

Vaccination of African Penguins (*Spheniscus demersus*) against high pathogenicity avian influenza

Supporting Information

Randomisation and blinding

The 24 penguins selected for the study were randomly allocated to four treatment groups, with six birds in each group. Approximately equal numbers of males and females were added to each group.

The birds were each assigned a random number using the Microsoft Excel random number generator function (=RAND()). The birds were sorted from highest to lowest random number and the females were assigned, in turn, to treatment groups, in order (1 to 4). The males were then assigned in the same way, starting with the group where the females had left off. This was done by the primary investigator.

Double blinding, of the handlers who monitored the penguins daily and the investigator, who performed the data analysis, was incorporated initially but was not maintained when some birds became ill.

In order to blind the handlers and the principal investigator, the treatment (vaccine and dosing schedule) applied to each group was not predetermined or visible at administration. A random number was allocated to each treatment, in an Excel spreadsheet supplied to the SANCCOB veterinarian, and the order of the random numbers determined the title of the group (1-4). The treatment assigned to each group was to be kept by the vet in the spreadsheet until the data analysis was finalised. The syringes containing vaccine were labelled out of sight of the principal investigator and handlers, and labelled only with the treatment group number. The label hid any visible vaccine (VLP vaccine is greenish and the inactivated vaccine more white).

Supplementary Table 1: African Penguins vaccinated with clade 2.3.4.4b high pathogenicity avian influenza vaccines: an H5 avian influenza virus-like particle (VLP) vaccine (Group 1) and one (Group 2a) or two (Group 2b) doses of an inactivated H5N8 vaccine: mean log₂ H5 influenza subtype-specific antibody titre and standard deviations (SD).

	Days after primary vaccination										
	0	14	28	56	70	84	112	175	224	287	430
Mean (SD) log₂ titre											
Group 1	-	8.7 (2.1)	8.8 (2.9)	-	-	-	-	-	-	-	4
Group 2	-	5.3 (2.1)	6.4 (1.3)	4.2 (1.2)	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Group 2a	-	4.3 (2.1)	6.2 (1)	4 (0.8)	4.2 (0.8)	3.2 (1.1)	2 (1.2)	1.5 (0.6)	2 (0)	-	-
Group 2b	-	6.3 (1.8)	6.7 (1.6)	4.3 (1.5)	8.2 (1.6)	7.5 (1.3)	5.4 (1.1)	3.5 (1.5)	3.2 (2.2)	2.2 (0.8)	-