



A Serological Survey for Avian Infectious Bronchitis Virus and Newcastle Disease Virus Antibodies in Backyard (Free-range) Village Chickens in Mexico

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ABSTRACT

The commercial flocks in Yucatan, Mexico are free of Newcastle disease virus (NDV) in its velogenic viscerotropic form, but little is known about the disease status of backyard poultry. A seroprevalence survey in 30 villages using haemagglutination inhibition (HI) tests for infectious bronchitis virus (IBV) and NDV antibodies was carried out from December 1997 to June 1998. The seroprevalences were 56.5% (95% CI 50–63%) for IBV and 2.2% (95% CI 0.5–3.8%) for NDV. All the villages had chickens that were positive for antibodies to IBV and nine of the villages had chickens that were positive for antibodies to NDV. This suggests that IBV may be responsible for a large proportion of the respiratory disease observed in backyard chickens in Yucatan. The implications of these findings are discussed, including the highly susceptible status of the backyard chickens in Yucatan to NDV and the possibility of this virus being one cause of the syndrome known as *mortandad* by the local people.

Keywords: haemagglutination inhibition, infectious bronchitis, management, Newcastle disease, poultry, respiratory disease, seroprevalence, virus

Abbreviations: AAF, amnio-allantoic fluid; CI, confidence interval; ELISA, enzyme-linked immunosorbent assay; FMVZ-UADY, Faculty of Veterinary Medicine and Animal Production, Autonomous University of Yucatan; HAU, haemagglutination units; HI, haemagglutination inhibition; IB, infectious bronchitis; IBV, IB virus; M41, Massachusetts 41; ND, Newcastle disease; NDV, ND virus; SPF, specific pathogen-free; VLA, Veterinary Laboratories Agency

INTRODUCTION

The two viruses that were the subject of this survey are extremely important pathogens of poultry responsible for diseases resulting in severe production and economic losses.

Infectious bronchitis virus (IBV) is a member of the *Coronavirus* genus of the family *Coronaviridae* and is distributed worldwide (Cavanagh and Naqi, 1997). The first isolates were closely related to the Massachusetts strain, but subsequently several

different types were recognized in the United States, Europe and other parts of the world (McMartin, 1993).

Outbreaks of IB have been reported to occur in vaccinated flocks, and virus strains isolated from these outbreaks are often found to be a type distinct from the vaccine type (Cavanagh and Naqi, 1997).

Among respiratory diseases, IBV infections are known to be a frequent problem in every country that has a system of intensive poultry production. If IBV is not considered to be significant in a particular country, this is generally because other diseases, such as Newcastle disease, pose a greater threat (Cook, 1995b).

Avian paramyxovirus type 1 (APMV-1) or Newcastle disease (NDV) is also distributed worldwide and is one of the major disease problems where it occurs. NDV is placed in the order *Mononegavirales*, family *Paramyxoviridae*, sub-family *Paramyxovirinae*, in the genus *Rubulavirus* (Rima *et al.*, 1995).

Both diseases have been diagnosed in Mexico and, in the case of IBV, this is despite vaccination of commercial birds, showing its great economic importance (Quiroz *et al.*, 1993).

The state of Yucatan, in the south-east of Mexico, has a unique geographical position. Being part of the peninsula with the same name, it is an important feeding stop for migratory birds, including waterfowl, travelling between North America and Central and South America. Yucatan was officially declared free of NDV in its velogenic viscerotropic form in 1995 and it has so far maintained this status. Very little evidence of the presence of this virus has been found in backyard chickens, by isolation or serology, although a proper study has not been carried out. Despite the absence of virulent virus, it would seem likely that village chickens become infected with ND viruses of low virulence for chickens, since such viruses are thought to be enzootic in wild birds (Alexander *et al.*, 1997), which are in constant contact with free-range chickens. On the other hand, antibodies to IBV were found in a large proportion (80%) of samples obtained from backyard chickens from two Yucatecan communities and processed at the Immunology Department of the Faculty of Veterinary Medicine and Animal Production, Autonomous University of Yucatan (FMVZ-UADY). One IBV isolate of unknown pathogenicity, but antigenically different from 12 reference strains, was obtained from backyard chickens in Yucatan that were experiencing respiratory disease (Gutierrez-Ruiz *et al.*, 1998). Respiratory signs and egg-shell defects are commonly observed in backyard birds, suggesting active IBV infection.

The objective of this study was to determine the prevalence and distribution of antibodies against IBV and NDV in backyard chickens in the state of Yucatan, Mexico.

MATERIALS AND METHODS

Sample size and distribution

The approach followed to determine the sample size and design for the study was the cluster sampling method (Bennett *et al.*, 1991; Thrusfield, 1995; Cook, 1995a; Otte and Gumm, 1997), because the individual sampling frame was not known.

Owing to the characteristics of the backyard system, it is reasonable to consider all the backyard poultry in a village as one flock, with very little within-cluster variation, so a village was considered as a cluster.

IB and ND are very contagious diseases so, to obtain similar accuracy to that with random sampling (246 using 80% expected prevalence, 5% accuracy and 95% confidence), the sample size had to be increased four times, to 984 samples (Martin *et al.*, 1987).

The number of clusters (villages) to be sampled was mostly controlled by the resources available (petrol, materials, time, etc.). Thirty clusters were included in the present study. The aim was to sample 40 birds per village, resulting in 1200 samples, allowing around 20% more samples than required as backup.

With this information, the estimated design effect (D) and precision of the study were determined. For IBV the rate of homogeneity (roh) used for calculations was 0.20, as suggested by Otte and Gumm (1997). The design effect was calculated from the formula (Bennett *et al.*, 1991)

$$D = 1 + (b - 1) \times (roh)$$

where b is the average number of serum samples in each cluster.

The standard error (S) was calculated from the formula (Bennett *et al.*, 1991)

$$S = \sqrt{[p(1 - p)D/n]}$$

where p is the prevalence and n is the total number of samples to be obtained. The expected prevalence was 80%, as suggested by the preliminary study. S was determined as 3%, indicating that with this design there is a 95% certainty that the true proportion (prevalence) of chickens with antibodies to IBV will be within $\pm 6\%$ ($\pm 2S$) of the estimate.

For NDV, the rate of homogeneity or intracluster correlation coefficient was calculated in a previous study as 0.18 (Otte and Gumm, 1997). Using the same formulae as for IBV, the design effect and precision (S) were calculated as $D = 8.02$, and $S = 4\%$, using an expected prevalence of 50% to be on the safe side (Bennett *et al.*, 1991). With this design there is a 95% certainty that the true proportion of chickens with antibodies to NDV will be within $\pm 8\%$ ($\pm 2S$) of the estimate.

A total of 1122 sera were obtained between December 1997 and June 1998. For NDV, only 970 samples were tested owing to the limited amount of serum obtained in some cases.

The prevalences were determined by dividing the total number of positive samples by the total number of samples (Bennett *et al.*, 1991; Thrusfield, 1995). The confidence intervals ($p \pm 2S$) were calculated from the formula (Bennett *et al.*, 1991)

$$S = \left[\frac{c}{\sum X_i} \right] \sqrt{\left\{ \frac{[\sum Y_i^2 - 2p \sum X_i Y_i + p^2 \sum X_i^2]}{c(c-1)} \right\}}$$

where c is the total number of clusters, X_i is the number of samples from each cluster and Y_i is the number of positive samples from each cluster.

The design effect and roh were estimated using the formulae given by Bennett and colleagues (1991).

The clusters were selected randomly, using a random number table (Thrusfield, 1995) and picking the selected numbers from a numbered list of communities with over 50 inhabitants obtained from the most recent Mexican national census (INEGI, 1991). The state of Yucatan was divided into four regions (central, east, south-east and south) and clusters were selected in each region, using proportional stratification, taking a percentage of the samples according to the percentage of villages contained in each zone. Accordingly, 14 (46.6%) communities were sampled in the central zone, 6 (20%) communities were sampled in the east zone, and 5 (16.7%) in each of the south-east and south zones. The communities sampled in each zone are listed in Table I.

To select the samples within a village, two transects were chosen at random through the main square of the village, forming a cross. Although it was planned to select eight backyards per village, it usually took more than this to obtain the required number of samples, and, even so, 40 samples were not always obtained. In some cases, the nearest village to the one selected had to be sampled as well, considering both villages as one cluster. Taking the main square of the village as the reference, around 10 samples were collected from each of four directions. To achieve this, a predetermined number household (every X th) was chosen for sampling. In cases (around 40%) where the selected household did not keep chickens or the owners did not wish to collaborate, the nearest household to it was approached. Usually 5 birds were sampled within each selected back yard.

Sampling procedure

Between 0.5 and 2 ml of blood was obtained from the brachial vein of chickens over 12 weeks of age, using a 3 ml syringe and a 23 gauge needle. The blood was deposited in a 5 ml sterile glass tube, left horizontally for approximately 2–4 h in a cool box with coolant, and then placed vertically at 4°C overnight. To obtain the maximum amount of serum, the samples were centrifuged for 10 min at 1000g. The serum was then collected and aliquoted into labelled 0.5 ml plastic vials.

Test procedure

The HI tests were done following the procedures described for IBV (Alexander *et al.*, 1983) and for NDV (Council Directive 92/66/EEC, 1997), from a dilution of 1:2 to a

dilution of 1:256, in the virology laboratory of the FMVZ-UADY. The antigens were diluted to contain four haemagglutination units (HAU). The titres were expressed as \log_2 of the reciprocal of the highest dilution of serum giving 100% inhibition of 4 HAU.

IBV titres equal to or higher than $5 \log_2$ (1/32) and NDV titres equal to or higher than $4 \log_2$ (1/16) were considered positive.

Antigen procedure for IBV and NDV HIT

The antigens were produced at the VLA, Weybridge, UK, by inoculation of seed viruses (IBV-M41 and NDV-Ulster strains) into the allantoic cavity of 9- to 11-day-old specific pathogen-free (SPF) embryonated hens' eggs (EHEs). The EHEs were incubated for 36–48 h (IBV) or 48–72 h (NDV) and the live embryos were then chilled at 4°C for at least 6 h before the amnio-allantoic fluids (AAF) were aseptically harvested. The fluids were clarified by being centrifuged for 10 min at 5600g in a bench centrifuge (MSE, Mistral 1000). For IBV, the virus was pelleted by centrifuging the supernatant for 60 min at 4°C and 32 000g in an ultracentrifuge (MSE, HI SPIN 21) with an 8 × 50 ml rotor (Model 43114-143). The supernatants were discarded and the pellets were resuspended in a filtrate of *Clostridium perfringens* type A toxin to 1/100 of the original volume; the suspensions were then incubated for 2 h at 37°C. NDV was inactivated by adding formalin to the clarified AAF to give a final concentration of 0.1%, followed by incubation for 18–24 h at 37°C, with constant stirring.

RESULTS

Infectious bronchitis virus

All 30 communities sampled had chickens that were positive for antibodies against IBV by the HIT. The design effect (D) was 4.9 and the roh was 0.1. The prevalences in each community are presented in Table I.

Newcastle Disease virus

Nine (30%) of the 30 communities sampled had chickens that were positive for antibodies against NDV by the HIT. D was 3.5 and roh was 0.08. The prevalences in each community are presented in Table I.

DISCUSSION

Despite the great importance of backyard chickens to a large proportion of the rural population in Mexico and many other developing countries, very little has been done to determine the health problems of these animals. As all the communities sampled in this

TABLE I
Seroprevalence of avian infectious bronchitis and Newcastle disease viruses in backyard chickens in 30 communities of the state of Yucatan, Mexico, using Massachusetts 41 (IB) and Ulster (ND) virus antigens in the haemagglutination inhibition test

Village	Results with IBV antigen			Results with NDV antigen		
	Sera sampled	Sera +ve	Prevalence (%)	Sera sampled	Sera +ve	Prevalence (%)
<i>South zone</i>						
Tixcuytun	39	22	56.4	39	0	0
Xoy	31	20	64.5	30	0	0
Peto/Tadziu	38	11	28.9	30	1	3.3
Macmay/Catmis	41	29	70.7	30	0	0
Tixmeuac	39	15	38.5	30	1	3.3
Sub-total	188	97	51.6	159	2	1.3
<i>South-east zone</i>						
Sotuta	40	35	87.5	40	7	17.5
Tadzibichen	40	17	42.5	30	0	0
Kanxoc	41	23	56.1	41	0	0
Chankom	30	14	46.7	29	0	0
Kaua/San Lorenzo	30	20	66.7	30	0	0
Sub-total	181	109	60.2	170	7	4.1
<i>East zone</i>						
Espita	40	26	65.0	40	0	0
Chan San Antonio	38	17	44.7	30	0	0
Sucopo	40	13	32.5	30	0	0
Tixcancal	40	24	60.0	30	0	0
Yalsihon Buena Fe	40	25	62.5	30	0	0
Xbec	41	34	75.6	30	0	0
Sub-total	239	139	58.2	190	0	0
<i>Central zone</i>						
Baca	41	20	48.8	30	0	0
Chicxulub Pueblo	42	26	61.9	30	1	3.3
Kantunil	40	19	47.5	40	0	0
Chunchucmil	39	11	28.2	39	3	7.6
Dzoyaxche	27	21	77.8	27	3	11.1
Noc Ac	36	13	36.1	30	1	3.3
Oxcum	31	25	80.6	31	1	3.2
Cuzama	42	24	57.1	30	0	0
Ruinas de Ake	31	12	38.7	25	0	0
Suma	40	27	67.5	30	0	0
Telchaquillo	38	21	55.3	38	0	0
Tetiz	30	15	50.0	29	0	0
Xtepen	35	14	40.0	30	0	0
Yobain	42	41	97.6	42	3	7.1
Sub-total	514	289	56.2	451	12	2.6
Total	1122	634	56.5	970	21	2.2

study were positive for antibodies to IBV, and minimal vaccination is practised, it appears that IBV may be responsible for a considerable percentage of the respiratory disease that occurs in these birds, especially when combined with secondary bacterial pathogens, such as *Escherichia coli* or *Mycoplasma* (Honhold *et al.*, 1993; Rivera-Ortega, 1997).

The seroprevalence for IBV (56.5% 95% CI 50–63%) found in this study may be an under estimate, since only one serotype was used as antigen, although the M41 serotype usually gives more cross-reactions than other serotypes (Alexander *et al.*, 1976). In a survey performed on free-range chickens in California, 46.7% of 30 flocks examined and 21.8% of birds tested were positive for IBV antibodies by an ELISA test (McBride *et al.*, 1991). The seroprevalences were lower than in Yucatan, but the study conditions were different and biosecurity measures in California are much stricter than in the backyards in Yucatan.

The IBV seroprevalences observed in individual communities in Yucatan were very variable (28.2–97.6%); this could have several explanations. It might be expected that chickens located in towns around which commercial farms are situated would have higher prevalences owing to more frequent contact with vaccine strains used in commercial enterprises, but the results with naive backyard chickens indicate that this was not the case. All the commercial farms are located in the central zone of the state of Yucatan, but only small differences in seroprevalence were observed in the four zones into which the state is divided. A more likely explanation for the variation in seroprevalence is that, although active vaccination is not practised in villages against any disease, it is known that local governments every now and then introduce into their communities packages consisting of 6–10 chickens of commercial lines (broilers or laying types) whose origins are difficult to trace but which may be from the surplus at commercial hatcheries, where live IBV vaccines are usually applied to 1-day-old chicks. Another explanation could be that different IBV variants of differing pathogenicity are distributed in Yucatan, some of which have higher transmission rates than others. Also, the sampling design used in this study, although adequate to determine the overall seroprevalence in the state, is not necessarily the best to determine the actual seroprevalence in the individual communities; a much larger sampling number would be needed to achieve this. Any or all of these factors may have been involved in producing the observed differences in prevalence.

Very little is known about diseases in backyard chickens but, in a preliminary study carried out in Uganda, researchers found seroprevalences of 50–90% for IBV infections in village chickens from three different areas of the country (Mukiibi-Muka and Olaho-Mukani, 1998). This agrees with the findings of the present study in that IBV was widely distributed, not only where commercial chickens are present but also in free-range village chickens.

Despite the wide distribution of IBV, it does not follow that highly pathogenic types are present in village chickens in Yucatan; serology is unable to demonstrate which types of IBV are present in an area, although this knowledge is indispensable in determining which vaccines, if any, should be used. Seven isolates of IBV were obtained during the survey and they will be characterized in order to determine their importance in backyard poultry in Yucatan, Mexico.

The low seroprevalence (2.2%) (95% CI 0.5–3.9%) of NDV in backyard chickens in the state of Yucatan supports the status of the state as being free from the velogenic viscerotropic form of NDV.

Eleven of the 21 positive sera had ND HI titres of only 4 log₂ (1/16), with 5 of 9 towns having only one reactor. This suggests that these may have been false positive results or perhaps that the birds were derived from those distributed by the government and vaccinated with live attenuated vaccines as day-old chicks. Three towns had chickens with titres of log₂ ≥ 7, and, although the owners of these birds stated that they had not been vaccinated against NDV, there is a possibility that some of them had been vaccinated with more than one dose of vaccine before being introduced into the towns by the respective local governments. Another possible explanation of the results is that a slow circulation of field virus was occurring at the time of sampling, although no isolations were made. The low prevalence or absence in the HI test of detectable antibodies to NDV in the 30 communities sampled indicates that the backyard chickens in those communities would be highly susceptible to pathogenic NDV infections (Allan and Gough, 1974). In their serological study in Uganda, Mukiibi-Muka and Olaho-Mukani (1998) found that the birds from one village out of the three sampled did not have antibodies to NDV, which indicates that, even in enzootic areas, some villages can remain fully susceptible to infection with virulent NDV. A seroprevalence of 4% was found in free-range chickens in California using an ELISA test (McBride *et al.*, 1991). Conditions were different from those in Mexico but, despite there being no confirmation of transmission between commercial and backyard flocks, these researchers pointed out that the disease status could change at any time owing to lack of immunity and that therefore biosecurity measures should be reinforced (McBride *et al.*, 1991).

It is popular knowledge, as described by some researchers (Honhold *et al.*, 1993), that every now and then in many communities in the state of Yucatan there is a syndrome known as *mortandad*, whereby most birds in an entire village die in a short period of time, sometimes without signs of disease, although often respiratory and enteric signs are observed. The cause or causes of this syndrome have not yet been identified, but highly virulent NDV may be one of the agents responsible; certainly the serological evidence and the constant contact between backyard chickens and wild birds makes this a viable possibility.

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Etude sérologique des anticorps contre les virus de la bronchite infectieuse aviaire et de la maladie de Newcastle chez des poulets élevés en liberté dans des villages du Mexique

Résumé – Les poulets élevés commercialement au Yucatan (Mexique) sont exempt du virus de la maladie de Newcastle (NDV), tout du moins dans sa forme foudroyante et viscérale. Cependant peu de choses sont connues sur la situation pour les volailles gardées en arrière-cour.

La séroprévalence fut étudiée par test d'inhibition de l'hémagglutination pour ces deux virus dans 30 villages suivis entre décembre 1997 et juin 1998. La séroprévalence fut de 56,5% (CI 95%, valeurs entre 50 et 63%) pour l'IBDV et 2,2% (CI 95%, valeurs entre 0,5 et 3,8%) pour le NDV. Tous les villages eurent des poulets avec des anticorps contre le NDV et 9 villages en eurent contre le IBDV. Ceci prouve que l'IBDV est responsable pour une part importante des maladies respiratoires observées chez les poulets d'arrière-cour au Yucatan.

Les conséquences sont discutées dans cet article, prenant en compte la grande susceptibilité des poulets au virus de le NDV et à la possibilité que ce virus soit la cause du syndrome bien connu du nom de "mortandad" par les populations locales.

Resumen – Las parvadas comerciales en Yucatan estan libres del virus de la enfermedad de Newcastle (VEN) en la forma velogenica viscerotropica pero poco se sabe acerca de la situacion de la enfermedad en aves de traspatio. Un estudio de sero-prevalencia en 30 pueblos usando las pruebas de inhibicion para la hemaglutinacion para el virus de la bronquitis infecciosa (VBI) y VEN se llevo a cabo de diciembre de 1997 a junio de 1998. Las sero-prevalencias fueron 56,5% (IC 95%, 50–63%) para VBI y 2,2% (IC 95%, 0,5–3,8%) para VEN. Todos los poblados tuvieron pollos positivos a anticuerpos contra VBI y 9 de 30 poblados tuvieron pollos positivos a anticuerpos contra VEN. Lo anterior sugiere que el VBI pudiera ser responsable de una alta proporcion de las enfermedades respiratorias observadas en pollos de traspatio en Yucatan, Mexico. Se discuten las implicaciones de estos hallazgos incluyendo la alta susceptibilidad de los pollos de traspatio en Yucatan al VEN y la posibilidad de que este virus sea una de las causas del sindrome conocido como 'mortandad' por la gente local.