AN ATTENUATED VACCINE PROTECTS HOUBARA BUSTARDS (*CHLAMYDOTIS UNDULATA*) FROM SYSTEMIC POX

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ABSTRACT

The results of the efficacy of an attenuated Houbara bustard pox vaccine are reported. Ten Houbara bustards were vaccinated twice with a pox vaccine which strain was isolated from cutaneous lesions of a sick Houbara bustard. This virus was passaged 120 times in chicken embryo fibroblasts. All 10 birds developed serum neutralizing antibodies after the first vaccination, the titres of which further increased after a booster and challenge dose was administered. Two birds were kept as controls. All 12 Houbara bustards were challenged with 1 of 2 different poxvirus field strains; 6 birds receiving each strain. One strain originated from a cutaneous pox form and the second virus was isolated from internal pox lesions observed in Houbara bustard in Morocco. None of the 10 vaccinated birds developed any typical clinical signs or lesions. The control, which was challenged with the systemic poxvirus, died 14 days after the infection with typical visceral pox lesions. However, the other control, which was infected with the cutaneous poxvirus seroconverted but did not develop any pox lesions. The possible reasons for this failure are discussed.

INTRODUCTION

Pox is probably the oldest recognized avian viral disease. It has been reported in more than 70 species of free-living birds, representing 20 families and has a worldwide distribution. Avian poxviruses (APV) are members of the genus Avipoxvirus within the subfamily *Chordopoxvirinae* in the family *Poxviridae*. Several antigenically related but distinguishable APV were isolated from various avian species like falcon, Houbara bustard or Stone curlew (WERNERY et al. 2004). New laboratory technologies like PCR and REA will make it possible to clarify their status within the genus APV in the future.

Avipoxvirus is spread by biting insect vectors or by direct contact with infected birds or their fomites. Poxvirus infections may cause cutaneous, diphtheroid or systemic lesions. All forms have been described in Houbara bustards. The diphtheroid or wet and the cutaneous form have been observed by OSTROWSKI et al. (1996) and SAMOUR et al. (1996). The systemic form of Houbara pox has also been described, causing severe losses (BAILEY et al. 2000). The skin form rarely develops into a fatal disease but the wet and systemic forms are often associated with fatalities also due to secondary invaders. An outbreak of all three forms of Houbara-pox has recently been reported from Morocco (KINNE et al. 2007) with high fatalities caused by the systemic form. APV-infections also occur in other bustard species. Seidel (1995) has described the diphtheroid and cutaneous form in the Great bustard (*Otis tarda*). OSTROWSKI et al. (1996) believe, that a canary poxvirus vaccine may protect Houbara bustards from Houbara-pox. Here we report the efficacy of an attenuated Houbara bustard pox vaccine in an experimental challenge trial.

MATERIALS AND METHODS

Experimental birds

Twelve Houbara bustards (*Chlamydotis undulata*) were used for this pox vaccination trial. Six months earlier the birds were vaccinated against paramyxovirus-1 (PMV-1). The birds were kept in 2 insect-proof aviaries with 6 birds in each aviary. Blood samples were collected from the basilic vein (*Vena cutanea ulnaris superficialis*), and 10 Houbara bustards were subcutaneously (sc) vaccinated with the Central Veterinary Research Laboratory (CVRL) pox vaccine named DuHoPo (Dubai Houbara Pox) into the neck after some feathers were plucked to ensure a clear view of the skin area. Two bustards, which were not vaccinated, were used as controls. A booster dose was administered 21 days after the first vaccination, and blood samples were taken at the same time. Blood samples were again collected 21 days after the booster vaccination and 30 days after the challenge. Twenty-one days after the second vaccination, 6 Houbara bustards (5 vaccinates and 1 control) were challenged sc with 1ml of the fourth passage of a cutaneous Houbara poxvirus strain. The other 6 birds (5 vaccinates and 1 control) were also challenged sc with 1ml of the third passage of a Houbara poxvirus strain isolated from internal organs during a pox outbreak in Morocco in 2004 (KINNE et al. 2007). The bustards were inspected daily for 3 months for any clinical signs or skin lesions. They received water, low protein pellets (18% protein) ad libitum and fresh alfalfa.

Vaccine virus and challenge viruses

A poxvirus was isolated from pox-like skin lesions in 1998 from a Houbara bustard. The virus was then passaged 120 times on chicken embryo fibroblasts (CEF) according to KAADEN et al. (1996) and OIE-MANUAL (2004). Titre and sterility were tested after every 10th passage. The 120th viral passage was used as the attenuated DuHoPo. The titre of the attenuated pox vaccine was $10^{6.0}$ $TCID_{50}$/ml. One ml of DuHoPo was given sc twice within 3 weeks into the neck region of 10 Houbara bustards. Two different Houbara bustard poxviruses were used for the challenge of the vaccinated birds. One originated from pox-like skin lesions, from which the vaccine strain was prepared. The second was isolated from systemic pox lesions of Houbara bustard from a breeding centre in Morocco (KINNE et al. 2007). The challenge titre of the cutaneous poxvirus was $10^{2.0}$ $TCID_{50}$/ml, whereas the titre of the systemic poxvirus was $10^{5.0}$ $TCID_{50}$/ml.

Laboratory procedures

The Houbara bustard sera collected before vaccination, and 3 times after vaccination (21 days after first vaccination, 21 days after second vaccination and 30 days after challenge) were tested for antibodies by the serum neutralization test (SNT). The viruses used as

antigens were the systemic and the cutaneous pox strains. The titre was $10^{4.0}$ $TCID_{50}$/ml for both viruses. The serological results were compared with each other (Table 1).

One control bird died 14 days after being challenged with the systemic poxvirus, and one vaccinated Houbara bustard, which was challenged with the systemic Moroccan pox strain, became emaciated 2 to 4 weeks after the challenge. Due to detoriation of its health it was euthanized and necropsied. Samples from major organs (lung, liver, spleen, brain, pancreas, intestine, skin, pharynx) were taken from both birds for bacteriological, virological and histopathological examinations using routine methods. The second control, which received the 4th passage of the cutaneous field poxvirus, did not develop any skin lesions and seroconverted 30 days after the challenge infection.

For statistical analyses the paired t-test was used to compare the SNT results using the 2 different pox antigens. The homoscedatic t-test was used to compare the SNT results of the 2 different groups after challenge.

RESULTS

For this vaccination experiment in Houbara bustards, two different poxvirus strains were used. One virus was earlier isolated from scabs and identified by electron microscopy, virus neutralisation, PCR and pock-lesions on CAM as avipox. This virus was used for the challenge of 5 Houbara bustards and as vaccine strain for all 10 immunised Houbara bustards. The second poxvirus was isolated from pooled internal organs of Moroccan Houbara bustards, which had died from systemic pox.

The bird not vaccinated against pox and challenged with the Moroccan systemic poxvirus died 14 days after the challenge. The bird showed the same lesions of systemic pox as seen in Moroccan Houbara bustards (KINNE et al. 2007) including diphtheroid-necrotizing laryngitis (Fig. 1) and pancreatitis with granulomas (Fig. 2). From all organs, including the injection site (except the brain) the challenge poxvirus was re-isolated on CEF. The virus produced the same CPE 3 to 5 days after inoculation of tissue samples as the Moroccan isolate. Intracytoplasmatic, eosinophilic inclusion bodies were observed in most of the organs resembling Bollinger bodies (Fig. 3 and 4).

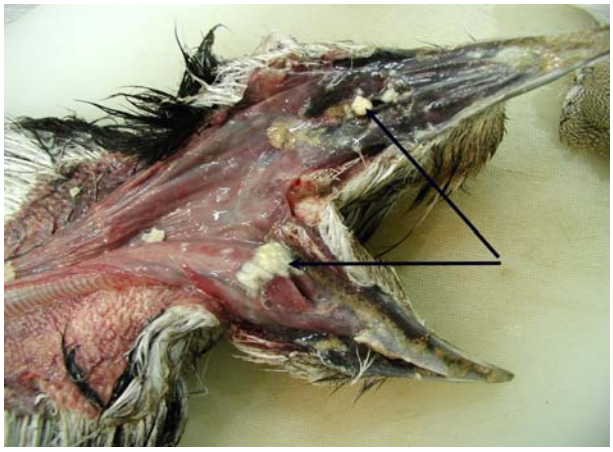

Fig. 1: Yellowish plaques indicating diphtheroid-necrotizing laryngitis of the non-vaccinated control bird (arrow), which was challenged with the systemic Houbara bustard poxvirus.

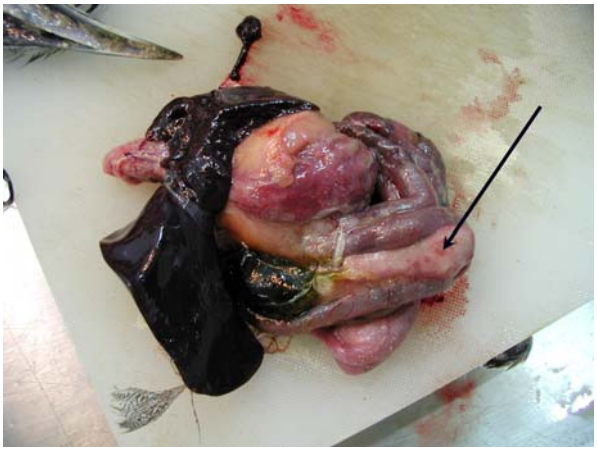

Fig. 2: Swollen pancreas and haemorrhagic pancreatitis (arrow) in the same control bird.

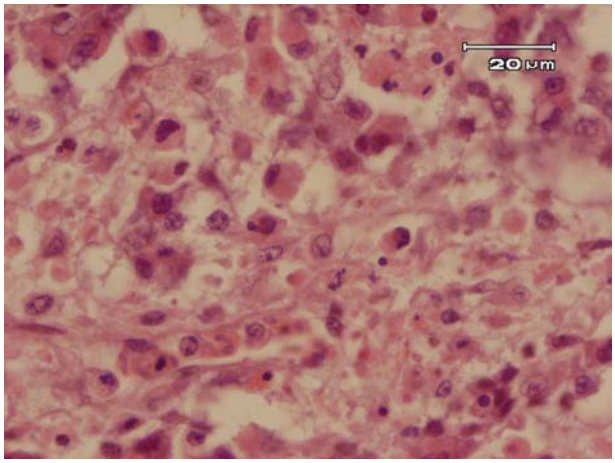

Fig. 3: Photomicrograph of the pancreas of the same control bird. Note the pancreatitis with numerous cells containing intracytoplasmatic eosinophilic inclusions (HE-stain; left picture).

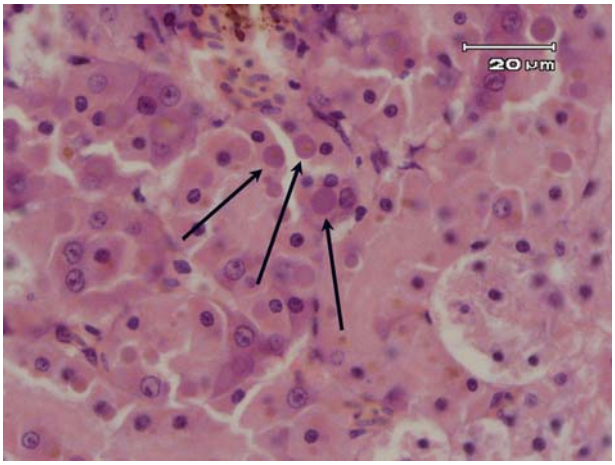

Fig. 4: Photomicrograph of the kidney of the same control bird. Note that numerous tubular cells contain large intracytoplasmatic eosinophilic inclusions (arrows, HE-stain; right picture).

The second control bird, which was challenged with the cutaneous pox strain, as well as all 10 vaccinated Houbara bustards, did not develop any clinical signs. All bustards were inspected daily for 3 months for any clinical signs and no pox-like lesions were observed at the injection site on the neck where feathers were plucked. However, one immunized bird, which lost weight after the challenge, was euthanized within 2 weeks. Necropsy examinations revealed no lesions in the pharynx, air sacs, lung, liver, pancreas or spleen. The Houbara bustard suffered from a perforated gizzard, which was caused by a sharp wooden stick. No pathogenic bacteria or virus were isolated from this bird.

The results of the pox-SNT are shown in Table 1. Before the start of the vaccine trial all birds were negative for pox serum antibodies. After the first vaccination the Houbara bustards seroconverted, and their antibody titres increased after the booster and challenge. The antibody titres were significantly higher ($P < 0.05$) when the viral antigen of the systemic pox was used in the SNT. Significantly higher SNT titres were also achieved in group 2 (cutaneous) compared to group 1 (systemic) using both antigens.

Table 1. Logarithmic SNT results achieved with 2 different antigens (cutaneous and systemic pox) in Houbara bustards after two vaccinations with DuHoPo and after challenge with cutaneous and systemic field Houbarapox strains.

Days	Antigen Used	Houbara bustards											
		1	2	3	4	5	6	7	8	9	10	C1	C2
1st vaccination													
21 days after 1st vaccination	Cutaneous*	2^4	2^4	2^4	2^5	2^4	2^5	2^4	2^4	2^3	2^3	-	-
	Systemic*	2^5	2^5	2^5	2^5	2^4	2^4	2^4	2^4	2^5	2^5	-	-
Booster vaccination													
21 days after 2nd	Cutaneous*	2^4	2^5	2^5	2^5	2^4	2^5	2^5	2^4	2^4	2^5	-	-
	Systemic*	2^6	2^6	2^6	2^5	2^6	2^6	2^5	2^5	2^5	2^5	-	-
Challenge													
30 days after challenge	Antigen Used	Group 1 (systemic)**						Group 2 (cutaneous)**					
		1	2	3	4	5	C1	6	7	8	9	10	C2
	Cutaneous*	2^5	2^6	2^6	2^5	2^5	D	2^6	2^6	2^5	2^5	2^6	2^6
	Systemic*	2^6	2^6	2^6	2^5	2^5	D	2^7	2^7	2^6	2^6	2^6	2^6

- = no antibodies D = died C = controls, C2 did not develop lesions

* paired t-test: significantly different ($p<0.05$), ** homoscedatic t-test: significantly different ($p<0.05$)

DISCUSSION

Different workers have described Houbarapox, and the systemic form especially causes severe losses (BAILEY et al. 2000). To avoid further outbreaks in breeding centres which over the last years have been established in different countries, a vaccination trial was set up to evaluate the efficacy of the CVRL DuHoPo. None of the 10 vaccinated birds developed any typical clinical signs or lesions after they were challenged with two different field strains after two vaccinations.

It is worthwhile mentioning, that only 12 Houbara bustards were used for this experiment. A larger number of birds would have made our results more comprehensive. However, Houbara bustards are classified as “vulnerable” by the World Conservation Union (IUCN; ANONYMOUS, 1992) and therefore difficult to obtain for pathogenicity trials. Vaccines may be prepared as live or inactivated products. DuHoPo is a live vaccine prepared from a pox isolate, which had caused the cutaneous form in a Houbara bustard. It has been modified by serial passage through culture media.

The Houbara bustards were subcutaneously (sc) vaccinated using a dose of 1ml, because this route was also applied with no side effects in falcons (Wernery, 2000). However, in falcons a dose of 0.25ml is recommended according to their lower weight. Even captive bred bustards can be very nervous birds and spreading out their wing for wing web injection can be very strenuous. Therefore the sc neck injection was the preferred route of vaccination. No swelling of the skin or scab formation at the site where the vaccine was applied was observed. This reaction often occurs after intradermal vaccination and is evidence of successful vaccination (OIE-MANUAL 2004).

Before vaccination, the birds did not possess any antibodies to pox antigens but 21 days after the first vaccination they had developed SNT antibodies. The antibodies increased in most birds after the second vaccination and again after the challenge infection. The antibody titres were highest when the systemic Moroccan pox strain was used as antigen in the SNT compared to the cutaneous pox antigen. This may indicate that 2 distinct poxviruses have been isolated. This hypothesis is also underlined by the fact that when the control was challenged with the systemic pox strain it only developed internal pox and not the skin form. Conversely our results clearly showed

that the DuHoPo, which was prepared from a cutaneous pox strain, protected the Houbara bustards against both the cutaneous and the visceral form of pox indicating cross reactivity between the 2 different strains. Tests are currently being carried out to compare both strains with each other using modern DNA technologies.

It is known that animals being vaccinated against pox with attenuated strains seroconvert, but do not produce high titres even after booster and challenge injections (WERNERY, 1994). The titre level is no real indication if vaccinated animals possess a sound immunity. Beside the humoral antibodies, the cell-mediated immune response (CMI) plays a greater role in the protection against pox infections (WERNERY 1994). However, CMI tests were not carried out.

One control died 14 days after receiving the third passage of the systemic field poxvirus strain. The lesions were identical to those found in the outbreak in Morocco in which more than 400 birds died in 2004 (KINNE et al. 2007). The second control, which received a cutaneous field poxvirus did not develop any skin pox lesions and seroconverted 30 days after the challenge infection. It is worthwhile mentioning that this bird, which was seronegative before challenge received a low virus dose which may explain why it did not develop pox. However, we were not able to increase the titre of the field strain even after passaging it through embryonated eggs. Passaging also has limitations because it is known that the virus will loose some of its pathogenicity (OIE-MANUAL 2004). In previous unpublished investigations in falcons it was also not possible to induce cutaneous pox lesions even with a much higher titre. The reasons for the failure are unknown.

So far no attempts have been made to re-isolate the virus after vaccination and challenge from pharyngeal or cloacal swabs. Also further vaccine safety tests should be carried out with at least six serial passages of the virus in SPF chickens to show, that there has been no revision to virulence before DuHoPo can be used in a broader vaccine trial.

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