Serological Survey of Dominant Viral Diseases (Newcastle Disease (ND), Infectious Bronchitis (IB) and Infectious Bursal Disease (IBD)), in Broilers Flocks in Northern Algeria

Omar SALHI[1,*], Djemel KHELEF[1], Chafik-Reda MESSAI[1], Aziz LOUNAS[2], Abdellah MOHAMED-CHERIF[1], Rachid KAIDI[2], Khatima AIT-OUDHIA[1]

[1]Laboratory HASAQ (Hygiène Alimentaire et Système Assurance Qualité), Higher National Veterinary School, Algiers, Algeria.
[2]Laboratory LBRA (Laboratoire de Biotechnologies liées à la reproduction), Institute of Veterinary Sciences, Blida, Algeria.
**corresponding author: dr.salhi-omar@hotmail.com*

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Abstract

The present study was conducted to survey about sero-epidemiological status of Newcastle disease (ND), Infectious bronchitis (IB) and Infectious bursal disease (IBD) on Algerian broiler chicken (30 flocks/1200 sera) using ELISA method and to assess the influence of some risk factors related to each disease. Among all investigated flocks, ND was the most seroprevalent disease (63.33%); however, IB and IBD showed less serological positivity (40% and 16.66% respectively). For ND, Cobb 500 Flocks were significantly more seropositive by 78% (p = 0.025) than other strains. Nevertheless, flocks with good hygiene were significantly less seropositive to ND by 26% (p = 0.022). For IB, the risk of seropositivity was significantly lower in spring by 40% (p = 0.036). Although, flocks with higher density or with more than 30 days old were more seropositive respectively by 47% (p = 0.041) and 45% (p = 0.019). At last, when broiler chicken were not boosted by IBD vaccine, flocks appeared to be more seropositive by 48% (p = 0.047); especially in spring by 45% (p = 0.048); or in farms with poor hygiene by 65% (p = 0.004); however, more than flocks 30 days old flocks were less seropositive by 30% (p = 0.009).

Keywords: Serological; Newcastle Disease; Infectious Bronchitis; Infectious Bursal Disease; broilers.

Introduction

The sector of broiler poultry is the largest and the most efficient meat production industry in the world (Gupta *et al*., 2014). Indeed, Algeria is one of the numerous countries where broiler production is threatened by a number of infectious diseases, especially viral, where the economic losses represent enormous bill with no reliable solution of any medication (Pradhan *et al*., 2014). Newcastle disease (ND) is the most economically important disease in poultry -particularly in developing countries- due to high mortality, and associated sanitary measures in poultry farms or slaughters (Ban-Bo *et al*., 2013). ND is caused by virulent strains of avian paramyxovirus type 1 (APMV1). This virus is highly contagious in all age groups and can infect many species of domestic and wild birds (Hasan *et al*., 2010). Infectious bronchitis (IB) is an acute, highly infectious and economically important viral disease in chickens caused by the infectious avian bronchitis virus (IBV) (Ahmed *et al*., 2007). A virus of the Coronaviridae family, IBV

is characterized by high genetic and pathogenic variability, and new strains continue to emerge. According to clinical signs, IB is generally divided into nephropathogenic and respiratory types and can spread through multi-age units (Abao *et al*., 2015). Infectious bursal disease (IBD) is a highly contagious acute viral disease of young chickens (3-6 weeks), which causes mortality or immunosuppression following damage to the bursa of Fabricius, resulting poor growth of young chickens and significant economic losses (Khan & Dana, 2005). The causative agent of IBD is an infectious bursal disease virus (IBDV), belonging to the Birnaviridae family. IBDV strains are classified into two distinct serotypes namely: pathogenic and non-pathogenic (Prandini *et al*., 2016).Various diagnostic methods like ELISA have been frequently used all over the world to detect viruses portage from the field samples (Desingu *et al*., 2014). The advantage of this test is to measure the serological reaction of a bird to the pathogen over a period of time (Auvigne *et al*., 2013). Risk factors related to biosecurity and farm practices appear to have a significant role in the severity of the disease observed in affected farms (Jaganathan *et al*., 2015). To our knowledge, this is the first research work using the ELISA method to study the main avian viral pathologies accompanied by clinical signs in broiler flocks in Algeria Therefore, the present study was conducted aiming at a sero-epidemiological survey for ND, IB and IBD in Algerian avian flocks using ELISA method and to assess the risk factors related to each disease.

Materials and methods

Ethical approval

Experimental procedures approved by the Institutional Committee for the Protection of Animals of the National Administration of Higher Education and Scientific Research of Algeria (98-11, Act of 22 August 1998).

Animals

The experiment was carried out at commercial farms in the central, east and west of northern Algeria (longitude 36° and latitude 3°), from July 2014 to June 2016 on thirty (30) broiler flocks with different strains (Arbor acres, Cobb 500, Hubbard F15) aged between four to seven weeks and containing 2,000 to 7,000 birds/farm. The studied flocks had been initially vaccinated for ND, IB and IBD with live vaccines through different protocols (Figure1). The analysed flocks were suspected to acquire a viral disease (ND, IB and IBD) after showing the characteristic clinical and necropsic signs.

Blood collection procedures

A total of 1200 birds were sampled randomly from 30 broiler flocks (20 samples/flock), according to our protocol, two samples were taken from each farm; the first was performed the first days after the appearance of the first clinical signs. The second one was done, two to three weeks later. Blood samples were collected from the wing vein, in dry tubes and centrifuged (5000 rpm for 10 min) at the same day to recover the sera that were stored in test tubes Eppendorf and frozen at -20°C until analysis.

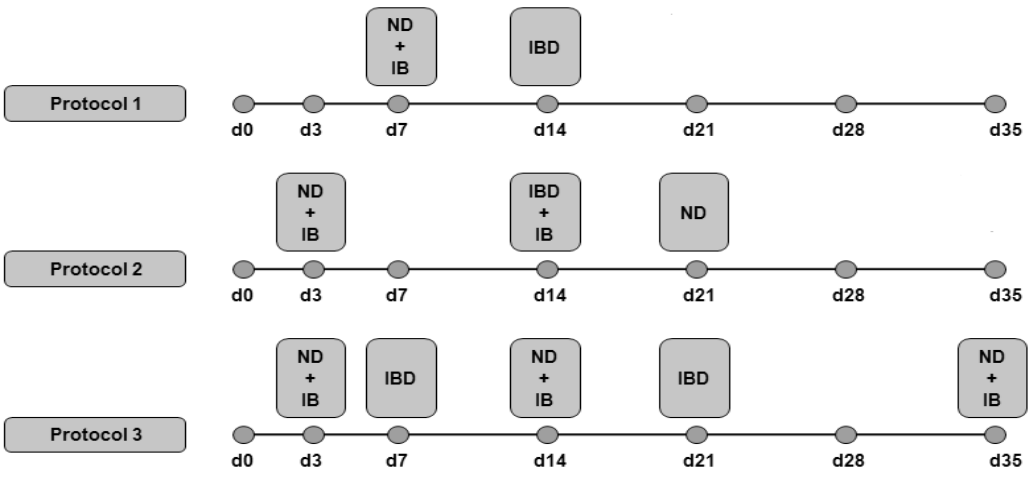

Figure 1. Schematic diagram of protocols vaccine used in the flocks (d: day of vaccine).

Serological Methods

An indirect ELISA technique was carried out using ID.vet Innovative Diagnostics kits (Montpellier, France): ID Screen® NDV Indirect, ID Screen® IBV Indirect and ID Screen® Indirect IBDV. The sera were diluted to 1 / 500th, then loaded to ELISA plates to start immuno-sorbent reaction as guided by manufacturer's manuals. ELISA plates were read by ELx800 spectrophotometer (DIALAB GmbH, Wiener Neudorf, Austria) equipped with the 450 nm filter; where the measured optical density (OD) was transformed into titrated 'antibody. The averages of the titers and the coefficient of variation (CV) were automatically calculated by band and by series of samples with the software provided by the laboratory (IDSoftTM, Montpellier, France).

Observation of risk factors

During our survey, we took into account several parameters such as: age of occurrence, stocking density, strain, hygiene, vaccination programs (age of vaccination, type of vaccine and method of administration) season, area and climate.

Statistical analysis

Firstly, descriptive statistics were used to characterize flocks according the different factors. Thus, statistical analyses were performed with SAS (Version 9.1.3; SAS Institute Inc., Cary, NC). Before fitting statistical analysis, examination of the distributions of antibody titers indicated using (PROC UNIVARIATE, Shapiro–Wilk test) that most could not be considered normally distributed. If the variable does not fit the normal distribution, adjustments such as logarithmic, squared, Square root transformations are possible tools. Antibody titer of each disease through the time was analyzed by fitting a mixed general linear model using the MIXED procedure of SAS to evaluate seropositivity between the first and second serum collection. Then, the effect of probability of seropositivity was assessed using mixed-effects multivariable models (PROC GENMOD), using a normal distribution and log it link functions, and flocks as a random affect. Variables offered to the model included area, vaccination protocols, season, strains, climate, hygiene, density and age. Variables of age, size of flock, season, climate and hygiene were dichotomized on ≤30 vs.>30 days groups for age; ≤10 vs. >10 birds/m^2 groups for density; autumn vs. summer and spring groups for season and dry vs. wet groups for climate. Before including in mixed model, initial screening of variables was performed using a manual backward stepwise procedure with significant variables ($P < 0.1$) remaining in the model. This procedure was repeated for each disease. Finally, sensitivity and specificity of detecting diseases according to clinical and necropsic signs was calculated using the diagnostic test evaluation of Win Episcope 2.0.

Results and discussions

Table 1 presents the results of antibody titers for ND, IB and IBD. Among total of 30 flocks, 19 (63.33%) were tested positive to ND; 12 (40%) flocks were tested positive to IB and 07 (16.66%) flocks were tested positive for IBD. For all mentioned diseases, it has been shown a low CV and significant difference ($p < 0.0001$) in antibody titer between the first and the second sample ; respectively for ND (LSM± SE, 1989.06 vs 4511.00 ± 258.07, CV (29-40%); IB (LSM± SE, 1935.22 vs 4665.89± 369.25, CV (11-25%) and IBD (LSM± SE, 2062.20 vs 4168.00 ± 313.03, CV (33-45%).

We observed that the use of necropsic and clinical signs to diagnosis the three diseases was matched to our serological findings (table 2), conducting to a very high specificity (100%). In other words, all birds suspected of having ND, IB or IBD had specific antibodies. However, the sensitivities were 85.0, 75.0, and 71.4% for ND, IB, and IBD respectively. So far for this three diseases, necropsy and clinical diagnosis were particularly reliable.

Table 1. Serological results

Pathology	Antibody titers		CV (%)	SE	P	Seropositivity (%)
	Mean 1	**Mean 2**				
ND	1989.06	4511.00	29-40	258.07	<0.0001	51.11
BI	1935.22	4665.89	11-25	369.25	<0.0001	31.11
IBD	2062.20	4168.00	33-45	313.03	<0.0001	17.77

The factors influencing the seropositivity of ND, IB and IBD are shown in Tables 3, 4 and 5 respectively. For ND, Cobb 500 strain were significantly more seropositive by 78% (OR = 1.78, p = 0.025) compared to Hubbard-F15 strains. However, this difference was not evident between Arbor acres and Hubbard-F15 (p = 0.729). On the other hand, flocks with good hygiene were significantly less seropositive by 26% (OR = 0.74, p = 0.022) compared to those where hygiene was poor (Table 3).

For IB, when the flocks were sampled in spring, seropositivity was 40% lower (OR = 0.60, p = 0.036) compared to the summer. Flocks with density superior than 10 birds/m^2 were significantly more seropositive by 47% (OR = 1.47, p = 0.041) than those with density inferior or equal than 10 birds/m^2. Therefore, flocks with more than 30 days old birds were seropositive by 45% (OR = 1.455, p = 0.019) those less aged of 30 days (Table 4).

For IBD, when the vaccination protocol 2 was applied, flocks were significantly more seropositive by 48% (OR = 1.48, p = 0.047) compared to protocol 3 and when flocks were sampled in spring, the seropositivity was 45% higher (OR = 1.447, p = 0.048) compared to summer. In addition, flocks with poor hygiene were more seropositive by 65% (OR = 1.65, p = 0.004) compared to those with good hygiene. More than 30 days old birds were

Table 2. Diagnostic sensitivity (%) and specificity (%), with 95 percent confidence intervals (CI) and true Prevalence of test based on lesional signs of detecting ND, BI and IBD.

Pathology	Sensitivity (%) (95%CI)	Specificity (%)(95%CI)	True Prevalence (%) (95%CI)
ND	85.0 (69.4,100)	100.0 (100.0, 100.0)	64.5 (47.7, 81.4)
BI	75.0 (50.5,99.5)	100.0 (100.0, 100.0)	40.0 (22.5, 57.5)
IBD	71.4 (38.0,104.9)	100.0 (100.0, 100.0)	23.3 (8.2, 38.5)

Table 3. Effects of risk factors on the seropositivity for ND

Factors	Value	Prevalence	Estimate	SE	0R	95%CI	P
Protocols of vaccination*	1	21.0	-0.39	0.25	0.67	0.41-1.10	0.11
	2	47.3	-0.08	0.20	0.92	0.61-1.39	0.70
	3	31.5			Ref		
Season	Autumn	21.0	0.07	0.18	1.08	0.75-1.54	0.66
	Spring	10.5	-0.09	0.21	0.90	0.59-1.38	0.66
	Summer	68.4			Ref		
Strain	Arbor acres	36.8	-0.05	0.16	0.94	0.67-1.3	0.72
	Cobb 500	21.0	0.57	0.25	1.78	1.07-2.9	**0.02**
	ISA	42.1			Ref		
Climate	Wet	52.6	-0.19	0.17	0.82	0.58-1.17	0.28
	Dry	47.3	Réf				
Hygiene	Good	15.7	-0.29	0.24	0.74	0.46-1.19	**0.02**
	Intermediate	26.3	0.12	0.19	1.13	0.77-1.67	0.51
	Bad	57.8			Ref		
Density (birds/m^2)	>10	57.8	0.06	0.19	1.07	0.73-1.56	0.72
	≤10	42.2			Ref		
Age (day)	>30	73.6	-0.01	0.15	0.98	0.71-1.34	0.90
	≤30	26.316			Ref		

Vaccination protocol, 1: primo vaccine without booster vaccine;2: primo vaccine with one booster vaccine; 3: primo vaccine with two booster vaccine

less seropositive by 30% (OR = 0.69, p = 0.009) compared to younger birds namely less than 30 days old (Table 5)

The aim of our study was to evaluate the immune status by screening sero-prevalence of ND, IB and IBD in Algerian broiler chicken. In fact,

Table 4. Effects of risk factors on the seropositivity for IB.

Factors	value	Prevalence	Estimate	SE	0R	95%CI	P
protocols of vaccination*	1	41.6	0.43	0.33	1.54	0.79-2.99	0.19
	2	41.6	0.14	0.24	1.15	0.71-1.88	0.55
	3	16.6			Ref		
Season	Autumn	8.33	-0.24	0.19	0.78	0.53-1.13	0.19
	Spring	0.00	-0.49	0.23	0.60	0.38-0.96	**0.03**
	Summer	91.6			Ref		
Strain	Arbor acres	41.6	-0.03	0.18	0.96	0.67-1.37	0.85
	Cobb 500	25.0	-0.31	0.33	0.73	0.37-1.41	0.35
	ISA	33.3			Ref		
Climate	Wet	75.0	-0.09	0.22	0.91	0.58-1.42	0.67
	Dry	25.0			Ref		
Density (birds/m²)	>10	83.3	0.38	0.21	1.47	0.96-2.25	**0.04**
	≤10	16.7			Ref		
Age (day)	>30	100.0	0.37	0.16	1.45	1.06-1.99	**0.01**
	≤30	0.00			Ref		

Vaccination protocol, 1: primo vaccine without booster vaccine; 2: primo vaccine with one booster vaccine; 3: primo vaccine with two booster vaccine

Table 5. Effects of risk factors on the seropositivity for IBD.

Factors	Value	Prevalence	Estimate	SE	0R	95%CI	P
protocols of vaccination*	1	28.5	-0.08	0.29	0.92	0.52-1.63	0.77
	2	57.1	0.39	0.20	1.48	0.98-2.22	**0.04**
	3	14.2			Ref		
Season	autumn	14.2	-0.20	0.15	0.81	0.60-1.09	0.16
	Spring	28.5	0.37	0.19	1.44	0.98-2.12	**0.04**
	Summer	57.1			Ref		
Strain	Arbor acres	57.1	0.22	0.14	1.25	0.94-1.65	0.11
	Cobb 500	0.00	-0.07	0.25	0.92	0.56-1.54	0.77
	ISA	42.85			Ref		
Climate	Wet	71.4	0.12	0.18	1.13	0.79-1.63	0.48
	Dry	28.5			Ref		
Hygiene	Bad	57.1	0.50	0.17	1.65	1.16-2.34	**0.004**
	Intermediate	14.2	0.01	0.14	1.02	0.77-1.34	0.88
	Good	28.5			Ref		
Density (birds/m²)	>10	57.1	0.21	0.17	1.24	0.88-1.73	0.20
	≤10	42.9			Ref		
Age (day)	>30	42.8	-0.36	0.14	0.69	0.52-0.91	**0.009**
	≤30	57.1			Ref		

Vaccination protocol, 1: primo vaccine without booster vaccine;2: primo vaccine with one booster vaccine; 3: primo vaccine with two booster vaccine

Immune status in response to viral diseases is estimated by measuring the serological response objectified by detection of specific antibodies produced either in response to infection or following vaccination (Picault *et al*., 1993; Brigitte *et al*., 1997). At last, the protected farms must have a higher average of titres than the protection threshold for all the analysed dates without being very high compared to those resulting from the vaccination; although in the absence of specific clinical signs (Gardin *et al*., 2002). In contrast, our sampled herds were suspected to be infected with one of the viral diseases (ND, IB or IBD), based on typical clinical and necropsy signs and showed high morbidity and mortality with a high level of antibody titers. Indeed, outbreaks have been reported in the vaccinated populations despite the fact that vaccination is widely applied (Van Boven *et al*., 2008). Thus, Clinical and necropsy manifestations of affected birds can help the diagnosis of a disease, but a laboratory analysis is needed to confirm it (Hasan *et al*., 2010). Within the scope, ELISA test does not distinguish post-vaccine antibodies from post-infectious antibodies when vaccinated with an inactivated vaccine; instead, the vaccines used for the three diseases (ND, IB, IBD) were live vaccines for all the farms. Thus, the absence or presence of clinical signs and the type of vaccine used should be taken into account (Van den Berg *et al*., 2000). In the present study, we took paired samples to screen the serology status of a disease (the first sample was taken at the beginning, the second, two to three weeks later). In fact, the appearance of antibodies between two successive sera (usually taken within a period of 10 to 21 days), indicated that the first contact with the vaccine took place around the period when the first sampling was applied. Since the obtained concentration of antibodies increased between the 02 sera collected, this would indicate that we had a stimulation of the immune system and could be due to a recent infection or to a symptomatic viral reactivation (Alexander *et al*., 2004; Lopez, 2006).

As we assessed the factors affecting ND, farms with the Cobb 500 strain were significantly more seropositive. Some breeds or strains are inherently resistant or less affected by a pathogen that may be lethal to other individuals of the same species (Zekarias, 2002). Local chickens appear to be somewhat more resistant to Newcastle disease than exotic or imported birds (Tewari *et al*., 1992). While, Martin and Spradbrow (1992) reported that native poultry has a higher resistance to ND than commercial breed. A serological survey conducted to determine the prevalence rates of Newcastle disease virus antibodies in different breeds of chickens reared in different systems showed no race-specific trends in farm, backyard and post-harvest systems and intensive (Higgins & Shortridge, 1988). Discrepancies of opinions about the relative susceptibility of native and commercial breeds are noted; at present, the importance of breed sensitivity in the epidemiology of Newcastle disease in free range poultry is not clear (Awan *et al*., 1994). Farms with good hygiene were significantly less seropositive, compared to those with poor hygiene. It is clear that good hygiene and biosecurity measures aim at preventing the introduction of viruses into poultry farms and reducing its economic losses (Alexander *et al*., 2004).

The present findings noted an effect of the season on IB infection; sampling in spring appeared to be less sero-positive compared to summer. Seasonal cycles of infectious diseases have been variously attributed to changes in environmental conditions (Dowell, 2001; Lopez, 2006). Thus, the cold seems to have an effect on diseases caused by corona viruses such as IBV, (Holmes, 2003). Indeed, the spring season in Algeria is considered to be a cold period. In discordance to our findings, a high prevalence of IBV had been demonstrated in New Zealand, from samples collected during the cold period (Ramneek *et al*., 2005) and was probably due to ineffective environmental factors such as poor ventilation due to the need to conserve heat (Ahmed *et al*., 2007). The impact of the season remains unclear. It may be due to environmental changes, changes in host physiology, or alterations in the virus (Dowell, 2001; Lopez, 2006). Additionally, Flocks with density superior than 10 birds/m^2 were significantly more seropositive to IB than those with density inferior or equal than10 birds/m^2. Overpopulation seems to be one of the factors favoring introduction and implantation of the virus (Ban-Bo *et al*., 2013). The clinical impact of these variants to IB appeared to be largely dependent on the breeding conditions of the birds, that is, the stocking density and the technical and health management (biosecurity). More than 30 days old birds were seropositive than younger ones. IB is a highly contagious

acute respiratory viral disease in chickens of all ages (Abao *et al*., 2015). Mortality may occur in young and old chickens due to respiratory or renal manifestations of infection, but clinical signs are more severe in young ones (Animas *et al*., 1994). However, the disease is more common between 7 days and 5 weeks (Ahmed *et al*., 2007).

For IBD, when the vaccination protocol was applied (a primary vaccination without booster), the farms were significantly more seropositive, compared to the vaccination protocol (primary vaccination + booster); these findings showed the importance of the vaccine booster. The success of vaccination also depends on the choice of vaccine strain and vaccination protocol (Van den Berg *et al*., 2000). The primo vaccinated batches with the inactivated vaccine are highly protected which underlines the importance of the primary vaccination (Brigitte *et al*., 1997). Also, when the farms were sampled in spring, the seropositivity was higher compared to the summer. IBD appeared with equal frequency regardless of the season (Diallo, 1978) or of month (Picault *et al*., 1993). In contrast, Raveloson (1990) showed for IBD a high prevalence during the wet and hot season. In addition, farms with poor hygiene were more seropositive compared to those with good hygiene. The prevention of IBD disease is based on hygiene and medical prophylaxis, for this purpose it is important to emphasize that no vaccine can solve the problem of IBD if the necessary precautions are not taken, such as the respect for all-in / all-out farming methods, cleaning and disinfection of farms and crawl space (Orsi *et al*., 2010). Birds older than 30 days were less seropositive than younger birds. IBD is a highly contagious acute viral disease of young chickens from 3-6 weeks old, when the bursa of Fabricius reaches its maximum development which coincides with the appearance of clinical signs during an illness (Van den Berg *et al*., 2000; Hasan *et al*., 2010; Gupta *et al*., 2014), then that infections before the age of 3 weeks are usually subclinical.

Conclusions

The serological survey conducted in this study provided an important scope about dominant viral diseases on broiler chickens, and revealed that the seroprevalence of ND, IB and IBD were 63.33, 40% and 16.66%, respectively. Clinical manifestations and postmortem findings of affected birds may aid to diagnose a disease but laboratory diagnosis is necessary for confirmation of the diseases. Further to that, the findings also suggest that risk factors related to biosecurity and farm practices appear to have a significant role in the severity of the disease observed in affected farms. If those factors are alleviated, the severity of the ND problems in farms would be greatly reduced.

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