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MATERNAL TRANSFER OF PARAMYXOVIRUS TYPE 1 ANTIBODIES AND ANTIBODY RESPONSE TO A LIVE NEWCASTLE DISEASE VACCINE IN KORI BUSTARDS

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ABSTRACT: Studies were conducted to monitor the decline of maternal antibodies to paramyxovirus type 1 (PMV-1) in kori bustard chicks (*Ardeotis kori*), and to determine the antibody response in birds with low levels of maternally derived immunity (MDI) after being given either one or five times the standard domestic fowl dose of a live PMV-1 La Sota vaccine intranasally. The results confirmed that PMV-1 antibodies were transferred to eggs and chicks derived from kori bustard hens immunized with inactivated vaccine 5 to 8 mo previously. The levels of inherited antibody in chicks varied considerably, with 21% of birds having no detectable antibodies at day 14. Chicks hatched from dams with high hemagglutination inhibition (HI) antibody titers had high titers of MDI. Mean antibody levels in seropositive chicks were $\log_2$ 6.3 and $\log_2$ 2.9 on days 14 and 42, respectively. The rate of decline of detectable antibodies (1 $\log_2$), was estimated to be 5.50 to 6.25 days and 12.25 days in 14 to 21 and 28 to 42 day old chicks, respectively. There was no significant HI antibody response in bustards given a primary vaccination of either one or five times the standard domestic fowl dose of live vaccine intranasally up to 3 wk post-vaccination. High levels of HI antibodies, $\log_2$ 7.4, were detected in birds given a secondary dose of inactivated vaccine, but not in birds given a secondary dose of live vaccine at 2 wk post-vaccination.

Key words: *Ardeotis kori*, kori bustard, maternally derived immunity, paramyxovirus type 1, vaccine.

INTRODUCTION

The free-living populations of many species of bustards (Gruiformes: Otididae) are declining and captive breeding and restoration programs have been established as a conservation measure. Newcastle disease virus is the type strain for avian paramyxovirus type 1 (PMV-1) and is an important pathogen of domestic poultry and exotic birds (Gerlach, 1994). The presence of PMV-1 in bustard collections and the commercial traffic of domestic and exotic avian species in the Middle East gives high priority to the need of developing PMV-1 vaccination regimens for captive bustards (Ostrowski et al, 1995; Bailey et al., 1996, 1997). However, the only vaccines that are currently available to protect bustards against PMV-1 are products developed for domestic fowl (*Gallus domesticus*), and there are few published reports of their efficacy in non-domestic avian species (Cornelissen, 1993).

It is known that successful vaccination of domestic fowl and turkey poults (*Meleagris gallopavo*) derived from immune flocks is hindered by maternally derived immunity (MDI) (Beard and Brugh, 1975). Vaccination schedules designed for bustards must therefore allow for the possibility that interference from MDI will occur in chicks where the breeding flock is vaccinated. However, the paucity of data on MDI means that the optimum time to give the primary vaccination to bustards is not known. Similarly, although some authors recommend that live PMV-1 vaccines should be given to exotic birds via the ocular or nasal route using five times the dose administered to domestic fowl (Gerlach, 1994), to the knowledge of the authors, there are no published reports of the antibody response of bustards to such a regimen.

The objectives of the current study were to (1) monitor the decline of maternal antibodies to PMV-1 in kori bustard chicks (*Ardeotis kori*), and (2) determine, in birds

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with low levels of MDI, the antibody response after being given either one or five times the standard domestic fowl dose of a live PMV-1 La Sota vaccine intranasally.

MATERIALS AND METHODS

Eggs and chicks used in the study were derived from a breeding flock of adult kori bustards maintained in a large (500 × 60 m) naturalistic aviary at the National Avian Research Center (NARC), Al Ain, United Arab Emirates (UAE). The adults had been vaccinated with inactivated oil emulsion PMV-1 vaccine (Newcavac Nobilis, Intervet, Cambridge, UK) subcutaneously (SC) in the inner crural area at a dose rate of 1.0 ml/kg before the current breeding season, and 2 yr previously. Eggs were laid and chicks were hatched 5 to 8 mo after the dams had been vaccinated. The chicks used in the maternal antibody and live vaccine trials were hand-reared, of mixed sex, and still growing during the trials. The birds were housed in isolated breeding and rearing facilities at the same NARC center. No cases of PMV-1 were diagnosed in the collection during the trial.

Eggs were collected from the aviary and artificially incubated. The identity of the dams, comprising six birds, was known for 11 of 14 chicks. Sera were collected when the dams were vaccinated before the breeding season and after the breeding season, 1 yr later, when they were revaccinated. It was not possible to collect sera from the dams during the breeding season.

Five infertile kori bustard eggs were submitted for post-mortem examination. The eggs had been incubated for 17 to 24 days before examination. Yolk and albumen, or a yolk-albumen mixture, in cases where the contents were disrupted, were collected from each egg. The samples were frozen at −20 C and submitted to the Central Veterinary Research Laboratory (CVRL), Dubai, UAE, within 1 mo of collection. The hemagglutination inhibition (HI) antibody levels of each sample was measured adapting the method reported by Singh (1995). The sample (1 ml) was mixed with phosphate-buffered saline (1 in 2 volume) and chloroform was added to the suspension. The mixture was shaken, incubated at room temperature and then centrifuged at 1,500 g for 20 min. The clear supernatant, representing a dilution of 1:2 were analyzed using a HI test to detect PMV-1 antibodies according to the methodology described by Wernery et al. (1992). The HI test was carried out in round-bottomed microplates with 0.5 ml aliquots. The samples were titrated from $\log_2$ 1 to $\log_2$ 8 in 0.9% sodium chloride solution. The tests were done with 4 hemagglu-

tination units of antigen. Titers were expressed as the reciprocal of the highest dilution of yolk fluid giving 50% inhibition. For the antigen we used a Newcastle disease virus (120-95) which was isolated from a falcon and identified as PMV-1 by the Avian Virology Laboratory (Central Veterinary Laboratory, Weybridge, UK). Specific pathogen free eggs from Lohmann Co. (Cuxhaven, Germany) were used for the production of hemagglutinin. The ND strain was inoculated into the amnion-allantoic cavity of 9 day embryonating eggs. The amnion-allantois fluids were harvested 96 hr after inoculation and frozen in aliquots at −80 C. The titer was found between $\log_2$ 9 and $\log_2$ 11.

Sera were collected from 14 chicks to monitor the levels of maternal antibodies. Blood samples were collected at 14, 21, 28, 35, 42, 49, 56 and 63 days of age. Additional opportunistic samples were collected at 77 to 91 days of age in three chicks.

The live vaccine used was an attenuated freeze-dried PMV-1 vaccine (Lentogen Lasota, IVAZ, Padova, Italy) manufactured for the vaccination of domestic fowl. The freeze-dried vaccine was dissolved in 30 ml of distilled water and was administered by the intranasal route (IN) using a 1 ml syringe and a 0.46 mm diameter irrigating catheter.

The birds were divided into three groups. Group 1 comprised nine birds, aged 77 to 118 days (mean ± SE = 95.6 ± 6 days), weighing 2,000–3,100 g (2,517 ± 181 g). Birds in this group were given a standard domestic fowl dose (one drop) of live vaccine. Group 2 comprised seven birds, aged 49–86 days (63 ± 5.2 days), weighing 1,200–2,700 g (1,757 ± 200 g). These birds were given five times the standard domestic fowl dose (five drops) of live vaccine. At day 21 after live vaccination, five birds were given an oil emulsion PMV-1 vaccine (Newcavac Nobilis, Intervet, Cambridge, UK) SC in the inner crural area of the leg at a dose rate of 1.0 ml/kg. Two birds were given the same dose of live vaccine and were housed separately from the five birds given inactivated vaccine. Group 3 (controls) comprised three birds, aged 43–73 days, weighing 640–3,000 g, and these birds were not vaccinated.

The groups were housed separately. Blood samples were collected at 0, 7, 14, and 21 days after the first vaccination from all birds and at 35 days post-vaccination in Groups 2 and 3. No side-effects associated with vaccination were observed in the birds.

Blood samples were collected from the *vena basilica*, 0.2 to 0.5 ml each time in the maternal antibody trial and 1 ml each time from the bustard dams and in the vaccine trial. Samples were collected in 1.3 ml serum gel tubes (Sar-

TABLE 1. Paramyxovirus type 1 hemagglutination inhibition (PMV-1 HI) antibody levels (log₂) in kori bustard dams and in yolk and albumen or a yolk-albumen mixture (mix) derived from infertile eggs.

	PMV-1 HI titer (log₂)				
Dam ID (Egg ID)	Dam pre-breeding season	Dam post-breeding season	Yolk	Albumen	Mix
429 (96/18)	1	0	0	0	nd
623 (96/26)	4	8	nd[a]	nd	5
623 (97/01)	4	8	5	3	nd
632 (96/09)	4	8	nd	nd	4
618 (97/02)	5	8	8	5	nd

[a] No data.

TABLE 2. Paramyxovirus type 1 hemagglutination inhibition (PMV-1 HI) antibody levels (log₂) in kori bustard dams before and after the breeding season and in their chicks.

	PMV-1 HI titer (log₂)				
Dam ID	Dam pre-breeding season	Dam post-breeding season	Chick 1	Chick 2	Chick 3
429	1	0	0	nd[a]	nd
628	3	8	7	5	8
623	4	8	5	5	nd
632	4	8	4	5	8
637	5	8	8	nd	nd
638	5	8	8	nd	nd

[a] No data.

stedt, Numbercht, Germany). Sera were frozen at −20 C and submitted to the CVRL within 1 mo of collection, where they were analyzed using the same HI test to detect PMV-1 antibodies as previously described for the yolks derived from the infertile eggs (Wernery et al., 1992).

Cloacal swabs were collected from all birds used in the vaccine trial at the same time that blood samples were collected. Swabs were immediately placed in virological isolation media and frozen. Virological isolation was attempted within 2 mo of collection according to the methods described by Wernery et al. (1992). The cloacal swabs were thawed and removed from the media. After centrifugation at 3,000 rpm for 20 min to clear, 0.5 ml of the supernatant from the suspension was added to 4.5 ml of Minimal Essential Media 199 (MEM, Flow Laboratories, Irvine, UK) and filtered through a 450 μm membrane filter. The filtrate was then applied to permanent chicken embryo fibroblast (CEF) cell cultures (Kaaden et al., 1982), which were observed for the onset of cytopathic effect for 5 days.

Statistics comparing HI antibody titers before and after vaccination in Groups 1 and 2 were carried out using a Student's unpaired t-test (Microsoft Excel Analysis Toolpak, Microsoft Corporation, USA). A value of $P \leq 0.05$ was considered be statistically different.

RESULTS

The results of monitoring antibody levels in kori dams and their infertile eggs are presented in Table 1. Eggs with high HI antibody titers were produced from dams with high antibody titers. In one case a seronegative egg was produced by a dam (ID 429) with low levels of HI antibody.

The results of monitoring antibody lev-els in the bustard dams and their chicks are presented in Table 2. The mean HI titer of the six dams of 11 of the 14 chicks before the breeding season was log₂ 3.7 ± 0.62 (range log₂ 1–5). The mean HI titer of the same birds, 12 mo later, at the end of the breeding season was log₂ 6.7 ± 1.3 (range log₂ 0–8). In all these cases, chicks that were hatched from dams with high titers, also had high titers of MDI at day 14. The dam (ID 429) of one seronegative chick at day 14 and one seronegative infertile egg, had the lowest antibody titer before being vaccinated and was also seronegative at the end of the breeding season.

Three of the 14 chicks (21%) were seronegative at 14 days and remained seronegative for the remainder of the study. The identity of the dams of two of these chicks was unknown, but the dam (ID 429) of the remaining seronegative chick had the lowest HI titer (log₂ 1) of the six dams that were sampled before the breeding season.

Of the remaining 11 chicks, a general decline in titer was apparent and antibody levels ranged from log₂ 4–8 at day 14 to log₂ 0–4 at day 63. The mean levels of maternally derived HI antibody to PMV-1 present in these 11 seropositive bustards is presented in Table 3. The rate of decline of detectable antibodies against PMV-1, 1 log₂, was estimated to be 5.50 to 6.25 days

TABLE 3. Decline of maternally derived Paramyxovirus type 1 hemagglutination inhibition (PMV-1 HI) antibody levels in kori bustard chicks after hatching.

Age	14 days	21 days	28 days	35 days	42 days	49 days	56 days	63 days
HI titer	6.3 ± 0.47[a]	5 ± 0.47	3.9 ± 0.38	3.5 ± 0.19	2.9 ± 0.34	2.2 ± 0.3	1.89 ± 0.45	1.83 ± 0.6
Sample size	11	9	10	8	11	11	9	6

[a] Log_2 mean ± SE.

in 14- to 21-day-old bustard chicks and approximately 12.3 days in 28- to 42-day-old chicks. The opportunistic samples collected from three birds at 77 to 91 days of age were seronegative.

The results from the vaccine trial for Groups 1 and 2 after being given live vaccine are shown in Table 4. There was no significant increase in HI antibody titer in Groups 1 or 2 at 7, 14 and 21 days after being given the first dose of live vaccine (Students t-test, $P > 0.05$ at 7, 14 and 21 days). Two weeks after being given inactivated vaccine the five birds in Group 2 had a large increase in HI antibody titer (7.4 ± 0.2, range log_2 6–8). The two birds from Group 2 that were given a second dose of live vaccine were seronegative 2 wk later. Chicks from Group 3 (controls) remained seronegative throughout the trials. Cloacal swabs collected from Groups 1, 2, and 3 chicks did not yield PMV-1 virus.

DISCUSSION

The transfer of antibody from hen to the chick through the yolk of fertile eggs is well-recognized in the domestic fowl (Grun and Wogan, 1963). The results from the current study confirm that PMV-1 antibodies are transferred to eggs and chicks derived from kori bustard hens given in-

activated vaccine 5 to 8 mo previously. The use of material derived from infertile eggs represents a non-invasive technique to monitor the vaccination status of birds maintained in captive breeding programs.

Although all dams had been vaccinated, the levels of inherited antibody varied considerably, with 21% of bustard chicks having no detectable antibodies at day 14. It is known that maternal antibody titers in domestic fowl eggs and chicks are related to antibody levels in the dam (Schmittle, 1950), and the low levels in some chicks in the current study may have been related to a poor immune response by their dams. Although it was not possible to monitor titers of the dams during the breeding season, so that chick and maternal levels could be correlated, in six chicks it was possible to measure antibody levels in their dams at the beginning and at the end of the breeding season. In all cases chicks hatched from dams with high titers also had high levels of MDI. The dam of one seronegative chick at day 14 and one seronegative infertile egg, had the lowest antibody titer before being vaccinated and was seronegative at the end of the breeding season.

In domestic fowl, MDI antibody levels of log_2 3–5 at week 2 declined to log_2 1.6–

TABLE 4. Paramyxovirus type 1 hemagglutination inhibition (PMV-1 HI) antibody in Groups 1 and 2 of kori bustards after vaccination with live vaccine at day 0.

Group	Time after vaccination (days)			
	0	7	14	21
1	0.22 ± 0.15[a]	0.22 ± 0.22	0.33 ± 0.24	0.33 ± 0.17
2	1.0 ± 0.31	0.29 ± 0.18	0.86 ± 0.34	1.71 ± 0.35

[a] Titer (log_2 mean ± SE).

3 by week 3 (Warden et al., 1975; Gough and Allan, 1976). In comparison with domestic fowl of the same age, antibody levels were higher and appeared to decline more slowly in the bustards. Mean antibody levels in the bustards were $\log_2$ 6.3 and $\log_2$ 2.9 on days 14 and 42 respectively. The rate of decline of detectable antibodies against PMV-1 (1 $\log_2$) was estimated to be 5.50 to 6.3 days in 14- to 21-day-old bustard chicks and approximately 12.3 days in 28- to 42-day-old chicks. The results for 14- to 21-day-old bustards were similar to those reported for the domestic fowl where the estimated half-life of HI antibodies are 4.5 to 6 days (Allan, 1973; Rehmani and Firdous, 1995). It is known that maternal antibodies have a longer half-life in psittacine chicks (Gerlach, 1994), and the slower rate of decline in the older bustard chicks suggests that there are species differences in the rate of decline of MDI.

Gough and Allan (1976) consider that a mean antibody titer of $\geq\log_2$ 4 protects domestic fowl against PMV-1 for up to 2 wk of age. In MDI domestic fowls challenged with PMV-1 survival was 82% in birds with a mean antibody titer of $\log_2$ 5.3 compared with 30% in birds with a titer of $\log_2$ 3.3 (Rehmani and Firdous, 1995). If the level of protection by MDI in bustards is similar to those of domestic fowl (Gough and Allan, 1976; Rehmani and Firdous, 1995), i.e., $\geq\log_2$ 4, our results indicate that unvaccinated MDI bustards would be protected for up to 4 wk. Ultimately, challenge studies on bustard chicks would be needed to assess the protection of MDI.

Although Gerlach (1994) and Cornelissen (1993) recommended the use of live PMV-1 vaccines at five times the normal dose in exotic species, there does not appear to be any published data on serological responses to this regimen. Domestic fowl given live PMV-1 vaccines by aerosol or intraocularly do not develop measurable increase in HI antibodies until 7 days post-vaccination and levels of $\log_2$ 4.3 to 6.3 are detectable in sera by days 14 to 17 (Gough

and Alexander, 1973; Gough and Allan, 1973). Although there were no side-effects in bustards given either one or five times the standard domestic fowl dose of live vaccine intranasally, in comparison with reports from domestic fowl, there was no detectable HI antibody response, even in the two birds given a second dose of live vaccine. The low levels of MDI in Groups 1 and 2, $<\log_2$ 0.22 and $<\log_2$ 1.0 respectively, when the chicks were first vaccinated, suggests that bustards do not develop a serological response to a primary dose of live vaccines. However, because the viability of the live vaccine used in the current study was not confirmed either by virus titration or by inoculation of chickens as positive controls, care should be taken in drawing conclusions from these negative results. In the domestic fowl intranasal vaccination induces both local and humoral antibody response and the local immunity is an important component of the protection against challenge by PMV-1 (Zakay-Rones et al., 1971). The effect of live vaccines on local immunity in bustards is unknown and further investigations are warranted.

High levels of HI antibodies, $\log_2$ 7.4, were detected in sera from five Group 2 birds, 14 days after being given inactivated vaccine. These findings concur with a previous study, where kori bustards previously given four doses of live vaccine developed an antibody titer of $\log_2$ 7.3 4 wks after being given the same inactivated vaccine (T. A. Bailey, unpublished data). The HI test is a reliable and practical serological indicator of immunity (Meulemans, 1988) and Philips (1973) reported that field challenge by PMV-1 would not kill chickens with HI antibody titers greater than or equal to $\log_2$ 5. Ultimately, challenge studies on vaccinated bustards using field strains of PMV-1 would be needed to assess vaccine efficacy.

The objective of a vaccination program against PMV-1 in birds, particularly in areas where the disease is enzootic, must be to minimize the susceptible period be-

tween waning passive immunity and the establishment of satisfactory active immunity (Box et al., 1976). Results from the current study show that maternal antibodies to PMV-1 are transferred from vaccinated bustard dams to their chicks, but the amounts vary between individuals and some chicks may therefore be at risk to PMV-1 infection. From our results we conclude that in areas where there is a high risk of infection with PMV-1 early in life, primary vaccination of bustard chicks derived from a vaccinated flock should be carried out at 3 to 4 wk of age. Where the risk of infection is not so great, vaccination at 6 to 8 wks may be preferable. Although there was no detectable HI antibody response in chicks given a primary or secondary doses of live vaccine, high levels of HI antibodies can be expected when birds are given a secondary dose of inactivated vaccine.

ACKNOWLEDGMENTS

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Received for publication 16 October 1997.