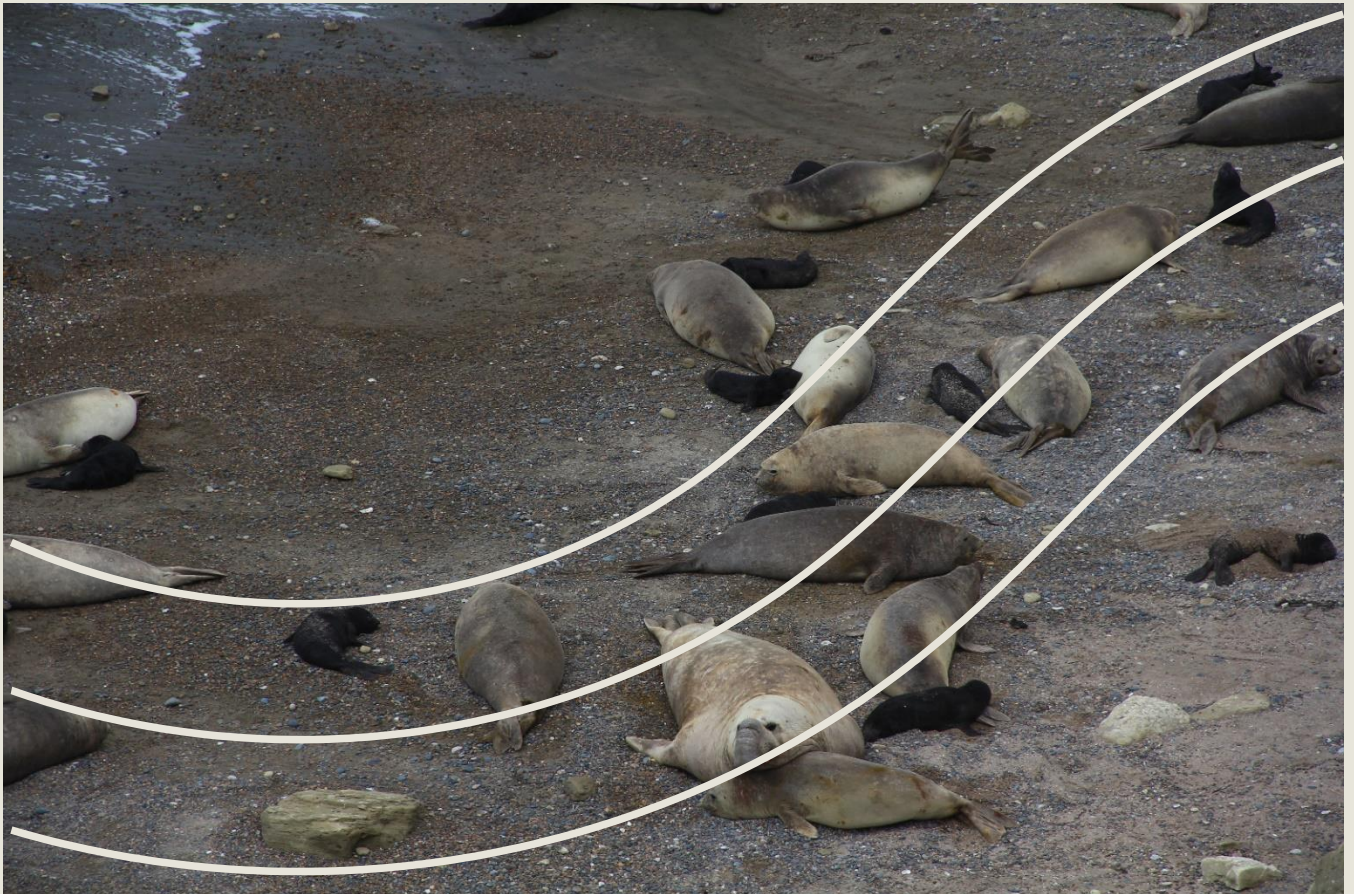


Practical guide for authorised field responders to HPAI outbreaks in marine mammals

With a focus on biosecurity, sample collection for virus detection and carcass disposal



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Section 1. Introduction

Since its emergence in 1996, high pathogenicity avian influenza (HPAI) caused by the H5N1 subtype, has evolved into a global panzootic, impacting Africa, Asia Pacific, the Americas, Europe, and the Middle East. Beyond domestic poultry and captive birds, it now threatens wild and domestic mammals, as well as humans. Since 2021, the HPAI H5N1 clade 2.3.4.4b strain has caused significant mortality in wild birds around the world and marine mammals in South America (Gamarra-Toledo *et al.*, 2023, Ulloa *et al.* 2023, Campagna *et al.*, 2023, OFFLU *ad hoc* group on HPAI H5 in wildlife of South America and Antarctica, 2023). Though these outbreaks have been linked to few human infections of mild to severe symptoms (Castillo *et al.*, 2023), all H5N1 strains (and some other subtypes) should be considered to pose zoonotic risks. Thus, this virus poses a risk to animal health, public health, and biodiversity.

Thus, early detection, immediate notification, and timely response, along with relevant biosecurity measures, are fundamental in devising containment and prevention strategies to protect animals, humans, and biodiversity.

To provide important guidance for such strategies, the current recommendations were developed. These recommendations stem from recent HPAI outbreaks in the marine mammals of South America, with potential global applicability that accounts for local conditions and differential epidemiological situations.

Section 2. Disease Ecology: HPAI in marine mammals

2.a) Background

The ongoing H5N1 panzootic originated in the Northern Hemisphere birds in 2021 and rapidly spread across the world. Between December 2022 and March 2023 HPAI H5N1 virus, clade 2.3.4.4b, was reported from more than 24 European countries with large mortality events observed in France, Belgium, Netherlands, and Italy. Confirmed cases have been reported from pinnipeds, both otariids and phocids (e.g., seals), and mustelids (mink, ferrets, weasels, badgers, river otter, sea otters, etc.). Though cases have been reported from small toothed whales (parvorder Odontoceti), no cases have been reported from baleen whales (parvorder Mysticeti) (OFFLU, Dec 2023).

In this ongoing panzootic which began in 2021, the virus appears to have retained its preferential binding for avian-like receptors, but several mutations associated with potentially increased zoonotic and mammal transmission risks were detected (Vreman *et al.*, 2023). Noteworthy examples include potential mink-to-mink transmission of clade 2.3.4.4b H5N1 viruses from a fur farm in Spain, HPAI H5N1 virus clade 2.3.4.4b associated with meningoencephalitis in a stranded harbour porpoise (*Phocoena phocoena*) from Sweden (Thorsson *et al.*, 2023), that was closely related to strains responsible for a concurrent avian influenza outbreak in wild birds. This case highlights the potential risk for virus spillover to mammalian hosts (Thorsson *et al.*, 2023; OFFLU, 2023).

The risk for humans in Europe is assessed as low for the general population, and low to moderate for occupationally or otherwise exposed people (EFSA, 2023). Considering the situation in North and South America, the H5N1 (clade 2.3.4.4b) virus was also detected in North America in late 2021 (one *Tursiops* spp. dolphin in Florida). After introduction from Eurasia the virus spread across North America in wild birds, spilling over into poultry farms and infecting an alarming number of wild terrestrial mammals. In June 2022, in the northeastern United States and eastern Canada, the spillover of HPAI H5N1 into marine mammals (Harbor seals, *Phoca vitulina* and Grey seals, *Halichoerus grypus*), was reported coincident with H5N1 in sympatric wild birds (Puryear *et al.*, 2023). Starting in late 2022, HPAI H5N1 virus spread southward in the Americas, extending from Mexico to southern Chile in about 6-8 months. Cormorants (Phalacrocoracidae or *Leucocarbo bougainvillii*), Peruvian boobies (*Sula variegata*) and Peruvian pelicans (*Pelecanus thagus*) were three of the most frequently infected wild bird species in the region with hundreds of thousands of deaths being reported in Peru, cormorants (>254,000), boobies (>235,000) and pelicans (>57,000). Recently, over 7,000 frigatebirds were affected in Ecuador (OFFLU, Dec 2023).

In South America, Leguia *et al.* (2023) reported the genomic characterisation of HPAI/H5N1 in five species of marine mammals and seabirds in Peru (dolphins, sea lions, sanderlings, pelicans, and cormorants) (Leguia *et al.*, 2023). Starting January 2023, an increase in mass marine mammal strandings and mortality caused by HPAI H5N1 spread along the coast of Chile, reaching small odontocetes, mustelids and especially impacting South American sea lions (*Otaria flavescens*) (Ulloa *et al.*, 2023) and also in Perú (Legia *et al.*, 2023). In Argentina, the first case in South American sea lions was reported in August 2023 and the virus then moved north along the Atlantic coast, affecting pinnipeds (includes seals, sea lions, etc). in Argentina, Uruguay, and Brazil. Over this time, the number of dead and/or sick pinnipeds with clinical signs compatible with H5N1 has been increasing, with mass impacts in South American sea lions. Recent figures from Chile reported over 20,000 South American sea lions, Peru over 10,000, Argentina over 1,300, Uruguay over 800, and Brazil over 550. (OFFLU, Dec 2023). In Argentina, a mass mortality of Southern elephant seals (*Mirounga leonina*) with over 17,400 specimens was reported, and fewer cases (13) in fur seals (*Arctocephalus australis*) (Campagna *et al.*, 2023). The introduction of HPAI H5N1 to two remote biological diversity hotspots including the Galapagos Islands and South Georgia (sub-Antarctic) towards the end of 2023 indicates the ongoing expansion of the virus (Promed Avian influenza (145; 158); OFFLU, Dec 2023).

2.b) Transmission

Reports of HPAI H5N1 in terrestrial mammals hypothesised to be linked to feeding on infected wild birds have been published (Vreman *et al.*, 2023). While this data may explain cases observed in terrestrial mammals including carnivores and scavengers, it may not be sufficient to explain transmission in marine mammals such as pinnipeds or cetaceans (includes whales, dolphins, and porpoises). Sea birds are not the preferred prey for sea lions, and it is very unlikely that thousands of sea lions have died because of feeding on infected wild birds. A different and more comprehensive hypothesis about the infection mechanism in marine mammals based on the sharing of foraging habitat between wild sea birds and marine mammals (including, sea lions, porpoises, mustelids and dolphins) who forage from the same school of fish) has been proposed (Ulloa *et al.*, 2023). This is in line with what Kaplan and Webby, (2013) pointed out for other species, indicating that, close contact of terrestrial bird or mammalian species with infected poultry/waterfowl or their biological products is suspected the major route of interspecies transmission. However, transmission from mammal to mammal must be considered before the recent death of over 17,000 newborn elephant seal pups verified in Argentina during their nursing stage (Campagna *et al.*, 2023). This is still a matter to be clarified in further studies.

2.c) Clinical signs and pathology

Clinical signs compatible with HPAI H5N1 documented in living pinnipeds (e.g. seals and sea lions) include dyspnoea (shortness of breath) and abdominal breathing, nasal discharge, coughing/sneezing, salivation, disorientation, decreased responsiveness to stimuli or approaches, abnormal postures, ataxia, incoordination, total or partial paralysis of the limbs, myoclonus, nystagmus, seizures, diarrhoea, emaciation, and abortions (Leguia *et al.*, 2023; Ulloa *et al.*, 2023, Puryear *et al.* 2023; Campagna *et al.*, 2023).

Multifocal suppurative to non-suppurative meningoencephalitis in sea lions as well as neutrophilic perivascular infiltration in seals have been reported in these cases as the main histopathological findings (Ulloa *et al.*, 2023). Additionally, in seals with respiratory signs, some cases had necrotising bronchiolitis, bronchitis, and/or interstitial pneumonia (Puryear *et al.*, 2023). Positive influenza virus type A antigen has been detected immuno-histologically in brain tissues of SA sea lions with neurological signs (Ulloa *et al.*, 2023). Pathological findings including neuronal necrosis and inflammation of the brain and meninges were described in a bottlenose dolphin (*Tursiops truncatus*) in Florida (Murawski *et al.*, 2023). Necrosis of different organs, such as adrenal glands, liver, spleen, and thymus, has also been reported in harbour seals, *Phocas vituline* (Stephane *et al.*, 2023, in press). Except for meningoencephalitis and concomitant neurological clinical signs reported in a harbour porpoise, *Phocoena phocoena* (Thorsson *et al.*, 2023), the above referenced clinical signs have not so far been widely reported in positive (H5N1) infected cetaceans.

Section 3. Recommendations: HPAI in marine mammals

3.a) Recommendations for the general public

- Become familiar with the clinical signs associated with avian influenza (HPAI) in marine mammals. (video of HPAI H5N1 clinical signs in South American Sea Lion South (*Otaria flavescens*) in Chile, available at: <https://zenodo.org/records/10624445>)
- Do not approach, and avoid direct contact with, wild animals such as wild birds and marine mammals (seals and sea lions, etc.), especially if they show any signs of sickness, strange behaviour or are dead. Also remember that wild birds and marine mammals can be infected without looking sick, so it would be better to avoid contact in any case.
- For better management of the panzootic it is important to allow wildlife time and space to recover and stabilise from this disease that has decimated several wild animal populations.
- Be a responsible pet owner, avoid direct contact between pets and wildlife whether they appear healthy, sick or are dead.
- Report the presence of abnormally behaving, sick or dead wild marine mammals or birds immediately to the relevant competent authorities.

3.b) Recommendations for Veterinary Services and Wildlife Authorities

- The access of people, dogs, and other animals to beaches where marine mammal or wild bird mortality events have occurred should be controlled by competent authorities according to the epidemiological context of the region/country.

- Surveillance, by screening for the virus in dead wild birds and marine mammals, and monitoring of viral mutation and adaptation to mammals, is of relevance for assessing risks to public health, animal health, and conservation.
- Countries with marine mammal populations should have the capacity for HPAI H5N1 diagnostic investigations in marine mammals (including pathology, sampling, and diagnostic laboratory capabilities). Upon request, assistance will be provided by WOAHP wildlife Collaborating Centre network, which includes marine mammal health specialists (contact at direccion_iusa@ulpgc.es or [contributors*](#)).
- Risk communication at different levels (international scientific communities, social media, print or radio media, among others) is highly relevant for disease response and control. Messages should not stigmatise wildlife nor encourage retaliation or other inappropriate actions disruptions. Messages should alert to the high conservation impact of this disease for affected wildlife populations.

3.c) Recommendations and procedures for professionals involved in response to suspected HPAI in marine mammals in areas not previously diagnosed as positive for HPAI

i. Living animals with clinical signs compatible with HPAI

- Prevent the public or their pets from approaching sick or dead animals on the beach.
- Use personal protective equipment (PPE) (detailed in section below) when working in a potentially contaminated environment or with potentially contaminated material.
- Disinfect or dispose of all response gear between handling birds and marine mammals.
- Do not transport animals suspected to be infected with HPAI to rehabilitation or primary care centres unless it has been agreed by the competent authority. Conversely, if animals are admitted or infection with HPAI is diagnosed after arrival, the centre should be considered an infected centre and quarantine procedures initiated.
- In cases where animals show neurologic clinical signs and/or where it is difficult to safely handle them or ensure their welfare, sedation can be considered for specimen collection, prior to euthanasia (<https://www.avma.org/sites/default/files/2020-02/Guidelines-on-Euthanasia-2020.pdf>). We suggest sampling every suspect animal until the first positive sample is confirmed and then continue with a sample size of ten samples per month to monitor the virus circulation (SERNAPESCA, 2024). Carcasses must be disposed properly and should consider international guidance and local health and environment regulations.
- According to local available resources within a mass mortality context, it may be necessary to use specialised third-party services to manage removal and disposal of carcasses since services normally charged with domestic/metropolitan cleaning usually lack appropriate training and equipment. Carcass disposal must be supervised by the responsible competent authorities (animal health, public health and environment) to make sure biosecurity procedures and PPE are correctly applied during removal, burial, incineration, composting, or whatever other method is used for disposal.

- The use of heavy machinery such as front loaders and backhoes can be considered in cases of mass mortalities and/or heavy/large specimens. In case of mortality in remote areas where the introduction of heavy machinery is not feasible, burial on site or non-intervention should be considered.
- Persons involved in HPAI mortalities should avoid contact with living, healthy birds and marine mammals for at least of two weeks after being in contact with live or dead HPAI infected animals.
- If the infection by HPAI was already diagnosed in a certain area, there may be no need to continue sampling unless it is for clinical, pathological and epidemiological studies or to determine if the virus has mutated so as to increase risk of transmission to humans.
- More recommendations can be found at: (<https://www.woah.org/app/uploads/2022/08/avian-influenza-and-wildlife-risk-management-for-people-working-with-wild-birds.pdf>).

ii. Euthanasia protocols

Euthanasia of sick animals and carcass removal should be considered where this may mitigate animal suffering and/or prevent transmission of virus from infected to uninfected animals and/or decrease the risk of exposure to scavengers and humans.

The euthanasia protocols will be chosen by professionals according to the criteria and current legislation in each jurisdiction. Unless animals are to be destroyed using firearms, prior chemical restraint or anaesthesia is recommended.

Pinnipeds are often large and potentially dangerous. Occupational health and safety are important aspects to consider together with the involvement of animal handling experts. Depending upon the species, chemical immobilisation of pinnipeds may require the use of specialised equipment, such as protective shields, prior containment in nets or with restraint boards and/or remote drug delivery using darts, rifle, blowpipes etc. The reality with a panzootic like HPAI is that it is rarely necessary to handle live or healthy animals in the field; most of the animals to be sampled or sedated are in very bad physical condition which makes them easier to handle (e.g. they are not responsive). More details or options on animal handling can be found in the “AVMA guidelines for the euthanasia of animals: 2020 Edition”. Also, NOAA Fisheries’ Marine Mammal Health and Response Program is a useful source of information and include a “[Marine Mammal Euthanasia Best Practices](#)” document that can be used as a guide to euthanasia of these animals.

Carcass disposal should occur under the direction of the competent authorities (e.g., animal health, environmental health, public health) who will provide guidance. A significant consideration is disposal of animals where chemical restraint and/or euthanasia has been used. In these cases, carcasses should not be left in place or buried in situ but preferably removed to a disposal pit/site deemed appropriate by the responsible authority to decrease the risk of environmental contamination and or exposure to non-target species or scavengers. Thus, euthanasia should only be considered when carcasses can be properly disposed of. Consideration should also be given to potential contamination of the environment from disinfectants used to clean the site.

iii. Dead marine mammals

- The approach to dealing with dead marine mammals should be under the guidance of the relevant competent authority.
- The public and their pets should be prevented from approaching dead animals and animal health operators must be equipped and appropriately trained in the use of personal protective equipment (PPE).
- According to the epidemiological context of the region/country and to the availability of experienced personnel in marine mammal post-mortem examination, a complete necropsy and/or a collection

of samples could be performed. If infection by HPAI was already diagnosed in a certain area, there may be no need to continue sampling unless it is for clinical, pathological and epidemiological studies or to determine if the virus has mutated so as to increase risk of transmission. If a dead animal is found in an area not previously diagnosed as positive for HPAI, or represents a new host species, then it is recommended to take samples, especially from animals in which there is prior knowledge that they had clinical signs, or from fresh carcasses.

Once an outbreak has been confirmed through diagnostic testing of samples collected from several animals in a specific area then decreased sampling can occur monthly (e.g. ten samples per month) to track the outbreak and the virus mutations and determine when the outbreak is resolving since there may be mortality due to other causes (such as shortness of food or biotoxin intoxication) that needs to be differentiated.

iv. Necropsy, sample collection and submission for diagnosis (RT-PCR and Sequencing; histopathological investigation) from dead /euthanised animals

Necropsy

- Performing a necropsy and sampling by a veterinary pathologist (trained for marine mammals) would be ideal. Alternatively, veterinarians or other authorised personnel (under national legislation) with expertise in marine mammals could perform a postmortem dissection and sampling following different marine mammal protocols (AMEVEFAS, 2017).
- When a complete necropsy is not a viable option sampling instead of necropsy can be considered. Recommended samples for collection include oropharyngeal tracheal and nasal swabs, and brain and lung tissue swabs. Rectal swabs can also be taken, however they have lower diagnostic sensitivity. The use of PPE, adequate dissection equipment, and disinfection and final carcass disposal protocol are mandatory.
- It is recommended to take photographs and videos during these procedures to complete reports with visual proofs and evidence.

Swabs

- To take oropharyngeal swabs it is necessary to extend the neck and open the mouth of the animal to adequately reach the oropharynx. Use long swabs and scrape the tissue or mucosa well before rinsing the swab in the transport medium (see “conservation of samples” below). For pinnipeds, oropharyngeal and tracheal swabs are better for HPAI diagnosis than nasal and rectal swabs.

Tissues

- When a complete necropsy and sampling is possible, follow protocols for marine mammals which can be found in international academics’ platforms. Such protocols could be provided by WOAHP upon request (contact: direccion_iusa@ulpgc.es or contributors*). A large set of tissues (including brain and lungs) should be taken, especially those presenting gross lesions. Samples should be stored frozen as well as in 10% neutral-buffered formalin for laboratory studies.
- When a complete necropsy is not possible, in addition to swabs (indicated above), tissues from brain and lung parenchyma is recommended for HPAI virus detection (even in autolytic carcasses). To prevent further dissemination and scavenging, brain samples can be taken through the foramen magnum after head disarticulation. Likewise, lung parenchyma can be sampled after removing skin,

muscles and cutting the ribs of the thoracic cavity or laterally or from the back without opening the chest. Brain and lung samples should be put in transport media, in 99% ethanol and/or frozen for PCR analysis. In these cases, and attending to tissue decomposition, samples can also be fixed in 10% neutral-buffered formalin for histological and immunohistochemical studies.

Personal protective equipment (PPE)s

Wear adequate PPE when working around sick or dead marine mammal or samples collected from them, including:

- Disposable long-sleeved, adjustable-end coveralls.
- Rubber boots (preferably white).
- Double disposable nitrile gloves.
- Respiratory protection (N95 FFP2 or KN95 well-fitted mask).
- Plastic goggles for eye protection.
- Practice good hygiene (thorough washing of hands) after removing PPE.
- Use > 60 % ethanol hand disinfectant after removing PPE.
- Do not reuse disposable material. All disposable material should be collected in a double bag for disposal. If available, it is recommended to assign a vehicle for exclusive use in activities related to HPAI. All the equipment must be washed and disinfected after its use. It is recommended to implement a rigorous cleaning and disinfecting procedure for all necropsy equipment after use. Bleach (sodium hypochlorite 3%) is an effective disinfectant against HPAI.
- Surfaces should be cleaned with soap/detergent and water to remove dirt, and then sprayed with or soaked in disinfectant (e.g., Safe4, 1% Virkon, 3% bleach, 60-90% ethanol, 60-90% isopropyl alcohol). Disinfectants should not be applied to the environment, sick animals, or carcasses.
- More information regarding risk management (Avian Influenza as zoonosis) can be obtained from WOAHA upon request or in its webpage.

v. Carcass disposal

- Carcass disposal should occur under the direction of the responsible competent authorities consistent with local legislation/regulations. Deep burial is preferred. Appropriately constructed and located burial pits can be isolated and covered with earth or sand for greater biosecurity.
- Where appropriate, carcasses can also be incinerated or composted.
- If animals are stranded in remote areas proceed to bury *in situ* according to local burial protocol. Please keep in mind that these procedures should be done away from resting or breeding sites of pinnipeds or seabirds to avoid stress or disturbance. If mass mortality occurs within breeding colonies, during breeding season and while animals are dying, the entry of machinery or even the access of specialised personnel to these areas is quite difficult and risky, further disturbing animals. The suggestion in these cases is to consider non-intervention.

3.d) Miscellaneous recommendations

Wildlife managers, environment services, Veterinary Services, public health authorities, and law enforcement officials, and any other involved authorised personnel should collaborate to:

- Try to keep a real time communication with updates of affected species, number of affected animals and diagnosis, whenever it is confirmed by the government laboratory.
- Report positive diagnostic findings for HPAI in any species and/or mass mortalities to WOAHA immediately and post information on government web sites. Information should be shared at the international level to inform risk analyses and early warning. Exchange and sharing information and best practices. For example, by using the FORA Framework (Fuzzy Management Methods) (Gozhyj *et al.*, 2018).
- Provide additional epidemiological, clinical, and pathological information when reporting diagnostic results or mass mortality events. This information contributes to greater knowledge of the disease in marine mammals which will inform surveillance and risk analysis.
- Share information on the management of sick animals and euthanasia, (based on welfare legal procedures in each country) to support other countries.
- Adequate support for national diagnostic capacity will facilitate fieldwork and surveillance. If national capacity is not available reference laboratories should be identified for shipment of diagnostic specimens
- Also remember to monitor populations of domestic and wild animals with access to carcasses, especially feral or unguarded dogs that may get infected.

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