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Field study on the use of inactivated H5N2 vaccine in avian species

SIR, – Avian influenza serovar H5N1 has become a major potential threat to both avian and human life in south-east Asia. The virus has been isolated in domestic poultry and ducks in Hong Kong, southern China, Vietnam, Cambodia, Thailand, Indonesia and Malaysia. It has caused human fatalities in many of these countries. The World Health Organization (WHO) has repeatedly warned of a pandemic in Asia.

In the midst of the outbreak of highly pathogenic avian influenza (HPAI) in Asia, we in the Singapore Zoological Gardens decided to vaccinate our high-risk species (Galliformes and Anseriformes) as well as the birds in open exhibits. However, the vaccine that is commercially available is an inactivated H5N2 vaccine and is manufactured for domestic poultry. We used Nobilis Influenza H5N2 (Intervet), which is an inactivated vaccine against avian influenza type A, subtype H5 from the strain A/Chicken/Mexico/232/94/CPA, maintained by Pronabive. Working seeds are obtained exclusively from Pronabive for vaccine production. The safety and efficacy of such a vaccine has not been tested in other birds.

We used the same vaccination protocol recommended for use in domestic poultry, giving a booster one month after initial vaccination. The birds were inoculated intramuscularly with a dose of 0·5-ml/kg bodyweight. We collected blood samples from the vaccinated birds before vaccination, and after vaccination at four weeks and six to eight weeks to test for the presence of antibodies.

Serum was tested by the standard haemagglutination inhibition (HI) test (OIE 2004) for antibody to H5 avian influenza using avian influenza A/Duck/ Singapore-Q/F119-3/97 subtype H5N3 as the virus antigen. If the birds were still negative after the booster, a second booster was administered and blood sampled two weeks after that. Three vaccinations was the maximum number administered even if the birds still remained seronegative. A re-evaluation was done six months later for some of the birds. The blood samples were all sent to the same laboratory, Agri-Food and Veterinary Authority of Singapore, for testing.

Sampling of the birds six months later was from a smaller population as some of the birds were culled, missing or had died from an unrelated cause. The ornamental ducks could not be retested due to logistical difficulty in trapping the birds. The pheasants, pelicans, cormorants and owls had been vaccinated more recently and had not passed the six-month stage at the time of writing.

The birds used in this study are listed in Table 1.

The vaccinated birds did not suffer any adverse reactions or deaths related to the vaccination.

We observed a variation in response between and within both species and taxonomic orders. Results (Tables 1, 2) indicated 100 per cent seroconversion after the booster in Egyptian geese, bar-headed geese, Radjah shelducks, the black swan, domestic ducks, the ringed teal and Canada geese (order Anseriformes), and peafowl, golden pheasants, the crested fireback pheasant and silver pheasants (order Galliformes). A partial response was recorded in spur-winged geese, white-faced whistling ducks, spotted whistling ducks (order Anseriformes), guineafowl (order Galliformes) and cormorants (order Pelicaniformes). Some species like the pelicans (order Pelicaniformes) and owls (order Strigiformes) failed to respond to vaccination at all.

Six groups of birds were blood sampled seven to eight months after the first and second vaccinations, and of these only the peafowls and bar-headed geese retained satisfactory seroconversion rates of 100 per cent; the guineafowl and Egyptian geese showed 80 per cent and 67 per cent retention, respectively. The

TABLE 1: Seroconversion rate* of different species at day 0, four weeks, eight weeks and six months following vaccination

Species	Population	Number (%) seroconverted					Comments
		Day 0 (first vaccination given)	Four weeks (booster given)	Number (%) requiring third vaccination	Eight weeks†	Six months	
Common peafowl (*Pavo cristatus*)	16	0/16	12/16 (75)	4/16 (25)	16/16 (100)	14/14 (100)	Two died from trauma
Egyptian goose (*Alopochen aegyptiacus*)	4	0/4	NS	0/0 (100)	4/4 (100)	2/3 (67)	One went missing
Magpie goose (*Anseranas semipalmata*)	2	0/2	NS	0/0 (100)	2/2 (100)	NS	Two went missing
White-faced whistling duck (*Dendrocygna viduata*)	6	0/6	3/6 (50)	3/6 (50)	5/6 (83)	NS	Unable to confine six months later
Bar-headed goose (*Anser indicus*)	16	0/16	15/16 (94)	1/16 (6)	16/16 (100)	11/11 (100)	Four went missing and one died due to infection
Radjah shelduck (*Tadorna radjah*)	6	0/6	5/6 (83)	1/6 (17)	6/6 (100)	NS	Unable to confine six months later
Spotted whistling duck (*Dendrocygna guttata*)	15	0/15	3/15 (20)	12/15 (80)	12/15 (80)	NS	Unable to confine six months later
Guineafowl (*Acryllium vulturinum* and *Numida meleagris*)	9	0/9	2/9 (22)‡	7/9 (78)	8/9 (89)	4/5 (80)§	‡Six samples haemolysed, §four died due to various reasons
Black swan (*Cygnus atratus*)	1	0/1	1/1 (100)	0/1 (0)	1/1 (100)	0/1 (0)	
Domestic duck (*Anas* species)	8	0/8	7/8 (88)	1/8 (13)	8/8 (100)	NS	Culled before six months
Spur-winged goose (*Plectropterus gambensis*)	5	0/5	0/5 (0)	5/5 (100)	4/5 (80)	0/5 (0)	
Domestic goose (*Anser* species)	5	0/5	0/5 (0)	5/5 (100)	5/5 (100)	NS	Culled before six months
Canada goose (*Branta canadensis*)	2	0/2	0/2 (0)	2/2 (100)	2/2 (100)	NS	Culled before six months
Ringed teal (*Callonetta leucophrys*)	1	0/1	1/1 (100)	0/1 (0)	1/1 (100)	NS	Unable to confine six months later
Golden pheasant (*Chrysolophus pictus*)	5	0/5	5/5 (100)	0/5 (0)	5/5 (100)	NYS	
Crested fireback pheasant (*Lophura ignita*)	1	0/1	0/1 (0)	1/1 (100)	1/1 (100)	NYS	
Silver pheasant (*Lophura nycthemera*)	2	0/2	2/2 (100)	0/2 (0)	2/2 (100)	NYS	
Pelican (*Pelicanus* species)	4	0/4	0/4 (0)	4/4 (100)	0/4 (0)	NYS	
Little black cormorant (*Phalacrocorax sulcirostris*)	3	0/3	0/3 (0)	3/3 (100)	1/3 (33)	NYS	
Owls (*Bubo bubo and Tyto alba*)	7	0/7	0/7 (0)	7/7 (100)	0/7 (0)	NYS	

* Minimum titre of 1/16 by haemagglutination inhibition test
† Some birds had a third vaccination at six weeks
NS Not sampled, NYS Not yet sampled (not reached six months)

TABLE 2: Avian influenza antibody titres in selected individual birds up to six months

Species	Titre at Four weeks after first vaccination	Two to four weeks after booster vaccination	Four to six weeks after booster vaccination	Six months
Egyptian goose	0	1/64	NC	0
Egyptian goose	1/16	1/128	NC	1/32
Egyptian goose	0	1/128	NC	0
Peafowl*	0	1/128	1/512	1/32
Peafowl	1/64	1/256	NC	1/64
Peafowl	1/128	1/128	NC	1/128
Peafowl*	0	0	1/1024	1/32
Peafowl	1/128	1/256	NC	1/32
Peafowl	1/64	1/256	NC	1/64
Peafowl*	0	1/32	1/128	1/32
Peafowl	1/32	1/128	NC	1/128
Peafowl	1/512	1/1024	NC	1/64
Peafowl	Unavailable	1/64	NC	1/64
Peafowl	1/256	1/512	NC	1/128
Peafowl	1/64	1/256	NC	1/32
Black swan	1/64	1/128	NC	0
Helmeted guineafowl	0	1/256	NC	1/32
Bar-headed goose	1/64	1/2048	NC	1/128
Bar-headed goose	1/128	1/2048	NC	1/32
Bar-headed goose	1/128	1/2048	NC	1/32
Bar-headed goose	1/256	1/512	NC	1/32
Bar-headed goose	1/64	1/256	NC	1/16
Bar-headed goose	0	1/128	NC	1/32
Bar-headed goose	1/128	1/1024	NC	1/64
Bar-headed goose	1/128	1/512	NC	1/128
Bar-headed goose	1/64	1/512	NC	1/32
Spur-winged goose*	0	0	1/16	0
Spur-winged goose*	0	0	0	0
Spur-winged goose	0	1/64	NC	0
Spur-winged goose	0	1/64	NC	0
Spur-winged goose	0	1/16	NC	0
Guineafowl	Haemolysed	1/32	NC	1/16
Guineafowl	Haemolysed	1/16	NC	1/16
Guineafowl*	Haemolysed	0	0	0
Guineafowl	Haemolysed	1/64	NC	1/16
White-faced whistling duck	1/16	1/64	NC	0

* Vaccinated three times
NC Not collected

black swan and spur-winged geese were negative at this time. Generally, titres at seven to eight months had decreased.

Factors affecting reaction to vaccination may have included natural biological variation between the species, and the health status and age of individual birds. The vaccine used in this study may not be the most suitable for some of the bird species included. The seroconversion of the bird collection as a whole was 84 per cent at eight weeks after the first vaccination (four weeks after the booster).

Assuming that a titre of more than 1/16 is protective, this implies only a one in five chance of an infection in one bird, and a one in 25 chance of it spreading to another bird in the same premises.

As this was an actual field study (without control birds) and not an experiment, there were certain limitations. For a more detailed study, a larger population of birds from different species with blood work done at routine intervals would be very useful, to compare species variation to antibody response. However, this study shows that wild species of birds do respond to vaccination against avian influenza, and consequently this information can be used to provide an option to protect birds other than domestic poultry against infection.

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Illegal import of equine wormers

SIR, – I am writing to highlight a growing issue for both the horse-owning public and livery yard managers who may be tempted to buy their supplies of equine wormers from foreign countries to save money. While the purchase of pharmaceutically licensed wormers from another country might appear cheaper, the activity is in fact illegal and subject to serious fines and possible prison sentences. In particular, livery yard owners who attempt to pass on the apparent cost savings to their clients are at serious risk of prosecution.

All equine worming products are government approved and licensed in each individual market by the central veterinary body to ensure safety of use and provide the purchaser with back-up and protection should a problem arise with a wormer. If, however, British horse owners use worming products that were originally approved in another country, they will have no local back-up and no protection from the high likelihood of counterfeit products.

Fort Dodge Animal Health is concerned that members of the horse-owning public are putting themselves at risk of serious legal consequences if they ignore this advice, and for very little gain. The company recommends that there should be a greater awareness of this problem and advises British owners to ensure that all purchases of wormers are made from licensed retail outlets or veterinary practices based in the UK.

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Symptoms or signs?

SIR, – Thanks to L. H. Thomas and colleagues for their interesting reply to my letter on symptoms and signs (*VR*, August 6, 2005, vol 157, p 180). However, I fear they have not addressed my main argument. I never asserted that animals have higher psychological functions such as self-awareness or time awareness. But this is not relevant to how they experience more basic functions such as pain. Pain is so important to survival, it has been highly conserved evolutionarily. Even if it is not experienced by animals in exactly the same way as in people, it is still likely to be an aversive sensation, and so we should work to reduce it. It is also undoubtedly subjectively experienced, and so should be classed as a symptom not a sign.

The references given below put both sides of the scientific and philosophical arguments regarding pain in animals. However, the answer to the question of whether animals feel pain in the same way as we do seems to be essentially unknowable, so it is sensible to err on the side of caution and treat or prevent it.

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