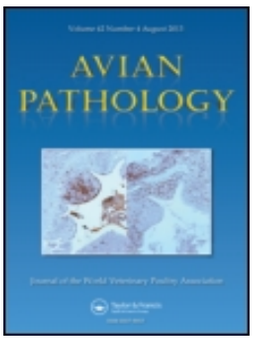

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ORIGINAL ARTICLE

Evaluation of a commercial vaccine against avian poxvirus in turkeys kept in the backyard system in the state of Yucatan, Mexico

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One hundred and sixty 1-month-old turkey poults were delivered to 40 households in four communities of the State of Yucatan, Mexico. The poults were divided into two populations, one vaccinated and the other non-vaccinated against avian pox. During three months, monthly visits were carried out in order to monitor the appearance of lesions suggesting avian pox in the birds delivered. Each turkey was clinically examined, searching for characteristic avian pox lesions that were classified according to the degree of severity observed. The true incidence rate and the cumulative incidence rate of avian pox were determined and the true incidence and cumulative incidence rates of mortality were determined and the relative risks calculated. The true incidence rates for avian pox in vaccinated and non-vaccinated birds were 1.5 and 1.47 respectively. The cumulative incidence rates were 0.94 and 0.90 for vaccinated and non-vaccinated birds, respectively. The comparison for the whole period between vaccinated and non-vaccinated groups did not show a significant statistical difference for mortality. However, when mortality was compared between vaccinated and non-vaccinated turkeys for each month of the study, there was a statistically significant difference for the first month (relative risk = 0.216, confidence interval 0.069 to 0.676). In addition, when the severity of pox lesions between groups was compared, statistically significant differences were found in favour of the vaccinated birds ($P < 0.0001$).

Introduction

Keeping animals in the backyards of houses is a common activity in rural communities of the State of Yucatan, Mexico (Rejón-Ávila *et al*., 1996). The turkey is among the most popular species kept in the system, being outnumbered only by chickens (Gutiérrez-Triay *et al*., 2007).

Just as in other animal production systems, in the backyard, diseases are among the most important constraints to production. Avian pox is one of the most important diseases affecting fowl in this system (Silva *et al*., 2009). The aetiological agent is a virus belonging to the Avipox genus of the *Poxviridae* family (Tripathy & Reed, 2003); the disease is characterized by nodular proliferative lesions in the featherless body parts of the birds (cutaneous form) and/or diphtheritic lesions in the upper respiratory tract, mouth and oesophagus (diphtheritic form). Mortality in the cutaneous form is usually low, contrary to the diphtheritic or combined form; however, mortality indices are also influenced by factors such as virulence of the agent, host susceptibility and environment amongst others (Bolte *et al*., 1999; Mockett, 2006).

Owing to the lack of an effective treatment, prevention is the best way to protect a flock against the disease, and vaccination has been the main tool used for such an aim (Bolte *et al*., 1999). However, there are reports of avian pox outbreaks in vaccinated flocks (Odoya *et al*., 2006). In clinical trials the lack of protection conferred by commercial vaccines has also been proved and this was attributed to the fact that a heterologous virus was infecting the chickens (Fatunmbi & Reed, 1996a). The results observed in this kind of study demonstrate the need for proving the protection that commercial vaccines can confer to flocks challenged with heterologous field viruses. Considering the above information, the objective of this study was to evaluate the protection conferred by an available commercial vaccine against avian pox in meat turkeys kept in the backyard of four rural communities of the State of Yucatan, Mexico.

Materials and Methods

Study area. Four rural communities were chosen by convenience, one in each of the four agricultural zones into which the state is divided, Molas in the Center zone, Xohuayan in the South zone, Xanlah in the Southeast zone and Chan San Antonio in the East zone.

Owing to the fact that we lacked information regarding the number and location of households raising turkeys in the communities, it was decided to include 10 households per community, assuring the collaboration of sufficient families to have enough data. The households were randomly

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selected using two transects and selecting five houses on each, which were selected using a systematic approach and a random number table. The only inclusion criterion was that the houses were keeping turkeys at the moment of delivering the birds.

Study population. A cohort study was used to determine the protection conferred by a commercial vaccine against avian pox in turkeys, evaluating raw mortality, cumulative incidence and severity of disease. A difference of at least 20% of disease between the vaccinated and the non-vaccinated groups was considered enough to determine whether the vaccination was advisable for backyard turkeys. Therefore, considering this percentage difference, a confidence level of 95% and a power of 80%, it was calculated that a total of 73 turkeys were needed for each group. To facilitate the distribution of the birds it was decided to include 80 vaccinated turkeys and 80 non-vaccinated turkeys.

For each household, four turkey poults were delivered (two vaccinated and two non-vaccinated). The turkeys were marked with plastic colour strips, secured to the leg. Four turkeys per household was considered an appropriate number for the families to cope with in terms of feeding, sheltering and other management practices favouring the stay of the birds in each community and therefore providing enough biological material to fulfil the study objective.

Vaccine. A commercial vaccine containing attenuated fowlpoxvirus, cultivated in specific pathogen free chicken embryos, produced by Avilab® (Tepatitlan, Jalisco, Mexico) was used.

Strategy. One hundred and sixty 1-day-old turkey poults were purchased from a commercial USA hatchery. They were housed in two different facilities of the Facultad de Medicina Veterinaria y Zootecnia, Universidad Autonoma de Yucatan (FMVZ-UADY); both groups received the same commercial food and water *ad libitum* and were looked after by different personnel. One group received vaccination against fowl pox by the wing web method at 2 days of age and was boosted 15 days later. After vaccination, birds were examined daily from day 5 to day 7 to observe the development of vaccine reactions. All vaccinated birds showed vaccine reactions while none of the non-vaccinated turkeys presented any lesions. Two weeks after the second vaccination, birds were delivered to the communities. No other vaccines were administered to the birds. Prior to delivery no diseases were observed on any of the birds, and no significant body weight difference was observed between vaccinated and non-vacinated birds.

After delivering the turkey poults, monthly visits were carried out for 3 months in order to collect information about the birds. The intervals were decided on the basis of preliminary trials, which showed that vaccinated and challenged turkeys developed lesions that lasted 30 ± 2 days while non-vaccinated birds presented lesions for 34 ± 3 days. All deaths were recorded and clinical examination of all birds was carried out, with special attention paid to observing possible pox lesions. When suggestive lesions were present, the bird was classified as mildly (10 or less small lesions), moderately (between 11 and 40 lesions up to 0.5 cm) or severely affected (uncountable lesions of diverse size) and assigned scores of 1, 2 or 3 respectively.

With the aid of sterile forceps and scalpel blades, samples from the lesions were collected for virus isolation; samples were placed in sterile test tubes and kept refrigerated in a cool box with ice packs. Samples were taken to the virology laboratory at the FMVZ-UADY where they were ground using a mortar and pestle and mixed with phosphate buffer saline, pH 7.2, containing penicillin, streptomycin and nystatin at a concentration of 10,000 IU/ml, 10 mg/ml and 5000 IU/ml respectively. The suspensions 10% (w/v) were centrifuged at 700×*g* for 5 min to remove large tissue particles. The supernatant was separated and allowed to stand for 1 hour at 37°C before inoculating 9-day-old to 11-day-old chicken embryos (Tripathy *et al*., 2000; Kulich *et al*., 2008), produced at the FMVZ-UADY. The inoculation route was the chorio-allantoic membrane. Inoculated embryos were incubated at 37°C for 5 to 7 days; they were chilled at 4°C for at least 6 h before opening and examining the chorio-allantoic membrane for lesions (Boyle *et al*., 1997; Tripathy *et al*., 2000).

Membranes with lesions suggestive of avian pox were processed for histopathology examination according to the methodology used by Allen (1995). The observation of cytoplasmic inclusion bodies was considered as confirmation of infection with avian poxvirus.

Statistical analysis. Cumulative incidence rates (CIRs) and true incidence rates (TIRs) for the vaccinated and non-vaccinated groups were calculated. Crude mortality rates for both groups of turkeys, general and by community, were also calculated. Using these rates as risk measures, the relative risks (RR) and 95% confidence intervals were determined using the Winepiscope 7.0 chi-squared test for incidence rates. Survival curves were constructed with SPSS software (IBM corporation, Armonk, NY, USA), which uses the Wilcoxon–Gehan test as tool to determine differences between groups.

To evaluate whether there was a significant difference of disease severity between vaccinated and non-vaccinated groups, the chi-squared test was used.

Results

Of 160 turkey poults delivered during this study, 137 (85%) became infected with avian poxvirus as determined by the presence of macroscopic lesions and virus isolation; from these, 71 were vaccinated and 66 were non-vaccinated.

The TIR for avian pox was 1.33 for vaccinated turkeys and 1.47 for non-vaccinated birds. The TIR for each month of observation is presented in Table 1.

The calculated CIR for avian pox was 0.90 and 0.94 for non-vaccinated and vaccinated birds respectively. Considering vaccination as the exposition factor, the RRs were not statistically significant for any of the monthly periods nor for the complete study (RR = 1.76, confidence interval = 0.95 to 1.22), and most new avian pox cases occurred during the first month of the study as shown in Table 1.

In Table 2 the CIR for mortality for each month and the whole period are presented. The CIR for the whole period was 0.40 for the vaccinated turkeys and 0.48 for the non-vaccinated

Table 1. *Cumulative incidence and incidence rates for avian poxvirus in free-range backyard turkeys in four communities of Yucatan, Mexico.*

Fowlpox vaccine	Age (months)	Number of birds that started[a]	Number of birds withdrawn[a]	Bird time at risk	Number of new cases[a]	CIR	TIR
Yes	2	80	3	45.5	66	0.83	1.47
	3	11	6	6.5	3	0.88	0.46
	4	2	0	1	2	0.94	2.00
	Total	–	–	53.0	71	–	1.33
No	2	80	13	42	63	0.79	1.50
	3	4	0	2.5	3	0.90	1.20
	4	1	1	0.5	0	0.90	0.00
	Total			45.0	66		1.47

[a]Corresponds to birds alive and not infected (started), birds that died (withdrawn) or birds that got infected (new cases) for each period of time.

Table 2. *Cumulative incidence and true incidence rates for mortality of turkeys vaccinated and non-vaccinated against fowlpox, raised in the backyard system in Yucatan, Mexico.*

Status	Age (months)	Number of birds that started[a]	Bird time at risk	Number of birds that died	Cumulative incidence rate	Incidence rate
Vaccinated	2	80	78.5	3	–	0.04
	3	77	69.0	16	–	0.23
	4	61	54.5	13	–	0.24
	Total	–	202.0	32	0.40	0.16
Non-vaccinated	2	80	73.5	13	–	0.18
	3	67	58.5	17	–	0.29
	4	50	46.0	8	–	0.17
	Total	–	178.0	38	0.48	0.21

[a]Corresponds to birds alive and not infected at the beginning of each period of time.

Table 3. *Relative risk and confidence intervals for the comparison of true incidence rates for mortality in vaccinated and non-vaccinated turkeys against fowlpox, in the backyard system in Yucatan, Mexico.*

	RR first month	CI (95%)	RR second month	CI (95%)	RR third month	CI (95%)
Vaccinated	0.216	0.069 to 0.676	0.805	0.407 to 1.591	1.384	0.576 to 3.327
Non-vaccinated	1	–	1	–	1	–

turkeys; the TIRs for mortality were 0.16 and 0.21 respectively. In Table 3 the RRs for mortality are presented for the 3 months of the study; only for the first month was there a statistically significant difference in favour of the vaccinated turkeys (RR = 0.216; 95% confidence interval = 0.069 to 0.676).

The mortality rates for vaccinated, non-vaccinated and total turkeys were 0.40, 0.48 and 0.44 respectively. The survival curve (Figure 1) shows that there was not a significant difference for vaccinated and non-vaccinated turkeys for the 3-month period of observation. A total 84.5% of vaccinated turkeys showing lesions had a score of one, and only 1.4% had a score of three, while 45.5% of non-vaccinated birds had a score of three. The complete distribution of turkeys according to the severity of lesions observed is presented in Table 4. A statistically significant difference (P = 0.0001) was found in favour of the vaccinated birds.

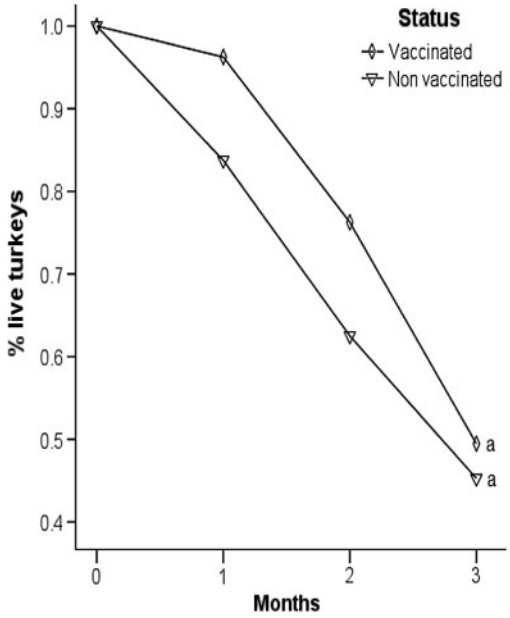

Figure 1. *Survival curve for mortality of turkeys, vaccinated and non-vaccinated against fowlpox, raised in the backyard system of Yucatan, Mexico (Wilcoxon–Gehan test: $P < 0.05$, degrees of freedom = 1; different letters indicate significant differences between curves).*

Discussion

TIRs for avian pox infection were similar for vaccinated and non-vaccinated birds, indicating that the vaccine did not prevent the disease. We observed that most turkeys were infected with avian pox within the first month after delivery to the communities. The TIR for the vaccinated group was 1.47 turkey-months, meaning that 147 birds become sick for every 100 turkey-months at risk; a similar value of 1.50 was obtained for the non-vaccinated group.

The CIR results showed that by the end of the first month 83% of the vaccinated turkeys and 79% of the non-vaccinated had presented with the disease. Although the disease was not observed in 100% of the birds from each group by the end of the third month, the probability of a turkey becoming sick was 0.94 for the vaccinated and 0.90 for the non-vaccinated groups. This suggests that most susceptible turkeys entering the backyard in the studied communities will become sick with avian pox in less than a

Table 4. *Severity of avian pox lesions observed in vaccinated and non-vaccinated turkeys kept in the backyard system of four communities of Yucatan, Mexico.*

Lesion score	1	2	3	Total
Vaccinated	60	10	1	71
Non-vaccinated	17	19	30	66

Lesion score: 1 is the least and 3 the most severe degree. $P < 0.0001$.

Table 5. *Number of turkeys with pox lesions that died, recovered or remained sick in the last 2 months of the study.*

	Vaccinated turkeys (n = 80)		Non-vaccinated turkeys (n = 80)	
Age (days)	61 to 90	91 to 120	61 to 90	91 to 120
Cases in previous month	66	29	63	19
Recovered	30	16	30	7
Dead	10	6	17	3
Still sick	26	7	16	9

month, reaching almost 100% of the population before the end of the fattening period (5 to 6 months), affecting negatively the benefits that turkey productivity in the backyard system provides to the families practicing this activity.

The similar CIR for vaccinated and non-vaccinated turkeys could be caused by factors such as vaccine strain, field virus or host characteristics (Tizard, 2004). Singh *et al.* (2000) have suggested two hypotheses about fowlpox outbreaks in vaccinated flocks; one is the emergence of variant viruses with poor cross-protection with strains used in vaccines (Fatunmbi & Reed, 1996b; Boyle *et al.*, 1997). The other hypothesis is the emergence of virus strains containing an integration of genes of the reticuloendotheliosis virus in their genome, which increases the virulence of those strains (Ramos *et al.*, 2002). Another possible explanation is that perhaps there is a high challenge in the backyard system due to poor hygienic conditions and keeping of different-age birds, together with possible immunodepression of birds resulting from transportation and the management conditions in the backyards such as poor housing and feeding.

When the severity of lesions was compared in vaccinated and non-vaccinated groups, there was a very significant difference in favour of the vaccinated birds, indicating that the vaccine did confer protection in these terms.

Considering that most new cases occurred during the first month, one could assume that the higher severity of avian pox observed in the non-vaccinated birds did influence mortality, because a significant difference was detected in this first month and the only difference between groups was the vaccine status; it has been stated that when the disease severity is high, the negative consequences on the flock, such as increased mortality and diminished weight gain, occur due to the difficulty in the ingestion of food and water (Tripathy *et al.*, 2000). In the same line of reasoning, most deaths occurring in the last month were probably attributable to causes other than avian pox, supported by the fact that very few cases of avian pox were observed at this time of the study, and that most turkeys that survived the first month, but presented with the disease during or after this period, remained alive until the end of the study, either recovered or were mildly ill (Table 5). Ostrowski *et al.* (1996) found that non-vaccinated birds inoculated with avian pox died between 7 and 20 days after challenge, contrary to the vaccinated birds that were mostly recovered by day 12. A similar report was made by Tripathy & Hanson (1978), who observed that sick birds were recovered between days 16 and 35 after challenge; furthermore, Boyle *et al.* (1997) found an inverse correlation beetween age and severity of lesions. These data reinforce the theory that observed mortality for months 2 and 3 after delivering the turkeys was probably caused by factors other than avian pox.

In the present study, vaccination against avian pox did not influence the incidence of the disease, but it reduced the severity of the clinical manifestation and the mortality at an early age in the studied populations. The vaccine used in this study is therefore recommended to reduce the negative impact of the disease in turkey production under backyard conditions.

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References

Allen T.C. (1995). Hematoxilina y eosina. In E.B. Prophet, B. Mills, J.B. Arrington, & L.H. Sobin (Eds.). *Metodos Histotecnologicos* (pp. 58–59). Washington, DC: Registro de Patologia de los Estados Unidos de America (ARP) y el Instituto de Patologia de las Fuerzas Armadas de los Estados Unidos de America (AFIP).

Bolte, A.L., Meurer, J. & Kaleta, E.F. (1999). Avian host spectrum of avipoxviruses. *Avian Pathology*, 28, 415–432.

Boyle, D.B., Pye, A.D. & Coupar, B.E.H. (1997). Comparison of field and vaccine strains of Australian fowlpox viruses. *Archives of Virology*, 142, 737–748.

Fatunmbi, O.O. & Reed, W.M. (1996a). Evaluation of a commercial quail pox vaccine (Bio-Pox Q) for the control of "variant" fowl poxvirus infections. *Avian Diseases*, 40, 792–797.

Fatunmbi, O.O. & Reed, W.M. (1996b). Evaluation of commercial modified live virus fowl pox vaccine for the control of "variant" fowl poxvirus infections. *Avian Diseases*, 40, 582–587.

Gutiérrez-Triay, M.A., Segura-Correa, J.C., López-Burgos, L., Santos-Flores, J., Ricalde, R.H., Sarmiento-Franco, L., Carvajal-Hernández, M. & Molina-Canul, G. (2007). Caracteristicas de la avicultura de traspatio en el municipio de Tetiz, Yucatán, México. *Tropical and Subtropical Agroecosystems*, 7, 217–224.

Kulich, P., Roubalová, E., Dubskáb, L., Sychra, O., Šmíd, B. & Literák, I. (2008). Avipoxvirus in blackcaps (*Sylvia atricapilla*). *Avian Pathology 2008*, 37, 101–107.

Mockett, A.P.A. (2006). Avipoxviridae. In F. Jordan, M. Pattison, D. Alexander & T. Faragher (Eds.). *Poultry Diseases* 5th ed (pp. 291–297). London: Elsevier.

Odoya, E.M., Abegunde, A., Agyogbo, B.G., Omotainse, S.O., Gwankat, E. & Okparapara, U.G. (2006). Outbreak of turkey pox disease in fowl pox vaccinated poults in Vom Plateau State of Nigeria. *African Journal of Clinical and Experimental Microbiology*, 7, 136–138.

Ostrowski, S., Dorrestein, G., Burger, L., Hemon, S. & Jalme, M.S. (1996). Cross-protection test of an avian poxvirus isolated from houbara bustards. *Avian Diseases*, 40, 762–769.

Ramos, E., Coroas, L., González, M. & Fernández, A. (2002). Detección de secuencias pertenecientes al virus de la reticuloendoteliosis en el genoma

de cepas vacunales y clones del virus de la viruela aviar. *Revista Salud Animal*, 24, 134–141.

Rejón-Ávila, M.J., Dájer-Abimerhi, A.F. & Honhold, N. (1996). Diagnóstico comparativo de la ganadería de traspatio en las comunidades de Texán y Tzacalá de la zona henequenera del Estado de Yucatán. *Veterinaria México*, 27, 49–55.

Silva, P.D., Batinga, T.D., Sales, T.S., Herval, E.F., Ramos, I., Maia, P. C. & Fernandes, L.M. (2009). Fowlpox: identification and adoption of prophylactic measures in backyard chickens in Bahia, Brazil. *Revista Brasileira de Ciência Avícola*, 11, 115–119.

Singh, P., Kim, T.J. & Tripathy, D.N. (2000). Re-emerging fowlpox: evaluation of isolates from vaccinated flocks. *Avian Pathology*, 29, 449–455.

Tizard, I.R. (2004). *Veterinary Immunology. An Introduction* 7th ed. Philapdelphia: Elsevier.

Tripathy, D.N. & Hanson, L.E. (1978). Pathogenesis of fowlpox in laying hens. *Avian Diseases*, 22, 259–265.

Tripathy, D.N. & Reed, W.M. (2003). Pox. In Y.M. Saif, H.J. Barnes, J.R. Glisson, A.M. Fadly, M.R. McDougald & D.E. Swayne (Eds.). *Diseases of Poultry* 11th ed (pp. 253–270). Ames: Blackwell Publishing.

Tripathy, D.N., Schnitzlein, W.M., Morris, P.J., Janssen, D.L., Zuba, J.K., Massey, G. & Atkinson, C.T. (2000). Characterization of poxvirus from forest birds in Hawaii. *Journal of Wildlife Diseases*, 36, 225–230.