

WILDLIFE CONSERVATION SOCIETY

Carbofuran poisoning at the interface between wildlife, livestock, and humans

Report

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Carbofuran poisoning at the interface between wildlife, livestock, and humans.

Introduction

Between January and August 2015, a series of animal mortality and human morbidity events in Preah Vihear Province, were detected by the LACANET wildlife disease surveillance network. Initial findings raised suspicions towards a link to pesticide use. Given the morbidity and mortality risks for humans, livestock, and endangered species, a thorough investigation was initiated. This document reports the results of this investigation.

Background

Surveillance System

The LACANET project is a European Union-funded project since 2014 with an objective to establish routine mechanisms for wildlife disease surveillance in Cambodia. The LACANET project partners in Cambodia include the Cambodian National Veterinary Research Institute (NAVRI located in the Ministry of Agriculture, Forestry, and Fisheries or MAFF), the Wildlife Conservation Society (WCS), and Institut Pasteur du Cambodge (IPC); all work together to ensure the reporting of wildlife mortality and the investigation of mortality events.

The surveillance network is structured around protected areas co-managed by MAFF's Forestry Administration (FA) or the Ministry of the Environment (MoE) and the Wildlife Conservation Society (WCS). Existing field capacity (wildlife monitoring teams and forest rangers) contribute to the reporting and submission of wild animals which are confiscated or found dead. The LACANET surveillance network also includes The Angkor Center for Biodiversity Conservation (ACCB) and several wildlife rescue centers that may receive confiscated, rescued, or sick animals and/or contribute to sample and carcass submission.

The surveillance system is in its first pilot phase in the WCS landscapes of the Preah Vihear Province and the Seima Protected Forest. Collaboration with other conservation NGOs such as the World Wildlife Fund (WWF) and Birdlife International (BI) expanded coverage to the Mondulkiri protected Forest and the Siem Pang District, respectively. Collaboration with ACCB allows a broader coverage of provinces where ACCB is operating (primarily Siem Reap, Preah Vihear, and Kampong Thom).



Case Detection and Sampling:

Unusual wildlife health events are detected by monitoring teams and FA/WCS forest rangers during research activities and law enforcement patrols, respectively. When sick or dead wildlife are found, the event is reported to the WCS site manager and technical advisor who then report to the WCS Wildlife Health and Health Policy (WCS WHHP) Program (based in Phnom Penh). The ACCB notifies cases directly to WCS WHHP.

Arrangements are then made to collect samples or ship entire carcasses to Phnom Penh, where necropsies are carried out and samples collected. Samples are then shared with IPC for pathogen screening, and/or with alternative laboratories when necessary.

As a result of WCS/FA/MoE presence in the protected landscapes and their interaction with the community, other adverse health events involving humans or livestock are sometime reported or discussed with the field staff.

Chronology of Events and Field Investigation Activities

Detected Cases, Investigation, and Response:

- **January 22nd 2015:**

One Woolly-necked stork was found dead next to a trapeang (water hole) near Tmabauy Village, Pring Thom Commune, Chamksan District, Preah Vihear Province (Fig. 1). Another woolly neck stork was spotted on the same trapeang with difficulty flying, but could not be captured. Dead fishes were also observed floating in the trapeang.



Figure 1: Dead Woolly-necked stork found near Tmabauy village

- **January 2015:**

Five cattle were sick (no deaths) after drinking water in the same area nearby Tmatbauy Village

- **February 8th 2015:**
Five villagers experienced vomiting, stomach cramps, dizziness within 5-10 minutes of drinking trapeang water (Trapeang Krosang), near Tmatbauy Village, Pring Thom Commune, Chamksan District, Preah Vihear Province. One villager from the same group who did not drink the trapeang water did not experience any symptom. As a response, the trapeang was emptied.
- **February 2015:**
A poisoned trapeang was found close to Tmatbauy village (Fig 2), as evidenced by the presence of dead fish in the water and poison pellets spread around the edge of the trapeang near the eco-tourism guesthouse, suggesting an intentional poisoning of the waterhole.



Figure 2: Poisoned waterhole close to Tmatbauy village

- **February 24th 2015:**
A Large-spotted Civet was found dead close to a trapeang (about 55m) (Fig. 3), within Hun Sen Park boundary, near Trapeang Pring, Chheab Mouv Commune, Chheab District. Bait was found as piles of rice mixed with purple poison pellets (Fig. 3), indicating a deliberate attempt to poison animals. Trapeang water was sampled. As a response, FA Patrol rangers cleared all dead fish from the area and used a generator to pump out all water



Figure 3: Large-spotted civets and poisoned bait

- **March 5th 2015:**
Two large-spotted Civet were found dead near to Doungphlet Village, Chheab District, Preah Vihear Protected Forest.

- **March 6th 2015:**
The Cambodian Government's Zoonoses Technical Working Group (including NaVRI, FA, Ministry of Health, IPC, WCS, World Health Organization, US-CDC, Food and Agriculture Organization) was briefed about this issue with a request for support towards the investigation of suspect poisoning in wildlife, livestock and humans.
- **March 7th 2015:**
The MoE and the Commune Council held a village meeting in Tmatbauy Village, in response to previous events at this location.
- **March 8th 2015:**
Suspected poison collected by WCS/FA patrol team in the field (bait) was compared to pesticides found on Tbeng Meanchey market (Fig. 4). This led to the suspicion that carbofuran was related the cluster of cases



Figure 4: Bag of pesticide found on the market matching the poisoned bait

- **August 23rd 2015:**
A slender-billed vulture was found sick with abnormal behavior including head-dropping and inability to fly in Trapeang Ropov, Western Siem Pang (Fig. 5). It died within two days.

These events are summarized in a timeline (Annex A) and geographically located in the following map (Fig. 6).



Figure 5: Slender-billed vulture found sick in Trapeang Ropov

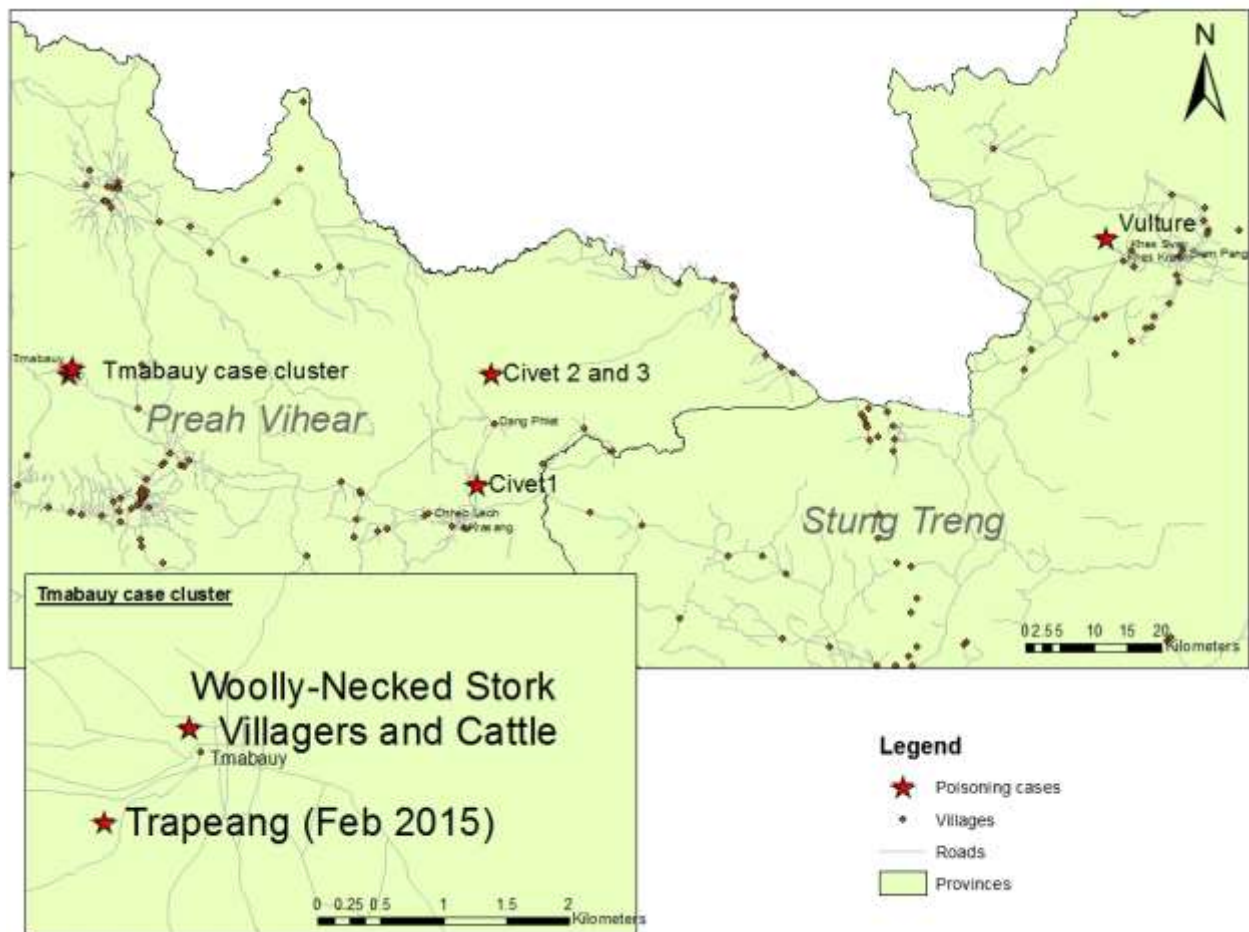


Figure 6: Geographical distribution of detected cases between January 2015 and August 2015 in Cambodia



Wildlife Clinical and Post-Mortem Examination Results

Species (Protection status)	Clinical Signs	Post-Mortem Findings
Woolly-necked stork (Vulnerable)	Weak, difficulty to fly	Lung congestion. Abundant fish bones in crop. *
Large-spotted civet (Vulnerable)	NA	Stomach contained remains of rodent, birds and rice. Presence of rice is consistent with the bait found near the Trapeang (no other human settlement around as potential source of rice). *
Large-spotted civet (Vulnerable)	NA	Stomach contained remains of rat and large quantity of fish bones. No other significant observation. *
Large-spotted civet (Vulnerable)	NA	Stomach full of fish bones. No other significant observation. *
Slender-billed vulture (Critically endangered)	Abnormal behavior, head dropped, unable to fly	Large quantity of meat in crop (feeding by field team in days preceding death) but rest of digestive track empty. Congestion visible over cerebellum.

* Advanced decomposition of the carcass limited the possible necropsy findings

Although many of the affected animals were recovered in an advanced state of decomposition, no gross pathology findings suggested any particular infectious cause. The moderate congestion of some organs (including brain) and the presence of abundant food (including poison bait) in the crop, gizzard, or stomach were all consistent with the poisoning hypothesis.

Relevant Pathogen Laboratory Testing Results

To exclude any important pathogens as potential causes for mortalities, additional testing was carried out by IPC on samples collected during the necropsies.



Species (Protection status)	Pathogen	Test result
Woolly-Necked stork (Vulnerable)	Flaviviridae	Negative
	JEV	Negative
	Influenza virus	Negative
Large-spotted civet 1 (Vulnerable)	NA	
Large-spotted civet 2 (Vulnerable)	Coronavirus	Negative
Large-spotted civet 3 (Vulnerable)	Coronavirus	Negative
Slender-billed vulture (Critically endangered)	Influenza virus	Negative
	West Nile virus	Negative

Toxicology Laboratory Testing Process and Results

Laboratory Identification and Export Process:

Given the strong suspicion of poisoning for these 5 cases of sick and dead wild animals, and the additional symptoms observed in the villagers and livestock and the information obtained during the field investigation. Decision was made to test the related samples for the presence of toxic residues. In particular, given the evidence found in the field for the use of poisoned baits and the resemblance of the poison granules to carbofuran pellet found on the local market, for the presence of carbofuran residues in these samples was prioritized.

Attempts were made to find a laboratory which could identify carbofuran residues in water and stomach content samples; these would confirm that the deaths were likely caused by the pesticide. In Cambodia, none of six laboratories (the most likely to be able to conduct this analysis) had protocols in place to identify carbamate residues in water or biological samples. Some of these laboratories had the appropriate equipment, but the individuals initially trained for such tasks, were no longer working for the laboratory. Additionally some of these laboratories simply did not have a protocol in place for this type of analysis.

Appropriate export authorizations were obtained from FA and MAFF to send samples to the Agri-food and Veterinary Authority (AVA) of Singapore, Pesticide Residues Section. The steps for this process were as follow (see also Annex A):

- October 27th 2015: Initial request from WCS to FA
- December 23rd 2015: Letter forwarded from FA to MaFF
- January 23rd 2016: Permitting letter from MaFF to the Convention on International Trade in Endangered Species (CITES) Cambodia office
- February 9th 2016: Permitting letter from MaFF to FA
- February 23rd 2016: Request letter from WCS to Custom & Excise of Cambodia
- March 4th 2016: Samples sent via World Courier



Toxicology Laboratory Analysis:

The AVA Laboratory used the following procedure for sample analysis. Digestive tract content samples were extracted with acetonitrile, and water sample was extracted with dichloromethane then solvent exchange into acetonitrile. The sample analysis was conducted on LCMSMS system (Liquid chromatography-tandem Mass Spectrometry) to detect the carbofuran residues. Gas Chromatography-tandem Mass Spectrometry technique (GCMSMS) was also used to look for additional pesticides.

Results are summarized in the table below:

Origin/Species	Sample Description	Test Results (ppm)	
		N-Methylcarbamate Pesticides (NMCs group tests)	Organophosphorus group tests
Woolly-necked stork	Gizzard Content	Carbofuran: 1.10	Not Detected
Large spotted civet 1	Stomach Content	Carbofuran: 0.70	Not Detected
Trapeang Water	Water sample from trapeang close to death location of civet 1	Carbofuran: 1.42; Carbofuran, 3-hydroxyl: 0.98	Not Detected
Large spotted civet 2	Stomach Content	Carbofuran: 351.74; Carbofuran, 3-hydroxyl: 0.30	Not Detected
Large spotted civet 3	Stomach Content	Carbofuran: 1630.70 Carbofuran, 3-hydroxyl: 0.70	Not Detected
Slender-billed vulture	Crop Content	Carbofuran: 0.08	Not Detected

The following comments can be made of these results:

- Carbofuran, the highly toxic N-methylcarbamate insecticide, was detected in all six samples. Carbofuran is one of the most toxic carbamates. In general, the amount of a chemical that is lethal to one-half (50%) of experimental animals fed the material, is referred to as its acute oral lethal dose fifty, or LD50. Carbofuran has an acute oral LD50 for several type of birds in the low ppm levels . (e.g., the acute oral LD50 for Japanese quail is as low as 2 ppm [1]). This means one granule of pesticide is often sufficient to kill a small bird. Bird deaths have also occurred when predatory or scavenging birds have ingested small birds or mammals which had eaten carbofuran pellets [2]. The oral LD50 for rats is 5 ppm, and for dogs is 19 ppm. [3]. It is also extremely toxic to fish with a 96-hour LD50 for fish is 150 ug/L.
- The carbofuran level in the stomach contents of the Large spotted civets 2 and 3 were 352 ppm and 1631 ppm respectively, which is considered extremely high, and certainly lethal.



- The carbofuran levels in the gizzard contents of the Woolly-necked stork and in the stomach content of the Large spotted civet 1 were at low ppm level (0.7 ppm and 1.1 ppm, respectively). The decomposition of bodies accelerate the decomposition of carbofuran, through microorganism activity. Both animals were in extremely poor condition upon recovery, and it is not surprising to observe lower levels of carbofuran. These results, and the contextual information indicate that carbofuran is still a very likely cause of death for these two animals.
- The level of carbofuran and 3-hydroxyl carbofuran (toxic metabolite) in the water sampled in the trapeang where civet 1 died, was found to be at 1.42 ppm and 0.98 ppm, respectively, which is considered to be high. Typically, carbofuran residues in irrigation water arising from the normal agricultural use of carbofuran would have been at low part per billion (ppb) levels. This explains the fish die-off observed on the trapeang, confirms that the death of civet 1 was very likely caused by carbofuran, and illustrates clearly the risk for livestock and humans using the trapeang after similar intentional poisoning acts.
- The low value found in the Slender-billed vulture are likely related to the fact that the sample sent to the laboratory was the meat found in the crop, but this meat was fed by the rescue team after the animal was found sick. The toxicology testing may therefore have detected the residues that were in the crop from an exposure to carbamate preceding the feeding of meat. This is supported by the consistent neurological symptoms observed on the live animal. In conclusion, the exposure to carbofuran is likely to have caused the condition of the vulture leading to its death.

Additional Suspect Poisoning Events

- Field reports from **WCS, Birdlife International, ACCB, and WWF** suggest that the practice of intentional poisoning of wild animals is frequent. Following the cluster of cases described earlier in this report, these four NGOs prepared a local communication campaign in the area most affected to encourage villager to stop these dangerous hunting practices. They are planning additional communication campaigns to keep addressing these issues.
- The **Cambodia Vulture Conservation Project (CVCP)** reported over 51 suspected poisoning of vulture deaths between 2005 and 2015 (including 35 vultures in four different events). These were secondary poisonings related to vulture consumption of poisoned cattle and dogs; vultures were found dead around the cattle and dog carcasses. It is noteworthy that all three species of vultures in Cambodia are critically endangered.



- A **Giant Ibis** found dead on its nest in November 2014 outside of Tmabauy Village, Pring Thom Commune, Chamksan District, Preah Vihear Province. The bird could not be necropsied and no sample was available, but the field team had suspicions that the animal had been poisoned from the nearby trapeang.
- The **Angkor Center for Conservation of Biodiversity** (ACCB) reported on April 24th 2016 (see Annex A) the mortality of three wild birds close to a man-made water hole containing insecticide granules (similar to commercial product described earlier). No necropsy was performed and samples were not obtained but the field observations indicated that the bird deaths are certainly related to the intentional use of pesticides for hunting purpose.
- There is very **limited data** available on the consequence of such pesticide misuse in livestock and humans. Given this report and the frequency of this practice, it is likely to be an underestimated issue.



Discussion and Conclusions

Between January and August 2015, a cluster of five cases of Carbofuran-poisoned wildlife was confirmed by field observations, necropsy findings, and toxicology laboratory testing. These findings are significant as poisoning cases of wildlife, human, and livestock in Cambodia, while likely frequent, are rarely confirmed and documented. In some cases, as described here, wildlife mortality appears to be secondary to the intentional poisoning of a waterhole. It is not clear what the motivations for such practices are (bushmeat consumption, wildlife trade, pest control), but the consequences on the environment and wildlife are severe. There is growing concern in the conservation community over such practices, as it is considered as a key threat to wildlife and biodiversity conservation. Additionally, we believe that both humans and livestock were also likely poisoned from consuming the contaminated water of these poisoned trapeangs.

This investigation revealed that Carbofuran, an extremely toxic carbamate, was the cause of the observed wildlife mortality and likely for the human and livestock morbidity. Due to the very high toxicity of this pesticide and its metabolites, lethal doses can very easily be reached in the environment, in water and in animals, resulting in a large number of primary and secondary poisonings. Due to this high acute toxicity, particularly in birds, and the high ecotoxicity of this pesticide, it was banned from many countries in the world (US, Canada, Europe).

Delays in addressing these cases was a major concern, as more than one year passed between the February-March 2015 cluster and the final laboratory results (see timeline in Annex A). These delays were for the most part due to the difficulty to find a laboratory that could analyze the samples in Cambodia and then internationally, and to the time to obtain the appropriate sample export permit once the laboratory was identified. These delays were not compatible with a rapid response and appropriate management of the case.

Although we were able to document these events and provide definitive confirmation of the cause of death, their high frequency and widespread distribution, along with the large number of suspected (and often unconfirmed) cases, should draw immediate attention and concern among the conservation community, public health sector, and animal health sector. The detection of wildlife mortality has served as a sentinel event for larger environmental contaminations with consequences on the entire ecosystem, including humans and livestock. This illustrates the need for continued wildlife disease and mortality surveillance as piloted through the LACANET project. There is still much to learn about the extent of these poisoning practices and more generally about the use of pesticides in rural areas, and their impact on the health of wildlife, livestock and local communities. Building appropriate knowledge and sound epidemiological data, and taking action to address these issues will require a concerted effort from multiple stakeholders and ministries. The next section summarizes certain of our recommendations to reach these objectives.



Recommendations

- Better documentation of chemical poisoning cases in wildlife, livestock and humans is crucial to better estimate the magnitude of the issue of pesticide misuse through enhanced and integrated surveillance strategy
- Coordination between health sectors (public, livestock, and wildlife health) for case investigation is absolutely necessary: cases notified by any of these three sectors should be followed by actions of all three sectors to adequately assess the environmental and health impacts
- Training should be provided to relevant staff of the different sectors for investigation of suspected poisoning cases, including for data collection, field investigation, and sample collection
- Increasing local laboratory capacities for the detection of toxic residues in water and tissue samples is of paramount importance to allow an appropriate investigation of suspected poisoning cases. Such analytical capacity is essential to ensure appropriate assessment and response to this critical issue. The designation of a reference laboratory (preferably where equipment is already present), and the transfer of knowledge to relevant staff to ensure the durable availability of these skills in Cambodia should be considered as high priorities. In particular, where equipment is already available (Camcontrol, DGA, and possibly others as should be informed by a laboratory equipment survey), SOPs should be developed to deal with samples from human or animal origin for residue testing. Personnel should be trained accordingly, and appropriate agreements should be in place to ensure the prompt processing of requests from livestock, human or wildlife health sectors.
- Until laboratory capacities are developed, interim solutions should be in place to allow the processing and analysis of any sample collected during field investigation of suspected poisoning cases. In particular, faster procedures for sample export should be in place when samples need to be sent abroad for analysis, in order to ensure timely confirmation of suspected cases.
- Lists of authorized pesticides should be reviewed and a ban should be considered of commercial product containing carbofuran, to align with EPA decisions in the US, and other bans in effect in Canada and Europe.
- Legislation regarding the use of pesticide for intentional wildlife poisoning/hunting should be reviewed and clarified, and legal actions should be taken against people using these methods. More generally, the legal framework around pesticide use and their ecotoxicological effects should be reviewed in light of current practices in Cambodia, to reduce the environmental and health impact of pesticide residues. Enforcement of the law should then be maintained, in particular through routine monitoring of environmental contamination with pesticide residues and other chemicals, including contamination of agricultural products.
- Educating on pesticide safety and on the risk of pesticide misuse is also an important step to reduce the occurrence of these cases, including on the risk of primary and secondary poisoning (in particular through consumption of contaminated water or bushmeat). For this purpose, it is



essential to build on existing training programs or communication campaigns established by various governmental (MAFF, DGA) or non-governmental organizations (CEDAC, Fintrac, RDIC, WCS, BirdLife international, WWF, and others). In particular, communication campaigns being developed by conservation NGOs regarding the public health risk of bushmeat consumption and intentional wildlife poisoning for consumption, would greatly benefit from a MoH endorsement and active participation.

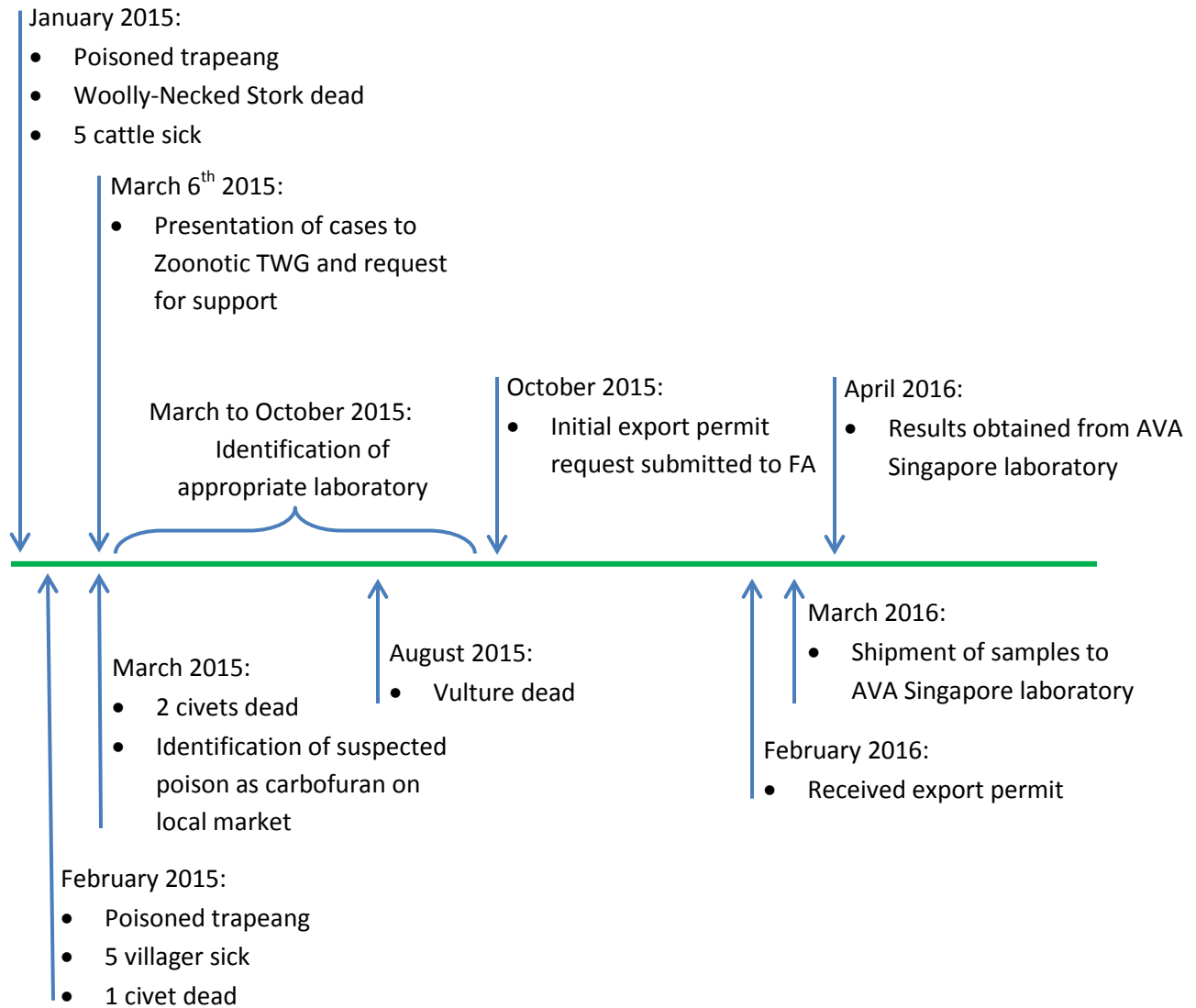
- Actions are required to better assess and understand the burden associated with poisoning in Cambodia. Retrospective studies on potential poisoning cases should be implemented based on existing public health surveillance as a first step. Further surveillance of chemical (and in particular pesticide) related health consequences need to be enhanced in the framework of a One Health approach, to address the current lack information on the impact on public health, livestock health, wildlife health and environmental health.

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ANNEX A: Overall timeline



ANNEX B

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Photos ▾





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Poisoning incident

Updated last Sunday ↻

The local man who has been employed in the past by ACCB to monitor and guard vulture nests recently informed ACCB Vulture Conservation staff that he had heard a report that poison was being used at a dry forest waterhole. When the staff members arrived they observed two small man-made holes, one of which still contained a purple granular substance. It is not possible to tell for certain what kind of poison it was, but termite-poison is thought to be by far the most commonly misused for this type of illegal activity. It seems the poison was being mixed with water and then birds that came to drink would immediately die of poisoning and be eaten by the poisoners. Frogs that drank would die, subsequently poisoning further birds to be collected for

cooking. ACCB staff recovered two dead adult Crested Serpent Eagles (*Spilornis chila*) only 5 m from the poison, and around 15 m away were a dead Red-billed Blue Magpie (*Urocissa erythroryncha*) and a Golden-fronted Leafbird (*Chloropsis aurifrons*). It is hard for us to imagine that anyone would want to poison an animal and then eat it, but those people obviously do not realise that this could be dangerous. In this case the poisoning was supposedly committed by illegal loggers camping out in the forest. Poisoning as a way of hunting is becoming one of the primary threats to many types of wildlife in Cambodia, and elsewhere around the world.

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