadelphia, was more pathogenic in the monkeys than the 28H strain that was isolated from the first outbreak in 1989 in Reston (Geisbert T, personal communication).

d) Rapid changes in the virulence of a number of Filovirus strains have been demonstrated following relatively few passages. Several studies have demonstrated the possibility of rapid change in the virulence of the *Zaire ebolavirus* with regard to guinea pigs and newborn ICR mice (Connolly et al., 1999; Chepurinov et al., 2001; Chepurinov et al., 2003) indicating that the *ebolavirus* is able to adapt in a number of species. The lethality of *Zaire ebolavirus* and *Sudan ebolavirus* strains for guinea pigs can be increased within a few passages by selecting viruses from certain organs, thus demonstrating relatively rapid adaptive selection for lethality.

**Answer 3:** It is difficult to extrapolate directly from experience either with other Filovirus infections in humans or with *Reston ebolavirus* infections in non-human primates. However, a wide range of clinical outcomes has been demonstrated following Filovirus infections in humans and relatively minor genetic differences between strains of the same Filovirus species can result in significantly different clinical outcomes. It cannot, therefore, be ruled out that some *Reston ebolavirus* infections could cause disease in humans.



**Question 4. *Is there evidence from outbreaks of other strains of ebola that some groups of patients present with more severe forms of disease than others? If so what can be extrapolated that is of relevance to Reston ebolavirus?***

Some data, based on observations, were noted by the group:

- During the *Sudan ebolavirus* outbreak in Gulu, Uganda, 2000–2001, it was observed that the case fatality ratio among HIV positive individuals appeared to be much higher (CFR 90%) than among HIV negative individuals (CFR 53%).
- Mortality tends to be higher among pregnant women than among non-pregnant women. This was demonstrated during outbreaks of *Sudan ebolavirus* in Gulu, *Zaire ebolavirus* in Gabon and Congo 2001–02 and *Marburg virus* in Uige, Angola 2005.
- A higher case-fatality ratio was observed among young children than adults during the outbreak of *Marburg virus* in Uige, Angola, 2005 (children under 1 year of age (CFR 87%), children under 15 years old (CFR 83%) adults (CFR 74%)).

In a study on the human leukocyte antigen–B (HLA-B) locus role in the outcome of *Sudan ebolavirus* disease, statistically significant associations were found between certain sets of alleles and either fatal or non-fatal disease outcomes. Alleles B\*67 and B\*15 were associated with fatal outcomes, whereas B\*07 and B\*14 were associated with non-fatal outcomes (Sanchez et al., 2007; Sanchez et al., 2004).

It was also noted that *ebolavirus* infections have been associated with opportunistic infections (Kalongi et al., 1999), even in patients with no pre-existing or underlying medical conditions. Hence for patients with pre-existing immunodeficiency, infection with *ebolavirus* is likely to further compromise their status.

**Answer 4: Based on observations during *ebolavirus* outbreaks and laboratory demonstration of the immunosuppressive nature of *ebolavirus* infection in a variety of species, it is possible that *Reston ebolavirus* infection could result in different clinical manifestations in different population groups, particularly with regards to differences in immune and pregnancy status.**

**Question 5. *What is the host range for Reston ebolavirus? Should we consider its host range more extensive than the one known today?***

The currently known host range for *Reston ebolavirus*, that has been documented by serology and virus isolation during outbreaks, includes:

- primates (serology, PCR, virus isolation)
- pigs (serology, PCR, virus isolation)
- humans (serology, PCR, virus isolation)
- dogs (1 positive serology during 1996 investigations in the Calamba facility in Philippines)

A number of other species may also serve as hosts for *Reston ebolavirus*, including:

- bats – not proven at this stage, however based on evidence from other Filovirus strains it is presumed that bats could act as a reservoir species for *Reston ebolavirus*
- guinea pigs – demonstrated in laboratory experiments
- rabbits – demonstrated in laboratory experiments
- mice – demonstrated in laboratory experiments

Mosquitoes (*Culex*) and other insects (cockroaches, leafhoppers, ants, spiders, centipedes) pigeons, reptiles (tortoise, snakes, geckos, frogs), molluscs (achatine snails) and plants (33 varieties belonging to 24 different species of plants including tomato, cucumber, wheat, cotton, lupin, maize, tobacco etc) – were experimentally inoculated with *ebolavirus* and were resilient to the virus (Swanepoel et al., 1996).

In vivo and in vitro work has demonstrated that:

- the host range of the *ebolaviruses* is extensive as evidenced by the wide range of cell lines that could be infected with the virus (Van der Groen et al., 1978) and in a wide variety of vertebrates but not invertebrates that can be infected in the laboratory setting (Fischer-Hoch et al., 1992; Swanepoel et al., 1996; Turell et al., 1996).
- *Reston ebolavirus* can be grown in a wide range of cell lines originating from different species.
- However caution was expressed in extrapolating conclusions from these findings.

**Answer 5: There is limited information about the host range for *Reston ebolavirus* and further research in this area is needed. However, based on current knowledge, non-human primates, pigs, bats and humans should all be considered susceptible to infection with *Reston ebolavirus*.**

**Question 6: *From existing evidence, what are the routes of transmission for human infection of Reston ebolavirus?***

**Question 6a. *Is there any evidence that bats are the reservoir host for Ebola Reston?***

There is currently no direct evidence indicating that bats are the reservoir host for *Reston ebolavirus*. Previous studies conducted in the Philippines were small (n = less than 20 bats on the two occasions that bats were captured and tested) and testing relied on serology and antigen capture.

In Africa, the Old World fruit bats of the family *Pteropodidae* are considered natural hosts for *ebolavirus* and *Marburg virus*:

- fruit bats, belonging to the genera *Hypsignathus*, *Epomops* and *Myonycteris*, were found PCR positive for *ebolavirus* in Gabon-Congo concomitantly with outbreaks of Ebola haemorrhagic fever (Leroy et al., 2005);
- fruit bats belonging to the genus *Rousettus* were found PCR positive for *Marburg virus* during an outbreak in Durba, Democratic Republic of the Congo, 1999 (Swanepoel et al., 2007) and the virus was isolated from the same genus of bat during an outbreak in Kitaka cave, Uganda 2007 (Towner et al. 2009);
- initial human cases of *Zaire ebolavirus* during the outbreak in Luebo, DRC 2007, were associated with contact with fruit bats belonging to the genera *Hypsignathus* and *Epomops* (Leroy et al. 2009)

**Answer 6a: *Bats are a potential host reservoir for Reston ebolavirus. However studies using appropriate capture techniques and laboratory diagnostics are urgently needed to confirm this.***

**Question 6b. *Is there evidence that bats represent a common source of infection for monkeys and pigs?***

Between 1989 and 1996, the monkey export facility in Calamba, the Philippines, was the only facility to report *Reston ebolavirus* infection. Calamba monkey facility was built in an orchard with numerous fruit trees where fruit bats ate during the night. The other monkey export facilities in the Philippines did not have any fruit trees on their premises or in the vicinity and were shown not to have been infected with *Reston ebolavirus*.

The pig farm affected in 2008 in Bulacan province, the Philippines, had numerous fruit trees (banana, mango, guava, palm) that had been planted between the different buildings of the farm. Fruit trees were also reported to be present on the affected pig farm in Pangasinan province, the Philippines (guava, banana, palm and Ipil ipil trees).

**Answer 6b:** At each of the facility or farms that experienced animal outbreaks of *Reston ebolavirus* in the Philippines (monkeys in Calamba and pigs in Bulacan and Pangasinan) the animals could potentially have been exposed, directly or indirectly, to fruit bats attracted to the fruit trees planted on the facility or farms.

**Question 6c.** *Is it plausible that Reston ebolavirus can be transmitted to humans by bats?*

The group raised the following points:

- Recent outbreaks of *Nipah virus* in Bangladesh have demonstrated that direct bat-to-human transmission of Nipah, a bat-borne virus, is possible (i.e. pigs are not required as intermediate hosts) (Luby et al., 2006).
- There is documented evidence from Africa of several separate events during which direct bat-to-human transmission of *Marburg virus* occurred or was suspected following visits to different bat caves (Kitum cave Kenya 1980 and 1987, Durba mine DRC 1999, Kitaka mine/cave Uganda 2007, Maramagambo Forest cave Uganda 2008) (Swanepoel et al., 2007).
- There was some anecdotal evidence of possible bat-to-human transmission of *Zaire ebolavirus* in the Luebo outbreak in 2007 (Leroy et al. 2009)

**Answer 6c:** Bat-to-human transmission of *Reston ebolavirus* has not been demonstrated. However it should be considered a plausible route of transmission.

**Question 6d.** *Is there evidence that humans have been infected with Reston ebolavirus transmitted by monkeys?*

*If so, by what route of transmission?*

Yes, there is very strong evidence that humans have been infected with *Reston ebolavirus* through contact with monkeys. In 1989/90 and in the 1996 outbreaks in the Philippines, all persons who tested positive for antibodies had been in daily contact with sick, infected monkeys.

In the 1990 outbreak in the USA, the 4 persons who tested positive had also had close contact with sick, infected monkeys:

- one person performed an autopsy on an infected monkey and suffered accidental inoculation
- the other three people entered monkey houses where there were sick, infected monkeys and used high pressure hoses to clean the monkeys' cages without using any respiratory or eye protection. In addition they regularly handled sick, infected monkeys.

In addition, there is a large volume of strong evidence indicating that other Filoviruses can be transmitted from non-human primates to humans (Leroy et al., 2009).

Close or direct contact with the infected blood, secretions, organs or other bodily fluids appears to be the most likely source of infection of *Reston ebolavirus* and other Filoviruses.

Bodily fluids of concern include:

- Faeces – *Reston ebolavirus* has been detected in the epithelial cells of the gut mucosa in infected primates. Marked ulceration of gut mucosa has been noted during autopsies of infected non-human primates indicating that viral shedding in faeces is likely;
- Blood – evidence from other Filoviruses indicates that contact with infected blood can result in transmission;
- Urine - evidence from Filovirus infection in humans and monkeys indicates that the virus is excreted in urine;
- Respiratory tract – no experimental inoculation has been performed for *Reston ebolavirus*. However, respiratory spread between non-human primates is thought to have occurred during the 1989/90 outbreak in Reston, USA. Of note, experimental infection by aerosol route with *Zaire ebolavirus* has demonstrated that small particle aerosols are infectious at very low doses.

Possible routes of entry include:

- Inoculation.
- Conjunctival/ocular mucosa – this has been demonstrated for *Zaire ebolavirus* in guinea pigs but no laboratory experiments have yet been performed for *Reston ebolavirus*.
- Oral infection – this has been demonstrated for *Zaire ebolavirus* in monkeys but no laboratory experiments have yet been performed for *Reston ebolavirus*.
- Respiratory – see above.

**Answer 6d: There is conclusive evidence that non-human primates can transmit *Reston ebolavirus* to humans. The most likely route of transmission is by direct contact with the blood, secretions, organs or other bodily fluids of infected animals. Possible routes of entry include inoculation, conjunctival contact and possibly inhalation.**

**Question 6e. *Is there evidence that humans have been infected with Reston ebolavirus by pigs?***

Yes, some evidence from the Philippines from serological testing in humans indicates possible pig-to-human transmission. However, a case control study and further testing are required.

Since December 2008, a total 102 people in the Philippines have been tested. This includes:

- 47 pig farm workers from the 2 affected farms of whom 4 tested positive for *Reston ebolavirus* antibodies (IgG).
- 48 pig slaughterhouse workers of whom 2 tested positive for *Reston ebolavirus* antibodies.
- 7 contacts of seropositive persons, all of whom tested negative.

All the persons who tested positive for antibodies deny any contact with bats or monkeys. All six people who tested positive had daily, occupational exposure to pigs.

In addition it should be noted that both the ill and the well pigs that tested positive for *Reston ebolavirus* had Ebola antigens in the alveoli in the lungs and intra-alveolar infiltration with *Reston ebolavirus* antigen, an atypical presentation for *Reston ebolavirus*. These findings are highly suggestive of potential respiratory transmission of *Reston ebolavirus* among the pigs and possibly from pigs to humans.

**Answer 6e:** Evidence suggests that pigs can transmit *Reston ebolavirus* to humans. The most likely routes of transmission from pigs to humans is by direct contact with the blood, secretions, organs or other bodily fluids of infected animals, and possibly, respiratory transmission.

**Question 6f. *Is there any evidence of human-to-human transmission of Reston ebolavirus?***

There is currently no evidence of human-to-human transmission of *Reston ebolavirus*. During the 1989/90 and 1996 outbreaks in monkeys of *Reston ebolavirus* in the Philippines, several (n=15) casual contacts of the individuals who tested positive for *Reston ebolavirus* antibodies, including administrative and maintenance support staff and family members, were tested for evidence of *Reston ebolavirus* infection and all were negative. Results from limited testing of close contacts during the 2008/9 outbreak in the Philippines, have thus far been negative (n=7).

However there is evidence from outbreaks in Africa that other Filoviruses can be transmitted from one viraemic human to another directly (through close contact with body fluids) and indirectly (through fomites especially blood stained articles). This transmission occurs during the later stages of the disease when virus titres are highest and when the patient has clinical conditions that allow the virus to exit the body (bleeding, diarrhoea, vomiting) (WHO, 2008).

There is evidence that indirect transmission via fomites can take place up to 9 days after the initial contamination of the fomite e.g. a patient is thought to have contracted *Zaire ebolavirus* after lying on a blood stained mattress that had been soiled 9 days earlier.

A number of documented transmissions of *Zaire*, *Sudan* and *Bundibugyo ebolaviruses* have occurred in health care settings involving transmission from patient to health care worker.

There is also evidence that Standard Precautions for infection control can dramatically reduce the risk of human-to-human transmission.

**Answer 6f:** While there is no evidence to date that human-to-human transmission of *Reston ebolavirus* has occurred, the expert group noted that this was potentially possible, particularly if an infected individual was to become viraemic and symptomatic.

This conclusion was based on:

- experience with other Filoviruses
- documented three-day viraemia in a human with confirmed *Reston ebolavirus* infection.

**Question 6g.** *Is there evidence of human infection of Reston ebolavirus documented via xenotransplantation or use of porcine sub-products in vaccines, cosmetics, medical products etc*

There is currently no evidence of transmission of *Reston ebolavirus* though these routes.

However, the following concerns were noted:

- the first documented cases of *Marburg virus* infection in humans occurred as a result of contact, in a laboratory, with either infected monkeys or with tissue collected from them in the preparation of cell lines for polio vaccine.
- pig heart valves are used in the Philippines for transplant purposes.
- pig placental products are used in a number of cosmetic products in the Philippines.
- the extreme pathogenicity for man of most known Filoviruses makes it essential that any possibility of their introduction into the vaccine supply (human or veterinary) is excluded.

**Answer 6g:** Porcine sub-products used for medical or cosmetic purposes should be considered potential sources of infection if they originate from areas where porcine infection with *Reston ebolavirus* has been reported. Further work is required to identify all such porcine sub-products, to assess the processing procedures and to identify the distribution of such products. It is essential that the possibility of *Reston ebolavirus* introduction into the vaccine supply (human or veterinary) is excluded.

**Question 6h: *Should any other potential route(s) of transmission be considered for Reston ebolavirus?***

The potential of transmission of *Reston ebolavirus* through the food chain (up to consumer) is considered to be possible and warrants an extensive risk assessment by an expert advisory group as a matter of urgency.

Furthermore, it was noted that there was documented or suspected evidence of transmission of Filoviruses from bushmeat (infected non-human primates, duikers, fruit bats) to humans during slaughter and preparation of the carcass for cooking. However, individuals who ate the cooked product did not seem to be at risk for ebola infection (Georges et al., 1999).

Transmission of Lymphocytic Choriomeningitis Virus (LCMV) and a LCMV-like arenavirus via organ transplantation has been documented in four clusters in USA (Amman et al., 2007; CDC 2008).

In the United States, transmission of *West Nile virus* (WNV), a mosquito-borne virus, by routes other than mosquito bites, has been reported including via blood transfusion, organ transplant, transplacental infection, and possibly breast milk (Allain et al., 2009).

**Answer 6h: In addition to the possible routes of transmission outlined in 6c to 6g above, the following potential routes of infection for humans should also be considered for *Reston ebolavirus*:**

- food processing throughout the food chain - from farm to fork: slaughtering animals, preparing meat and animal products;
- eating raw or semi-cooked pork products;
- close contact with semen from infected animals (pigs or monkeys);
- bite or scratch by an infected animal;
- exposure of people during culling of infected animals (pigs or monkeys);
- blood transfusion;
- transplacental infection;
- organ transplant.



**Question 7.    *Could Reston ebolavirus evolve genetically in nature and become more pathogenic to humans?***

The group thought this was possible, notably when there are interspecies transmission events. Selection resulting in increased pathogenicity after only a relatively small number of passages has been demonstrated in the laboratory with other Filoviruses.

Relatively small genetic differences have been shown to result in significant differences in clinical outcomes for a range of Filoviruses.

Within the Reston species, there is already a 5% genetic difference between the different strains that have been identified and it is likely that other strains exist but have yet to be identified. We do not know what the virulence factors are and are therefore not in a position to predict, based on genetic sequence information, which sub-strains would be pathogenic to humans and which would not.

Filoviruses are RNA viruses and as such are very 'plastic'. However, to date no significant drift in Filoviruses has been noted during human outbreaks in Africa. That said the maximum chain of transmission during these outbreaks is thought to have been less than 10 generations and the outbreaks have been relatively small in terms of number of humans infected. Also, infected humans died quickly resulting in limited opportunities for selection of mutants that might have a more pathogenic phenotype.

**Answer 7:    *Reston ebolavirus is genetically diverse. Minor changes in its genetic sequence could result in a virus with greater virulence for man. Evolution and production, through adaptation, of more virulent strains of Reston ebolavirus is considered a possibility. As we lack an understanding of virulence factors, predicting virulence based on genetic sequence information is currently not possible.***

**Question 8. *Does the presence of Reston ebolavirus in domestic pigs pose a greater or lesser potential for genetic evolution than a situation in which pigs were not infected?***

It was thought by the experts that *Reston ebolavirus* would evolve genetically more rapidly if there were interspecies transmission.

Four different strains of *Reston ebolavirus* have been isolated from the two farms that tested positive. All four strains vary by up to 3-5% from previous 1989/90, 1992 and 1996 strains.

The pig farm in Bulacan province tested positive in June 2008 and again in January 2009. The virus was still found to be circulating among pigs on the farm in Bulacan province at the time of the January 2009 sampling with no indication of clinical disease. Antibodies were found in animals born after June 2008 (with the caveat that these could be maternal antibodies) but, more importantly, RT-PCR positive animals were detected and a *Reston ebolavirus* was isolated from one of them. The *Reston ebolavirus* isolated in January 2009 has a 1% genetic variance from the strain isolated in June 2008. These results suggest that the virus can potentially circulate for a long time among pig populations and has the potential for genetic change.

The group concluded that the higher the prevalence of virus infection and transmission in pigs in the Philippines, the greater the opportunity for evolution in the virus.

**Answer 8: *The presence of Reston ebolavirus in domestic pigs is thought to pose a high potential for genetic evolution because:***

- in the absence of biosecurity measures at pig farms, it provides an opportunity for recurrent interspecies transmission from fruit bats or other possible reservoir(s) to pigs;
- due to the close and frequent level of contact between humans and pigs, as compared to humans and non-human primates, and in the absence of personal protective procedures, an increased opportunity for recurrent pig to human interspecies transmission is likely.
- the on-going circulation of the *Reston ebolavirus* in the pig population provides an unusual opportunity for continued genetic evolution to occur as a result of passage, adaptation and possible natural selection of this virus.

**Question 9. *Should Reston ebolavirus be considered potentially pathogenic for humans?***

Considering the response to questions 1 to 8, the expert group concluded that *Reston ebolavirus* should be considered potentially pathogenic for humans.

**Answer 9: *Yes, Reston ebolavirus should be considered potentially pathogenic for humans.***

### III. Conclusion

In light of the information and knowledge available at the time of the discussion, the group of experts came to the following conclusions:

- *Reston ebolavirus* should be considered potentially pathogenic for humans.
- Human infections have been proven and, in one case, demonstrated to be viraemic.
- The number of humans with proven infection that have been studied is too small (n=15) to draw any conclusions on possible clinical spectrum. As a range of presentations is possible for other Filoviruses, the working assumption, until proven otherwise, should be that this could be the same for *Reston ebolavirus*.
- In addition, host factors such as immunosuppression or pregnancy have been shown to affect the clinical outcomes with other Filovirus infections. To date, all individuals who have tested positive for *Reston ebolavirus* have been adult males with only one person having any underlying medical conditions. *Reston ebolavirus* infection in children, the immunocompromised, pregnant women or persons with underlying medical conditions therefore remains unstudied and unknown.
- It has been demonstrated for all Filovirus species that relatively minor genetic differences can result in marked differences in clinical outcomes.
- Marked genetic diversity in the *Reston ebolavirus* strains already exists and it is considered likely that other, as yet unidentified, strains could already be circulating in the reservoir host.
- The on-going circulation, and passage, of *Reston ebolavirus* in pigs in the Philippines, coupled with the likely introduction of different strains into the pig population from fruit bats or other possible reservoir(s) means that the situation is unpredictable.
- Knowledge of virulence factors is too limited to be able to predict, based on genetic information, which strains pose a threat to humans and which do not.
- It is plausible that if more people become infected with *Reston ebolavirus* we would see a range of clinical responses, including disease and possibly death.
- As with other Filoviruses, *Reston ebolavirus* should only be propagated in laboratories with the highest level of Biosecurity (BSL4). Other laboratories can handle specimens potentially infected with the virus, but should ensure adequate biosafety protocols (inactivation, etc...) are applied.

Given the likelihood of further infections and the potential pathogenicity of *Reston ebolavirus* for humans, public health authorities are urged to take several steps to reduce the risk of human infections:

- by implementing control measures to avoid pig-to-human infections;
- by determining the extent of infection among the national swine herd in the Philippines; and
- by reducing infection of pigs from fruit bats or other possible reservoir(s).



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