Bull Environ Contam Toxicol (2016) 96:25–30
DOI 10.1007/s00128-015-1688-0

Analysis of Insecticides in Dead Wild Birds in Korea from 2010 to 2013

Soohee Kim[1] · Mi-Young Park[1] · Hyo-Jin Kim[1] · Jin Young Shin[1,2] · Kyung Yuk Ko[1,2] · Dong-Gyu Kim[1,2] · MeeKyung Kim[1,2] · Hwan-Goo Kang[1] · ByungJae So[1] · Sung-Won Park[1]

Received: 29 January 2015 / Accepted: 27 October 2015 / Published online: 16 November 2015

Abstract Wild birds are exposed to insecticides in a variety of ways, at different dose levels and via multiple routes, including ingestion of contaminated food items, and dermal, inhalation, preening, and embryonic exposure. Most poisoning by insecticides occurs as a result of misuse or accidental exposure, but intentional killing of unwanted animals also occurs. In this study, we investigated insecticides in the gastric contents of dead wild birds that were suspected to have died from insecticide poisoning based on necropsy. The wild birds were found dead in various regions and locations such as in mountains, and agricultural and urban areas. A total of 182 dead wild birds of 27 species were analyzed in this study, and insecticide residue levels were determined in 60.4 % of the total samples analyzed. Monocrotophos and phosphamidon were the most common insecticides identified at rates of 50.0 % and 30.7 % of the insecticide-positive samples, respectively. Other insecticides identified in dead wild birds included organophosphorous, organochlorine and carbamate insecticides. However, there was limited evidence to conclusively establish the cause of death related to insecticides in this study. Nevertheless, considering the level of insecticide exposure, it is speculated that the exposure was mainly a result of accidental or intentional killing, and not from environmental residue.

✉ Sung-Won Park
pasawa@korea.kr

1 Animal and Plant Quarantine Agency (QIA), Anyang, Gyeonggi-do 14089, Republic of Korea

2 Present Address: Ministry of Food and Drug Safety, Cheongju, Chungcheongbuk-do 28159, Republic of Korea

Keywords Insecticides · Gas chromatography · Wild birds · Korea

Insecticides have been widely used in agricultural practices worldwide since the middle of the twentieth century to prevent the colonization and feeding of insects on plants and to inhibit outbreaks of diseases carried by insects to humans and animals (Peshin et al. 2014). In 1990, a WHO task group indicated that approximately one million people experience serious, unintentional poisoning with insecticides is happening each year (Jeyaratnam 1990; van der Hoek and Konradsen 2005). In the case of animals, poisoning is often caused by accidental exposure (Gupta 2011). Direct insecticide poisoning also occurs in wild animals via ingestion of insecticide-contaminated water, seeds, and foliage (Berny 2007). Three classes of insecticides have been particularly problematic namely, the organochlorine, organophosphorous, and carbamate insecticides. In Korea, annual agricultural use of pesticides has increased rapidly from the 1970s to 1990s, but has declined since 2001. The use of pesticide was decreased during 2010–2013 following the establishment of new eco-friendly approaches for insect pest control. Organophosphates were the most widely used among the top 50 pesticides between 2007 and 2011 (Cha et al. 2014). As of 2012, fenitrothion (14.2 tons) was the most commonly used, followed by iprodione (9.2 tons) and mancozeb (9.1 tons). Organophosphorous insecticides such as parathion, diazinon, dichlorvos, fenthion, phosphamidon, and monocrotophos are toxic owing to their ability to inhibit cholinesterase, thereby causing neurotoxicity. The presence of this enzyme in insects, birds, and other vertebrates confers an enormous toxicity potential of this class of insecticides with an enormous toxicity potential toward non-target species. Clinical signs result from the hyperstimulation of the parasympathetic autonomic nervous system, skeletal muscles, and the central nervous system (Mineau 2002). Carbamate insecticides are also cholinesterase inhibitors with a mechanism of action analogous to that of organophosphorous insecticide (Van Dyk and Pletschke 2011). Korea is on the East-Asian Australian flyway (Kang et al. 2010). Four hundred species of birds inhabit in Korea. Approximately 120 species of birds spend the winter in Korea, with 100 species inhabiting the country in the summer. Some bird species have experienced a reduction in numbers since the 1960s (Won 1983). This may be due to the destruction of habitats by industrialization, direct or indirect disturbance by humans, and an increase in the use of toxic contaminants such as organochlorine compounds and heavy metals (Lee et al. 1989). Recently, highly pathogenic avian influenza virus caused the deaths of several wild birds in Korea (Choi et al. 2010). In Korea, a variety of dead animals are submitted to the Animal and Plant Quarantine Agency from provincial veterinary service laboratories to diagnose diseases or determine the exact cause of death. Insecticide determination is performed based on analysis of the gastric contents of dead birds with suspected insecticide poisoning based on necropsy. In the present study, we identified the insecticides in the gastric contents of dead wild birds submitted to the Animal and Plant Quarantine Agency in Korea from 2010 to 2013. The objectives of the present study were to: (1) provide an updated estimate of the level of insecticide exposure of wild birds in Korea, (2) identify the insecticide class distribution and concentrations of insecticides in the gastric contents of dead birds, and (3) describe the temporal and regional distributions of the birds exposed to insecticides.

Materials and Methods

Chemicals All chemicals used were of analytical grade or higher and were obtained from Sigma-Aldrich (St. Louis, MO, USA) unless otherwise specified.

Sample Collection and Preparation A total of 182 stomach contents were collected from dead wild birds found dead in various regions and locations in Korea, such as mountains, and agricultural and urban areas, from 2010 to 2013. We identified the insecticides in the gastric contents of dead wild birds that were suspected to have died from insecticide poisoning based on necropsy. The analytical methods used in this study were developed and validated for 13 insecticides in previous studies, including 10 phosphorous (monocrotophos, phosphamidon, diazinon, EPN, fenitrothion, fenthion, methidathion, parathion, edifenphos, phorate), 1 organochlorine (endosulfan), and 2 carbamate (cabofuran and methomyl) insecticides (Kim et al. 2008; Ko et al. 2014; Kwon et al. 2004). The procedures of extraction and sample preparation were based on the modified QuEChERS technique (Ko et al. 2014; Wilkowska and Biziuk 2011). For extraction, 5 g of homogenized gastric contents was mixed with 15 mL of 1 % acetic acid in acetonitrile (v/v). After shaking and vortexing, 6 g of magnesium sulfate and 1.5 g of sodium acetate were added to the tube, and then the mixture was vigorously shaken. The acetonitrile layer was collected and cleaned using a dispersive cleanup kit from Agilent 5982-0029 (Little Falls, DE, USA). All samples were analyzed using gas chromatography with a flame photometric detector (GC-FPD) and mass spectrometry (GC–MS). The concentrations were calculated using the calibration curves obtained through the insecticide standard solution diluted from the standard solution. For each assay, both positive and negative controls were systematically included using blanks and fortified blanks. The limit of detection and limit of quantification values ranged from 0.03 to 5.84 mg/kg and from 0.09 to 17.70 mg/kg, respectively.

GC-FPD and GC–MS Conditions An Agilent Technologies 6890N gas chromatograph equipped with an FPD was used for separating and identifying the insecticides. Separation was achieved on a DB-5 column. The temperature of the column oven was set as follows: the initial temperature was 60°C, held for 1 min; increased to 260°C at a rate of 25°C/min, and held for 9 min. The GC–MS analysis was performed on an Agilent 7890A gas chromatograph (Agilent Technologies) coupled to an Agilent 5975C mass spectrometer (Agilent Technologies). The electrospray ionization source was used in ionization mode at an ionization energy of 70 eV. The temperature of the column oven was set as follows: the initial temperature was 60°C, held for 1 min; increased to 170°C at a rate of 25°C/min; increased to 175°C at a rate of 2°C/min; increased to 270°C at a rate of 10°C/min, held for 5.6 min.

Results and Discussion

A total of 182 dead wild birds of 27 species were submitted to our laboratory and analyzed in this study. The results of the analyses shown in Table 1 revealed that insecticides were identified in 60.4 % (110/182) of all samples. The pattern was similar across years, although the frequency of insecticide detection decreased slightly in 2013, with frequencies of 57.8 % (26/45), 61.8 % (55/89), 69.0 % (20/29), and 47.4 % (9/19) in 2010, 2011, 2012, and 2013, respectively. Of a total of 27 avian species examined, carcasses of 17 species were found to contain insecticide residues. Mallard (20.0 %, 22/110) was the species that most

Table 1 Numbers of wild birds analyzed and insecticides detected in the gastric contents in Korea from 2010 to 2013

Avian species		2010		2011		2012		2013		Total	
Common name	Scientific name	n, Req.[a]	n, Det.[b]	n, Req.	n, Det.	n, Req.	n, Det.	n, Req.	n, Det.	n, Req.	n, Det.
Baikal teal	*Anas formosa*			2	1					2	1
Black coot	*Fulica atra*			1						1	0
Bulbul	*Hypsipetes amaurotis*			1	1			1	1	2	2
Buzzard	*Buteo buteo*			1						1	0
Crane	*Grus vipio*			2		1	1			3	1
Crow	*Corvus corone*	2	2	3	3	2	1	2	2	9	8
Duck	*Anatidae* (*unclassified*)	10	5	7	6	4	2	1		22	13
Vulture	*Aegypius monachus*			8	3	2	2			10	5
Bean goose	*Anser fabalis*	16	8	8	8	3	3	3	1	30	20
Great tit	*Parus major*					1				1	0
Greenfinch	*Carduelis sinica ussuriensis*					1	1			1	1
Gull	*Larus canus*	1	1							1	1
Peregrine falcon	*Falco peregrinus japonensis*			1	1			1		2	1
Heron	*Ardea cinerea*			4						4	0
Kestrel	*Falco tinnunculus*			1						1	0
Loon	*Gavia stellata*	1								1	0
Magpie	*Pica pica serica*			1	1					1	1
Mallard	*Anas platyrhynchos*	2	1	21	16	5	4	2	1	30	22
Mandarin duck	*Aix galericulata*	3	3	2	2	1	1			6	6
Parrot	*Psittacidae*					1		1		2	0
Pheasant	*Phasianus colchicus karpowi*	1		1	1					2	1
Pigeon	*Columba rupestris*	1	1	1	1	2	2	4	3	8	7
Quail	*Coturnix japonica*					1				1	0
Spotbill duck	*Anas poecilorhyncha*			15	9					15	9
Stork	*Ciconia boyciana*			1						1	0
Swan	*Cygnus columbianus*	1		2				2		5	0
Thrush	*Turdus pallidus*			1						1	0
Unconfirmed	7	5	5	2	5	3	2	1	19	11	
Total	45	26	89	55	29	20	19	9	182	110	

[a] Number of birds submitted to analyses

[b] Number of birds with insecticide detections

often had insecticide residues in the gastric contents, followed by bean goose (18.2 %, 20/110). Overall, 20.0 % (22/110) of the insecticide-positive samples were from mallards and 18.2 % (20/110) samples were from bean geese. A relatively large proportion (10.4 %, 19/182) of the samples could not be positively identified to species. A more detailed analysis of the data according to insecticide class is shown in Table 2. These data showed that organophosphorous insecticides were responsible for most of the insecticide residues detected. Among all insecticide cases, 101/114 (88.6 %) were caused by organophosphorous, 11 (9.6 %) by carbamate, and 2 (1.7 %) by organochlorine insecticides. Two organophosphorous insecticides, monocrotophos and phosphamidon were the two most common insecticides identified in this study, accounting for 50.0 % (57/114) and 30.7 % (35/114) of all insecticide-positive cases, respectively. Other organophosphorous (i.e., diazinon, EPN, ethoprophos, fenitrothion, fenthion, methidathion, and phorate), organochlorine (i.e., endosulfan), and carbamate (i.e., carbofuran and methomyl) insecticides were also found in various concentrations in samples from the dead wild birds. In 4 of the 110 insecticide-positive samples, two different insecticides were detected simultaneously. The monthly distribution of avian carcass submission is shown in Fig. 1. The overall number of birds with insecticide detections was substantially higher in winter than in other

Table 2 Number and concentrations of insecticides detected in wild birds according to insecticide class: carbamates, organochlorines, and organophosphorous insecticides

	2010		2011		2012		2013		Total	
	n, Det.[a]	Range (mg/kg)	n, Det.	Range (mg/kg)	n, Det.	Range (mg/kg)	n, Det.	Range (mg/kg)	n, Det.	Range (mg/kg)
CA[b]										
Carbofuran	1	113.0	2	75.2–297.6					3	75.2–297.6
Methomyl	3	761.0–900.0	4	27.7–779.5	1	160.3			8	27.7–900.0
OC[c]										
Endosulfan	1	29.7	1	644.9					2	29.7–644.9
OP[d]										
Diazinon			1	133.0			1	105.1	2	105.1–133.0
EPN					1	5339.0			1	5339.0
Fenitrothion			2	1.0–101.4					2	1.0–101.4
Fenthion			1	669.3					1	699.3
Methidathion							1	452.0	1	452.0
Monocrotophos	12	0.6–7557.0	30	0.8–306.8	12	3.9–151.5	3	4.22–66.5	57	0.6–7557.0
Phorate	2	0.1							2	0.1
Phosphamidon	8	11.3–622.6	17	14.9–305.2	6	1.2–337.0	4	37.3–638.0	35	1.2–638.0
Total	27		58		20		9		114	0.6–5339.0

[a] Number of birds with insecticide detections

[b] Carbamates

[c] Organochlorines

[d] Organophosphorous

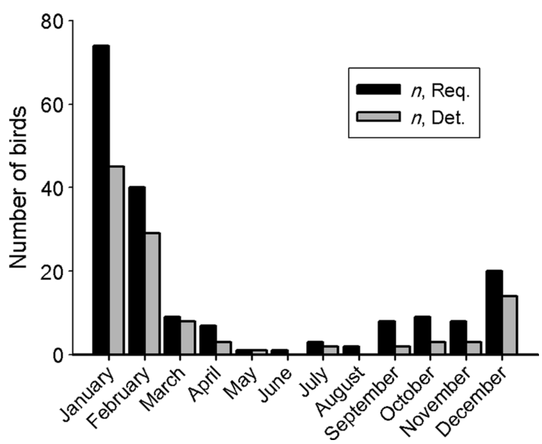

Fig. 1 Temporal distribution of insecticide-positive birds from 2010 to 2013 in Korea

Fig. 2 Map of the locations where the carcasses of wild birds were collected in Korea during 2011–2013

seasons, which correlated with the number of birds submitted over time. Figure 2 and Table 3 show the locations of the collection of the dead birds. Unfortunately, we have only partial information regarding the regional distribution of the dead birds. We could not obtain the regional information of the dead birds submitted in 2010. The highest number of

Table 3 Regional distribution of birds with insecticide detections from 2010 to 2013 in Korea

Region	2010		2011		2012		2013		Total	
	n, Req.	n, Det.	n, Req.	n, Det.	n, Req.	n, Det.	n, Req.	n, Det.	n, Req.	n, Det.
Busan			1	1	1				2	1
Incheon			1	1			3	2	4	3
Ulsan			3	2	1	1	3	2	7	5
Gwangju			1				2	2	3	2
Jeju-do			2						2	0
Gyeonggi-do			19	11	10	9	3	1	32	21
			1	1	1	1			2	2
Chungcheongbuk-do			3	3	3	2	1		7	5
			23	19	3	2	1		27	21
Gyeongsangbuk-do			5	4			1		6	4
			13	4					13	4
Jeollabuk-do			7	4	6	2	3	2	16	8
			6	4	4	3	2		12	7
Unknown	45	26	4	1					49	27
Total	45	26	89	55	29	20	19	9	182	110

dead birds submitted was in 2011, and most carcasses were found in Gyeonggi-do from 2011 to 2013.

The present study provides information on the insecticides found in the gastric contents of dead wild birds in Korea in 2010–2013. A total of 182 stomach contents from dead wild birds with suspected insecticide poisoning based on necropsy were analyzed. Based on the results of our 4-year study, it can be concluded that monocrotophos, an organophosphorous insecticide, was the most prominent compound detected in positive samples. The widespread use of monocrotophos is of particular concern because of its high avian toxicity. The LD50 values of monocrotophos are low for avian species, which are typically below 7 mg/kg (Goldstein et al. 1999). Fortunately, however, monocrotophos has a low environmental persistence as its half-life is only 7 days, similar to many other organophosphorous insecticides. Although monocrotophos has been banned as a registered insecticide in Korea since 2011, it can be used up to 2015 according to the sell-by date. Consistent with our results, previous studies showed that organophosphorous insecticides, including monocrotophos and phosphamidon, were most frequently identified in wild birds from 1998 to 2007 in Korea (Kim et al. 2008; Kwon et al. 2004). In Europe and the US, organophosphorous or carbamate insecticides and anticoagulant rodenticides are the most frequently reported causes of intoxication in wild birds (Berny 2007; Fleischli et al. 2004). Among a total of 394 species of wild birds found in Korea (Woo and Yoon 1989), 182 birds of 27 species were examined in this study. Waterfowl, including mallard and bean goose, which are known as winter migratory birds in Korea, were the most common birds found dead. As shown in Fig. 1, there were many more birds with insecticide detections in winter than in summer. In addition, this pattern of seasonal distribution is similar to the moving pattern of migratory birds in Korea. A variety of migratory birds, including mallard, mandarin duck (*Aix galericulata*), and bean goose, fly from Russia to Korea during the winter for the breeding season (Yeh et al. 2011). The locations of the collection of the carcasses of wild birds in Korea from 2011 to 2013 are evenly spread across the nation. Ingestion of grains that are intentionally treated with insecticides is considered to be the most common route of exposure to insecticides in wild birds (Hill and Fleming 1982; Quick 1982; Reece and Handson 1982). The dead wild birds submitted to us for analysis were suspected to have died from insecticide poisoning based on necropsy. Furthermore, the insecticide concentration level in the gastric contents identified in this study is somewhat higher considering the acute oral LD50 in birds. For example, the acute oral LD50 values in birds for monocrotophos and phosphamidon are 0.94–6.5 mg/kg and 3.6–7.5 kg, respectively. In most cases, the deaths of birds evaluated in this study appear to have been caused by the deliberate abuse of pesticides. Although we successfully identified the causative insecticides in some cases, it is often difficult to conclusively diagnose insecticide poisoning as a cause of death in wild birds, and not all cases can be solved. In general, the diagnosis of insecticide poisoning is based on a history of exposure, symptoms, post-mortem examinations, cholinesterase activity in the plasma or brain, and via direct insecticide analysis. However, symptoms of poisoning are not very specific and can vary substantially between species. A post-mortem examination is essential, as most animal deaths resulting from insecticide poisoning may not be readily apparent without it (Sheffield et al. 2012). External examination is also necessary to rule out natural or accidental causes (Sheffield et al. 2005). Occasionally, clinical signs such as internal bleeding induced by poisoning of anti-coagulant rodenticides may provide strong evidence to identify the class of compound involved. Alternately, analysis of brain cholinesterase activity is widely used as a diagnostic technique. However, diagnosis of inhibition of cholinesterase activity is interpreted based on the normal value for each species (Blakley and Skelley 1988), and it may be confused with changes in cholinesterase levels due to post-mortem alterations. Therefore, the findings reported by onsite investigators and complete histories of avian mortality events are essential for obtaining a correct diagnosis of insecticide poisoning (Kwon et al. 2004). Where no cause of death is apparent from a post-mortem examination, poisoning may be suspected. In such cases, the preferred tissue samples for the detection of insecticides in wildlife are proposed to be the stomach and gizzard contents (Brown et al. 1996; Mineau 2002). The results of this investigation describe the insecticide contamination status of wild birds in Korea. More attention should be paid to insecticide poisoning and future efforts to reduce the number of insecticide-related deaths are needed to help preserve wild bird populations.

Acknowledgments We thank the staff of the Avian Disease Division at the Animal and Plant Quarantine Agency (QIA). This research was supported by a grant from the Animal and Plant Quarantine Agency, the Ministry of Agriculture, Food and Rural Affairs of the Republic of Korea.

References

Berny P (2007) Pesticides and the intoxication of wild animals. J Vet Pharmacol Ther 30:93–100

Blakley BR, Skelley KW (1988) Brain cholinesterase activity in animals and birds. Vet Hum Toxicol 30:329–331

Brown P, Charlton A, Cuthbert M et al (1996) Identification of pesticide poisoning in wildlife. J Chromatogr A 754:463–478

Cha ES, Jeong M, Lee WJ (2014) Agricultural pesticide usage and prioritization in South Korea. J Agromedicine 19(3):281–293

Choi JG, Kang HM, Jeon WJ et al (2010) Characterization of clade 2.3. 2.1 H5N1 highly pathogenic avian influenza viruses isolated from wild birds (mandarin duck and Eurasian eagle owl) in 2010 in Korea. Viruses 5(4):1153–1174

Fleischli MA, Franson JC, Thomas NJ, Finley DL, Riley W (2004) Avian mortality events in the United States caused by anticholinesterase pesticides: a retrospective summary of National Wildlife Health Center records from 1980 to 2000. Arch Environ Contam Toxicol 46:542–550

Goldstein MI, Lacher TE, Woodbridge B et al (1999) Monocrotophos-induced mass mortality of Swainson's Hawks in Argentina, 1995–96. Ecotoxicology 8:201–214

Gupta PK (2011) Epidemiological studies of anticholinesterase pesticide poisoning in India. In: Satoh T, Gupta RC (eds) Anticholinesterase pesticides: metabolism, neurotoxicity, and epidemiology. Wiley, Hoboken, pp 417–431

Hill EF, Fleming WJ (1982) Anticholinesterase poisoning of birds: field monitoring and diagnosis of acute poisoning. Environ Toxicol Chem 1:27–38

Jeyaratnam J (1990) Acute pesticide poisoning: a major global health problem. World Health Stat Q 43(3):139–144

Kang HM, Jeong OM, Kim MC et al (2010) Surveillance of avian influenza virus in wild bird fecal samples from South Korea, 2003–2008. J Wildl Dis 46(3):878–888

Kim MK, Yun SJ, Kim DG, Bong YH, Kim H, Jang JH, Chung GS (2008) Determination of pesticides in dead wild birds in Korea (in Korean). Korean J Vet Res 48:131–137

Ko KY, Shin JY, Kim DG et al (2014) Determination of organophosphorus pesticides in stomach contents of postmortem animals by quechers and gas chromatography. J Anal Toxicol 38:667–671

Kwon YK, Wee SH, Kim JH (2004) Pesticide poisoning events in wild birds in Korea from 1998 to 2002. J Wildl Dis 40:737–740

Lee DP, Honda K, Tatsukawa R, Won PO (1989) Distribution and residue level of mercury, cadmium and lead in Korean birds. Bull Environ Contam Toxicol 43(4):550–555

Mineau P (2002) Estimating the probability of bird mortality from pesticide sprays on the basis of the field study record. Environ Toxicol Chem 21:1497–1506

Peshin SS, Srivastava A, Halder N, Gupta YK (2014) Pesticide poisoning trend analysis of 13 years: a retrospective study based on telephone calls at the National Poisons Information Centre, All India Institute of Medical Sciences, New Delhi. J Forensic Leg Med 22:57–61

Quick MP (1982) Pesticide poisoning of livestock: a review of cases investigated. Vet Rec 111:5–7

Reece RL, Handson P (1982) Observations on the accidental poisoning of birds by organophosphate insecticides and other toxic substances. Vet Rec 111:453–455

Sheffield SR, Sullivan JP, Hill EF (2005) Identifying and handling contaminant-related wildlife mortality/morbidity. In: Braun CE (ed) Techniques for wildlife investigations and management. The Wildlife Society, Bethesda, pp 213–238

Sheffield SR, Sullivan JP, Hill EF (2012) Identifying and handling contaminant-related wildlife mortality or morbidity. In: Silvy N (ed) Wildlife techniques manual, 7th edn. John Hopkins University Press, Baltimore, pp 154–180

van der Hoek W, Konradsen F (2005) Risk factors for acute pesticide poisoning in Sri Lanka. Trop Med Int Health 10:589–596

Van Dyk JS, Pletschke B (2011) Review on the use of enzymes for the detection of organochlorine, organophosphate and carbamate pesticides in the environment. Chemosphere 82:291–307

Wilkowska A, Biziuk M (2011) Determination of pesticide residues in food matrices using the QuEChERS methodology. Food Chem 125:803–812

Won PO (1983) Bird population in the Nakdong estuary-Assessment of ecological impact of Nakdong estuary barrage and land reclamation Project. Theses Collect Kyung Hee Univ 12:67–84 **(in Korean)**

Woo HJ, Yoon MB (1989) Wild birds of Korea. Academic Press, Seoul, pp 10–12 **(In Korean)**

Yeh JY, Kim HJ, Nah JJ et al (2011) Surveillance for West Nile virus in dead wild birds, South Korea, 2005–2008. Emerg Infect Dis 17:299–301