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End of studies project aiming to obtain
Doctor of Veterinary Medicine degree

Clinical, histopathological and Serological study on Newcastle disease

Presented by

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Dedications

To my dear parents, whose unwavering love and support have Figured me into who I am today. Your guidance and encouragement have been my guiding light.

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To my cherished family and friends, who have filled my life with laughter, warmth, and unforgettable moments. Your presence and belief in me have been my greatest blessings

-Abderrahmane

Dedications

I dedicate this thesis to my dear parents who have always been by my side and supported me throughout these long years of study.

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To my dear brothers, a source of hope and motivation.

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Abstract

The broiler poultry sector is very important for a growing number of countries, Algeria being one of them. However, its production is threatened by a number of viral diseases causing huge economic losses.

The present study was conducted to assess the clinical, serological and histopathological status of Newcastle disease (ND) in broiler farms (20 farms / 600 sera) using the ELISA method, and to evaluate the influence of certain risk factors associated with each disease.

Our results show that: of all the farms studied, ND was 60% seropositive. With regard to risk factors: farms with good hygiene were significantly less seropositive to ND by 26% ($p = 0.022$). However, farms with a higher density or older than 30 days were more seropositive by 47% ($p = 0.041$) and 45% ($p = 0.019$) respectively. Finally, when the broilers were not vaccinated against ND, the farms appeared to be 48% more seropositive ($p = 0.047$).

In conclusion, the serological survey carried out as part of this study provided an important framework on viral diseases, which are dominant pathologies in broilers. Thus, many factors are responsible for the appearance of these diseases.

Key words: clinical, histopathological, serological; Newcastle disease; broiler chicken, Algeria.

Résumé

Le secteur de volaille de chair est très important pour un nombre croissant de pays, l'Algérie en faisant partie dont sa production est toutefois menacée par un certain nombre de maladies virales causant des pertes économiques énormes

La présente étude a été menée dans le but d'évaluer l'état clinique, sérologique et histopathologique de la maladie de Newcastle (ND) en élevage de poulet de chair (20 élevages / 600 sérums) par la méthode ELISA et d'évaluer l'influence de certains facteurs de risque associés à chaque maladie.

Nos résultats montrent que : parmi tous les élevages étudiés, la ND présente une séropositivité de 60%. En ce qui concerne les facteurs de risque : les élevages ayant une bonne hygiène étaient significativement moins séropositifs à la ND de 26% ($p = 0,022$). Cependant, les élevages ayant une densité plus élevée ou âgés de plus de 30 jours étaient plus séropositifs respectivement de 47 % ($p = 0,041$) et 45 % ($p = 0,019$). Enfin, lorsque les poulets de chair n'ont pas fait un rappel vaccinal contre ND, les élevages ont semblé plus séropositifs de 48 % ($p = 0,047$).

En conclusion, l'enquête sérologique menée dans le cadre de cette étude a fourni un cadre important sur les maladies virales qui sont des pathologies dominantes chez le poulet de chair. Ainsi, de nombreux facteurs sont responsables de l'apparition de ces maladies.

Mots clés : clinique, histopathologique, sérologique ; maladie de Newcastle ; poulet de chair, Algérie.

ملخص:

يعد قطاع الدواجن اللاحم مهمًا جدًا لعدد متزايد من البلدان، ومع ذلك فإن إنتاج الجزائر مهدد بعدد معين من الأمراض الفيروسية التي تسبب خسائر اقتصادية فادحة لعدد متزايد من البلدان.

أجريت الدراسة الحالية بهدف تقييم الحالة السريرية والمصلية والنسيجية المرضية لمرض النيوكاسل (ND) في مزارع الدجاج اللاحم (20 مزرعة / 600 مصل) بطريقة ELISA وتقييم تأثير بعض عوامل الخطر المرتبطة بالمرض. كل مرض.

تظهر نتائجنا أن: من بين جميع المزارع التي تمت دراستها، ND لديه إيجابية مصلية تبلغ 60٪. فيما يتعلق بعوامل الخطر: كانت المزارع ذات النظافة الجيدة أقل إيجابية بشكل ملحوظ عند ND بنسبة 26٪ (ع = 0.022). ومع ذلك، كانت القطعان ذات الكثافة الأعلى أو التي يزيد عمرها عن 30 يومًا أكثر إيجابية بنسبة 47% (ع = 0.041) و45% (ع = 0.019)، على التوالي. وأخيرًا، عندما لم يكن لدى دجاج التسمين تطعيم معزز ضد ND، ظهرت إيجابية المصل في المزارع بنسبة 48% (ع = 0.047).

في الختام، قدم المسح المصلي الذي تم إجراؤه كجزء من هذه الدراسة إطارًا مهمًا للأمراض الفيروسية التي تعتبر الأمراض السائدة في دجاج التسمين. وبالتالي فإن هناك عوامل كثيرة مسؤولة عن حدوث هذه الأمراض.

الكلمات المفتاحية: سريرية، نسيجية، مصلية؛ مرض نيوكاسل. دجاج لاحم الجزائر..

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Summary

Introduction:

The broiler sector is the largest and most efficient meat production industry in the world **(Gupta, & al 2014)**. Indeed, Algeria is one of the many countries where broiler production is threatened by a number of infectious diseases, particularly viral, where economic losses represent a huge bill without a reliable solution of any medicine **(Pradhan, 2014).**

Newcastle disease (ND) is the most economically important disease in poultry - particularly in developing countries - due to mortality high and associated sanitary measures in poultry farms or slaughterhouses **(Ban-Bo, 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 numerous species of domestic and wild birds **(Hasan, 2010).**

Several methods are available to diagnose MN (PCR, serology, isolation viral). However, the specific treatment of the disease does not yet exist, prophylaxis being the only way of protection. Health prophylaxis varies depending on the situation in the country regarding the disease, according to the means available (human and financial) and according to the onset of the disease. **(Suzanne, 2015)**

Histopathology is the microscopic study of tissues and cells to examine the structure and pathological changes that can occur in the body of an infected animal by Newcastle disease. Histopathology is often used to confirm the diagnosis of Newcastle disease and to learn more about the tissues and organs of infected birds.

In Algeria, despite the existence of means to combat Newcastle disease, including vaccination, we have always witnessed clinical epidemics.

Risk factors related to biosecurity and agricultural practices appear to play an important role in the severity of disease observed on affected farms **(Jaganathan, 2015).**

This document is divided into 5 chapters:

* The first chapter concerns the introduction and bibliographicalsynthesis.
* The second chapter reports Medical Aspects.
* The third chapter reports the materials and methodsused.
* The results obtained are presented in the fourthchapter.
* The fifth chapter is devoted to discussions, conclusions and perspectives.

Chapter I :
Generalities

I. Chapter : Generalities :

1. Definition :

Newcastle disease, also known as "pseudo-fowl-pest," is a highly contagious infectious disease affecting birds, caused by an RNA virus. The disease was first described by (Kraneveld, 1926) in Java, Indonesia, and by (Doyle, 1927) in Newcastle-Upon-Tyne, England.

This notoriously contagious disease is listed among the diseases to be reported to (OIE, 2013).

The term "Newcastle Disease" (ND) was suggested by (Doyle, 1927) following the initial outbreaks in Great Britain. It was chosen as a temporary name to avoid confusion with other diseases, rather than a descriptive one (Doyle, 1935). Despite its temporary nature, the name persisted and became associated with the "avian paramyxovirus type 1" (APMV-1) (Alexander, 2008). Within two decades of its emergence, ND evolved into a panzootic disease, spreading across continents and affecting numerous animal populations (Mayer, 2014). A comprehensive review (Alexander, 2012) delved into the disease's history and research, emphasizing epidemiological and virological aspects. Suggestions were made for future studies, particularly in developing countries where ND poses significant threats to poultry.

The outbreaks of Newcastle disease have significant health and economic consequences for backyard chicken farming in developing nations. These birds serve as vital sources of animal protein and income for local populations (Alders, 2001). Conversely, in developed countries where vaccination and effective farming practices mitigate the disease’s impact, trade embargoes and restrictions during outbreaks lead to notable economic losses (Alexander,2000).

2. Importance:

A. **Medical:** the disease proves fatal for a high number of birds; a scourge for poultry farming.
B. **Economic:** considerable, due to deadly epizootic outbreaks with high morbidity and mortality rates ranging from 90 to 100%.
C. **Hygienic:** minor zoonosis; benign conjunctivitis spontaneously curable in humans. (Akakpo et al., 2013).

Figure 1: Conjunctivitis due to NDV in humans. (J Brugère-Picoux, maladies aviaire2015)

3. Etiology :

It is an enveloped ribovirus classified within the family Paramyxoviridae, genus Avulavirus. This genus includes nine serotypes originating from avian species. All strains of Newcastle disease belong to serotype one.

Nine serotypes of avian paramyxoviruses have been identified: APMV-1 to APMV-9(10). Among these, NDV (APMV-1) remains the most significant pathogen in poultry, while APMV-2, APMV-3, APMV-6, and APMV- 7 are recognized as causing diseases in poultry.

Paramyxoviruses are RNA viruses with a helical capsid symmetry. They are enveloped by a membrane derived from the plasma membrane of the infected cell, which is covered with spikes made of various glycoproteins.

- **Hemagglutinin-neuraminidase (HN):** facilitates the attachment of the virus to cellular receptors.
- **F glycoproteins:** facilitates the fusion of the viral envelope with the cell membrane, allowing the nucleocapsid and viral RNA to enter the cell (Brugere-Picoux, 1992).

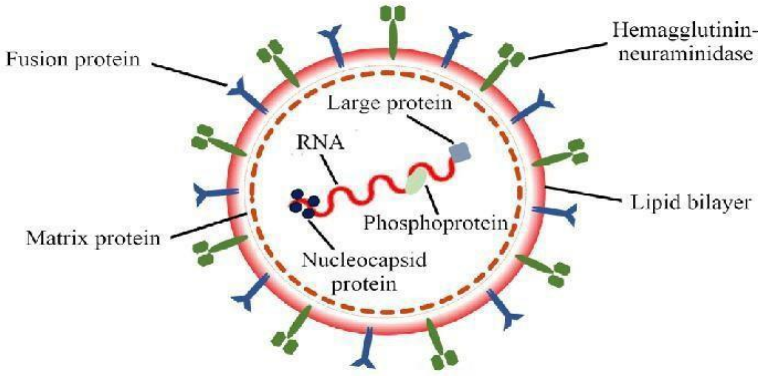

Figure 2: Schematic representation of Newcastle disease virus (NDV) structure

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4. Epidemiology:

Descriptive Epidemiology:

Newcastle disease is a cosmopolitan disease that affects both wild and domestic birds. Its evolution starts with an epizootic phase and then becomes enzootic (Kenzie, 2008).

Analytical Epidemiology:

Factors involved in the pathology are:

A. Intrinsic Factors:

* Species: Gallinaceous birds, especially chickens, are the most susceptible.
* Sex and age: While sex does not influence susceptibility, age is significant. Although the disease affects birds of all ages, mortality is higher in chicks (90 to 100 per 100), which can decrease if chicks are from vaccinated hens before three weeks of age. Chickens are more susceptible than adults.
* Breed: Breed does not affect susceptibility, but improved breeds tend to be more sensitive (Jaganathan, 2015).

B. Extrinsic factors :

These factors promote the outbreak of the disease by directly or indirectly affecting the birds' organisms.

* Livestock conditions: Overcrowding in very restricted poultry houses, absolute lack of hygiene, underfeeding, and parasitism predispose animals to disease. Sometimes, an overdose of live virus vaccine can also trigger thedisease.
* Climatic conditions: Cooling, drafts, rainfall on outdoor birds, in fields, or in

poorly protected poultry houses are stress factors that promote the outbreak of the disease. The season influences the course of the disease, which often takes on an epizootic nature during the dry and windy season (Jaganathan, 2015).

5. Pathogenesis :

The sources of infection include both sick and carrier wild and domestic birds, which excrete the virus through various secretions and excretions such as feces and ocular-nasal secretions. Additionally, carcasses, eggs, feathers, and similar items can also serve as sources of infection.

Chronic carriers can shed the virus for at least two months. The virus responsible for the respiratory form primarily resides in migratory aquatic birds in colder and temperate climates, whereas the virus causing the visceral form affects birds in tropical forest habitats. Transmission can also occur through contact with water, food, personnel, equipment, litter, incubators, vehicles, etc. Ticks and lice also contribute to the spread of the disease.

The infection spreads through airborne transmission, where viral particles in aerosols are inhaled, as well as through ingestion of contaminated food and water, and occasionally through direct contact with the skin. The virus replicates initially in lymphoid organs and vascular endothelium, leading to their alteration, and then spreads to other tissues based on the strain's specific affinity. The severity of the disease is linked to the cytopathic effect caused by infected cells, with potential complications from bacterial infections also noted (Bachir Pacha et al., 2013).

Chapter II :
Medical Aspect

II. Chapter II: Medical Aspect:

1. Symptoms: (Alexander1988):

The disease typically has an average one-week incubation period. Symptoms can vary based on the virulence and type of viral strain involved, as well as the individual receptiveness and resistance of those affected.

However, classically, four forms of the disease can be distinguished:

A. Hyperacute forms:

A Severe general impairment; sudden mortality within 1 to 2 days affecting over 90% of the population.

B. Acute forms:

Rapid incubation (4 to 5 days) initially, general signs appear: lethargy, ruffled feathers, often with edema, cyanosis, or hemorrhage of the caruncles, combs, and wattles.

Association or not of the different forms:

a) Digestive (greenish to hemorrhagic diarrhea).

b) Respiratory (ocular-nasal and tracheal catarrh, leading to significant dyspnea).

c) Nervous (convulsions, ataxia, paralysis of one or morelimbs).

After a few days, these symptoms progress towards death or a slow recovery associated with nervous sequelae (paralysis, torticollis) and significant drops in egg production in laying females.

C. Subacute and chronic forms:

Progressing slower than the previous form and less pronounced, typically presenting mainly respiratory symptoms (Rezki, 2014).

- These forms correspond to the prolonged presentation of acute symptoms with exacerbation of respiratory signs. Complications such as mycoplasmosis, colibacillosis, pasteurellosis, and chlamydiosis are frequent.
- There is a drop in egg production in laying hens.
- Rare occurrence of diarrhea andparalysis.

D. Inapparent forms:

– The existence of asymptomatic and unnoticed forms is certainly more common than one might assume (Alexander, 1988).

Figure 3: Neurotropic form of Newcastle disease (Ashraf, Asma & Mahboob, Shahid & Andleeb, Rahat & Ijaz, Muhammad & Shah, Muhammad. (2018)

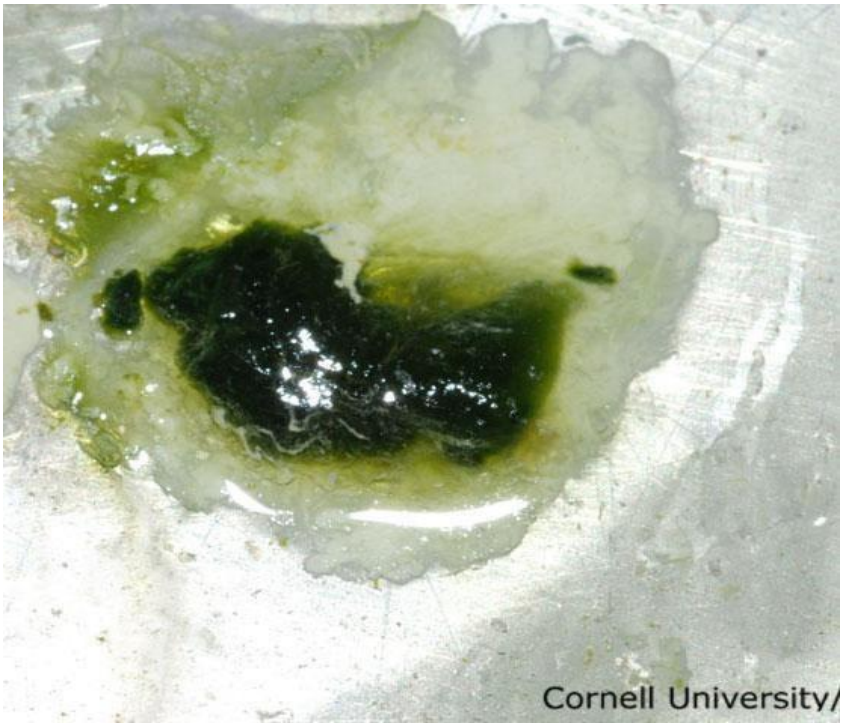


Figure 4: Dark green colored feces in hens infected with Newcastle disease (Cornell University)

Table 1: The five pathotypes of Newcastle disease (Young et al, 2002)

Pathotype	Description Of disease	Clinical signs and post mortem lesions
Viscerotropic Velogenic	Acute lethal infection in chickens of all ages.	Hemorrhagic lesions in the gastrointestinal tract
Neurotropic velogenic	Acute infection in chickens of all ages; high mortality	Respiratory and nervous sgns
Mesogenic	Less pathogenic with low mortality, usually in young chickens	Respiratory and nervous Signs
Lentogenic	Mild, inapparent infection; deaths confined to young chickens	Respiratory signs

Asymptomatic enteric (avirulent)	Avirulent infection; no mortality	NO signs or lesions

2. Lesions:

At autopsy, the lesions observed are either macroscopic or microscopic, varying widely depending on tissue tropism and strain virulence.

In cases of an acute form, which reveals more characteristic macroscopic lesions like catarrh and hemorrhagic septicemia, there are petechiae and hemorrhagic suffusions in the abdominal fat, proventriculus or gizzard, intestine, and epicardium.

Splenic hypertrophy is not always present in this condition. The identification at autopsy of the hemorrhagic triad—petechiae centered on the papillae of the gizzard, cloacal suffusion, and epicardial petechiae—forming a "hemorrhagic-necrotic" belt, is pathognomonic for the acute form.

Microscopic lesions are only visible in the laboratory; histological examination for the pneumotropic form shows tracheitis followed by mucosal hemorrhage and desquamation, while the neurotropic form exhibits endothelium flattening with neuronal degeneration. The most pathognomonic lesions of attacks by highly virulent viruses include hemorrhages in Peyer's patches and minimal lymphoid aggregates alone the intestine (Villate, 2001).

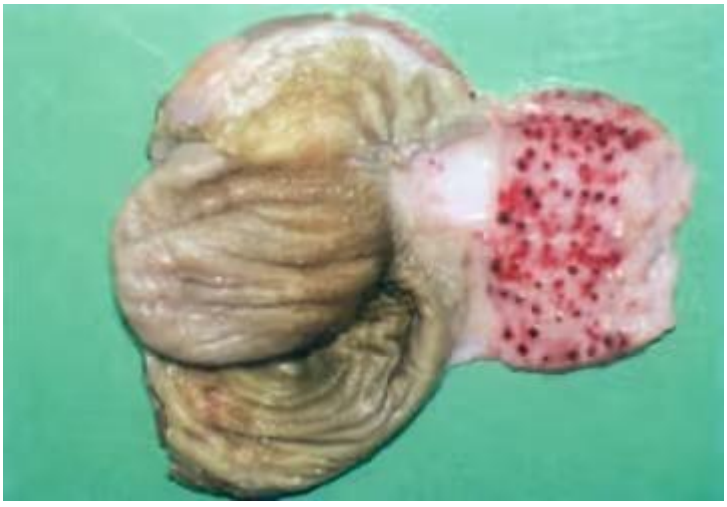


Figure 5: The haemorrhages of gizzard epithelium ("Diseases of poultry - A colour atlas" - Ivan Dinev & amp; CEVA Santé.)

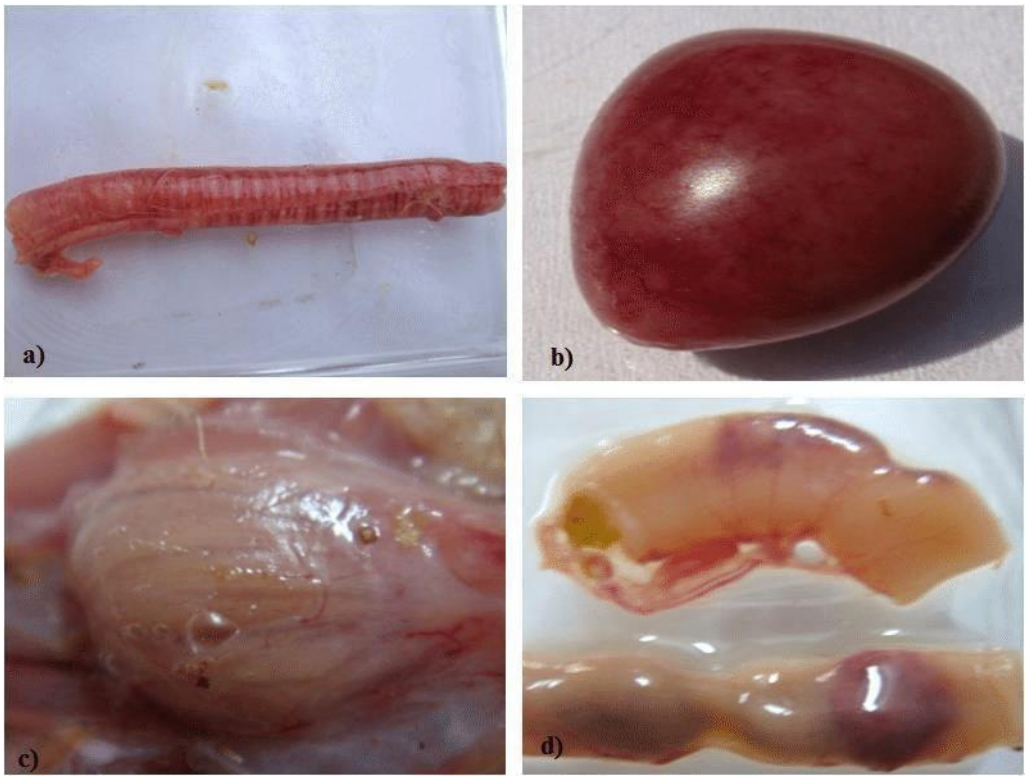


Figure 6: Post-mortem lesions of the birds due to VVNDV infection: Showing hemorrhages ulceration on proventriculus (a), trachea (b), spleen (c), bursa (d), small intestine. (Pathology and transmission of experimental velogenic viscerotropic Newcastle disease in wild pigeons, broiler and Aseel chicken, Murree et al. 2016)

3. Diagnostic:

Clinical diagnostic:

* General weakness, respiratory issues (noisy breathing, sneezing, nasal discharge), digestive problems (greenish diarrhea with occasional blood), and nervous signs (convulsions, paralysis).
* Autopsy reveals ulcerative and hemorrhagic lesions in the digestive tract and lymphoid tissues.
* Hemorrhagic lesions are found in the digestive tract, ovaries, cecal tonsils, heart, and muscles.
* Ulcerative-necrotic lesions affect lymphoid tissues along theintestine.
* Additional findings may include foamy mucus in the trachea, congestive lesions in the liver, spleen, and kidneys, as well as airsacculitis, catarrhal enteritis, and bronchopneumonia.
* Besides the hyperacute and acute forms, clinical diagnosis is challenging due to the variability in affected avian species, symptoms, and lesions expressed.

Laboratory diagnosis supported by appropriate sampling is always recommended (Alexander, 1988).

Laboratory diagnostic:

Based on the isolation and identification of the virus:

* Viral isolation can be done as early as the 8th day after the onset of the disease.
* Samples can be taken from live animals, spleen, bone marrow, and nervous system.
* Virus culture is performed by inoculating the treated samples into the allantoic sac of embryonated eggs. The obtained chorioallantoic fluid is mixed with bird red blood cells. In the presence of viruses, there is hemagglutination, and then the virus is identified by hemagglutination inhibition (Ichakou, 2004).

Experimental diagnostic:

A. Serological Diagnosis (within 24 hours):

* IHA or Hemagglutination Inhibition Test: commonly used to detect antibodies against PMV1 (cf. materials and methods).Detectable as early as the end of the first week. Antibodies peak at 2-3 weeks and disappear within a few months. Results interpretation can be challenging depending on vaccination or pathological history.
* HAP or Passive Hemagglutination (Alexander, 1988): Two other methods can also be used:
  - Serum neutralization: highly sensitive but delicate.
  - ELISA Technique: easy but requires expensive kits.

Antibodies are detectable only after 7 days of infection in chickens, which can pose interpretation problems. Antibodies peak at 2-3 weeks and disappear within a few months (Orne, 2001). Fifteen to twenty blood samples are to be collected in dry tubes. The analysis is carried out by:

Detection of antibodies by hemagglutination inhibition (H.A.I):

The H.A.I allows the diagnosis of Newcastle disease and provides information on the value of vaccine immunity (DE Langhe C 2006). It is a reference technique in serology. This method takes advantage of the fact that certain viruses, like the Newcastle disease paramyxovirus, agglutinate poultry red blood cells. If anti-virus serum (containing antibodies) is added to the viral preparation, agglutination is inhibited because the antibodies have bound to the viral antigenspermet, the diagnosis of Newcastle disease and provides information on the value of vaccine immunity (DE Langhe C 2006).

ELISA (Enzyme-Linked Immunosorbent Assay): Indirect ELISA:

With a correlation coefficient of 0.7498, the classic ELISA method remains close to H.A.I results. The L.S.I. laboratory thus provides tables for quantitative correspondence between indirect ELISA/I.H.A., establishing titration groups from one to 14. Different serological profiles are expected depending on the situations (Lemiere S, 2002).

* Competition ELISA or Blocking ELISA:

The samples (sera) always bind to the antigen and occupy antigenic sites. In the absence of antibodies in the sample, antigenic sites remain free.

The conjugate (peroxidase-labeled monoclonal antibody against N.D.V) added subsequently binds to the remaining free antigenic sites. Finally, the substrate allows for the coloring of antibodies against paramyxovirus. This method indirectly detects Newcastle disease virus antibodies in all bird species since it uses revealing antibodies binding to the viral antigen, not species-specific antibodies. It can the refore be applied to game birds. It reveals all complementary types of virus antibodies (Lemiere, 2002).

B. Molecular, by RT-PCR:

There are other highly reliable detection techniques now implemented by L.N.R. Molecular detection by RT-PCR is based on detecting virus genomefragments.

Nucleic acid sequences are then compared with those of the Newcastle disease virus already known internationally (Lemiere, 2002). Comparées avec celles du virus de la maladie de Newcastle déjà connues au plan international (Lemiere, 2002).

C. Histological diagnosis :

Histological analysis cannot definitively diagnose Newcastle disease but can raise suspicion of it.

For instance, examining the digestive tracts of experimentally inoculated chickens has revealed necrotizing pancreatitis (Monque Roque, 2011).

Additionally, cases of acute pancreatitis have been reported following infection with asymptomatic non-vaccine strains that have an intestinal preference (Russel, 1995).

Furthermore, both lentogenic vaccine and non-vaccine strains cause microscopic lesions in the respiratory tract, which can be observed through histology (Despordes, 2002).

Highly virulent strains can induce microscopic lesions in various systems including the nervous, vascular, lymphoid, and reproductive systems (Alexander, 1998).

For example, histological examination of the brain may reveal signs of neurotropic virus involvement, such as perivascular lymphocytic cuffs.

4. Differential Diagnosis:

None of the clinical signs or lesions described are specific to Newcastle disease. Therefore, any clinical diagnosis in the field must be confirmed in the laboratory. Clinical signs and the course of Newcastle disease virus can closely resemble those of numerous avian diseases:

* Highly pathogenic avian influenza (HPAI) caused by an Orthomyxovirus.
* Avian cholera caused by Pasteurella multocida, where diarrhea is abundant, and the liver is hypertrophied and yellowish.
* Acute form of infectious laryngotracheitis.
* Avianpox (diphtheriticform).
* Ornithosis (psittacosis or chlamydophilosis) affecting parrots andpigeons.
* Infectiousbronchitis.
* Pacheco's disease in parrots (psittacids).

- Infections in certain psittacids by avian paramyxoviruses types 3 and 5.
- Infectious bursal disease (Gumboro disease) with highly virulentstrains.
- Salmonellosis in pigeons.
- Typhoid, caused by Salmonella gallinarum, affecting adult birds. The liver is hypertrophied, congested, and greenish (Ichacou, 2004).
- Gumboro's disease: less contagious than Newcastle disease, is characterized by hemorrhagic lesions in the digestive tract and particularly in the muscle masses, along with hypertrophy of the bursa of Fabricius.When doubt persists, laboratory diagnosis can be employed (Ichacou, 2004).

5. Treatment:

There is no specific treatment for Newcastle disease due to its viral nature. However, bacterial complications in animals infected with less virulent strains can be managed with antibiotics (Ichakou,2004).

6. Prophylaxis:

A. Sanitary prophylaxis:

The most important points can be resumed as follows:

- Isolate all sick hens.
- Cull severely sick hens. Do not transport sick or dead hens to disease-free areas.
- Bury or burn all dead hens. If not possible for any reason, any unused parts of the hen should be buried or burned.
- Do not vaccinate hens showing signs of the disease.
- Advise farmers to contact veterinary services, extension agents, or local livestock assistants as soon as they notice any signs of disease (Alders and Spradbrow, 2000).

B. Medical prophylaxis:

It complements the previous measures and is based on animal immunization.

There are two types of immunization:

* **Passive immunization:** It is uncommon, random, and lesseffective.
* **Active immunity or vaccination:** Currently, there are two types of vaccines: live attenuated vaccines andinactivated vaccines (Ichakou, 2004).

Experimental Part

Chapter I :

Materiel & methods

I. Chapter I : Materiel & Methods :

1. Objective :

Our study focuses on a sero-epidemiological and histopathological investigation of Newcastle Disease, considered one of the primary viral avian diseases. This involves field surveys and laboratory analysis of samples, aiming to enhance productivity through improved health management in poultry. Specifically, our goal is to assess the presence of viral pathogenic constraints by evaluating the bird's immune status and implementing appropriate disease management strategies.

To achieve these objectives and establish a practical working protocol under local conditions, the experimental study is structured into several parts:

* A field survey conducted on selected farms using a questionnaire aimed at practicing veterinarians responsible for monitoring.
* Investigation to determine the circulation of Newcastle Disease Virus (NDV) by detecting antibodies in chicken serum (serological survey).
* Histopathological study on organs collected during the disease.
* Identification of risk factors associated with Newcastle Disease.

2. Duration of the Experimentation:

Serology:

This part was conducted at the Laboratory of Biotechnologies Related to Animal Reproduction (LBRA), University of Blida, over a period of 15 days (from March 1st to March 15th).

Figure 7: Biochemistry laboratory (Personal photo, 2024)

Histopathology:

The histopathology was conducted in the region of Algiers at the National School of Veterinary Sciences over a period of 15 days during March 2024.

Figure 8: Anatomopathology labratory (Personal photo, 2024)

3. Materiel:

Kit components:

Reactives:

* Microplates sensitized with purified ND antigen:

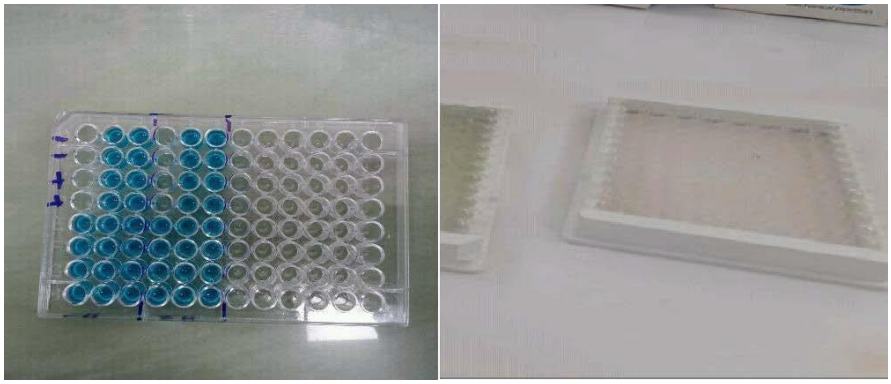


Figure 9: Microplates with purified ND antigen. (Personal photo, 2024)

* Positive control
* Negative control
* Dilution buffer 14
* Concentrated conjugate (10X)

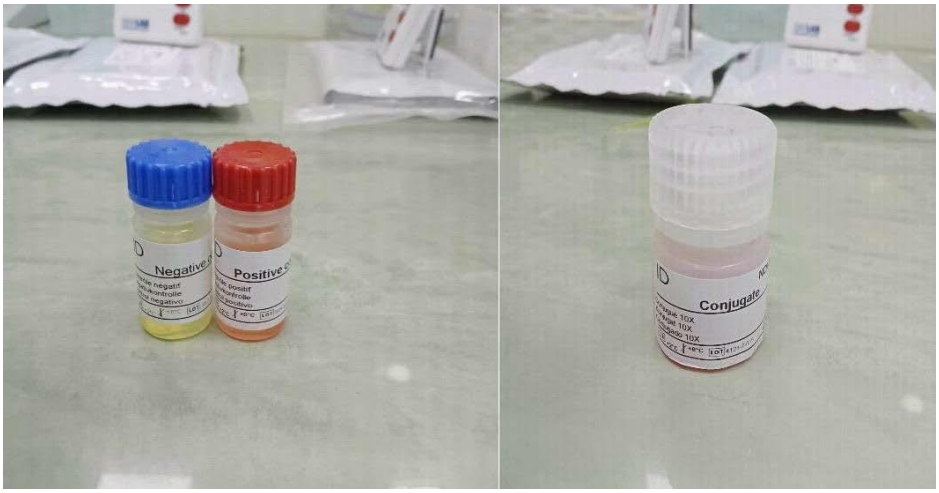


Figure 10: positive + negative control and Conjugate concentrate. (Personal photo, 2024)

- Dilution buffer 3
- Concentrated wash solution (20X)
- Substrate solution
- Stop solution (0.5M)

Figure 11: Development Solution & Stop Solution and Dilution Buffer (Photo personal, 2024)

- The conjugate, controls, and substrate solution should bestored at 5°C (±3°C).
- Other reagents can be stored between +2°C and+26°C.
- Components with the same designation (wash solution, diluents) canbe used across the entire IDvet range.

Material needed:

- Single or multichannel precision pipettes capable of delivering volumes of 5 µl, 10 µl, 100 µl, 200 µl.

Figure 12: Multichannel pipettes. (Personal photo, 2024)

4. Methods:

Serology:

Animal:

The subjects were sampled from thirty (30) private poultry farms of broiler chickens (W.Médea). The subjects originate from private broiler chicken production centers (private hatcheries).

These broiler chicken farms consist of different strains (Arbor Acres, Cobb 500, Hubbard F15) aged four (4) to seven (7) weeks and containing 4,000 to 10,000 subjects per farm (Figure 9).

Figure 13: Breedings sampled (Photo personnelle, 2024)

The farms under study had initially been vaccinated against Newcastle disease (ND) using live vaccines administered according to various protocols.

Subsequently, these farms were suspected to have contracted a viral disease (ND) due to the presence of characteristic clinical and necrotic signs.

Table 2 : The vaccines used (vaccine strain, type of vaccine, and method of administration)

Pathology	Vaccine strain	Vaccine type	Method of administration
Newcastle Disease	Clone 30 VG/GA	Live vaccines	Drinking water

Clinical study (Clinical diagnosis) :

The clinical diagnosis was established on the basis of the clinical history taken by farm managers, including the veterinarians in charge of follow-up, with clinical signs and lesions recorded during autopsy of affected chickens.

Sampling:

Samples were randomly collected from broiler chickens showing clinical signs suggestive of viral diseases like Newcastle disease (ND) and exhibiting characteristic necrotic lesions upon autopsy. A total of 600 samples underwent serological analysis at the Laboratory of Biotechnologies Related to Animal Reproduction (LBRA) at the Institute of Veterinary Sciences, University of Blida.

Upon notification of suspected cases by veterinarians, sampling was conducted within 1-2 days to gather initial samples and complete sampling forms. Each farm underwent two rounds of sampling: an early sampling at the onset of clinical signs (within 1-2 days), and a later sampling 2-3 weeks later to detect potential seroconversion.

Samples were taken directly from the wing vein within each farm (10 samples per farm, Figure 15), ensuring representative sampling.

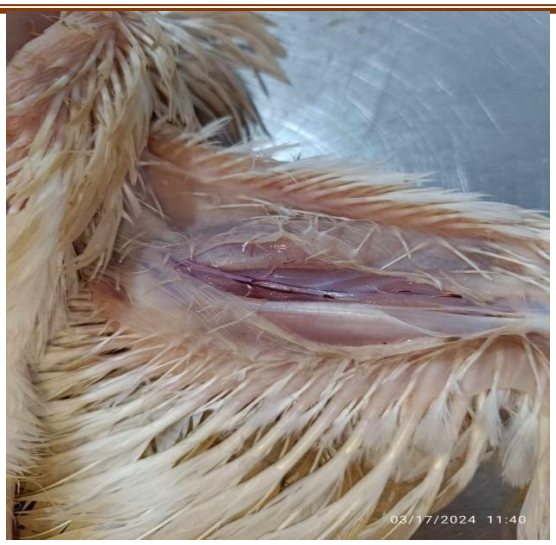


Figure 14: Sampling technique. (Personal photo, 2024)

Once blood samples were collected into pre-labeled dry tubes (approximately 3 ml per subject to facilitate multiple analyses from the same serum), they were immediately transported to the laboratory. Upon arrival, they underwent centrifugation on the same day (5000 rpm for 10 minutes) to obtain serum, which was then stored in labeled Eppendorf tubes and frozen at -20°C (Figure 16).

Blood before centrifugation Blood after centrifugation

Figure 15: The serum decantation steps. (Personal photo, 2024)

Once the expected number of serums (400 serums) was reached, the samples underwent serological examinations.

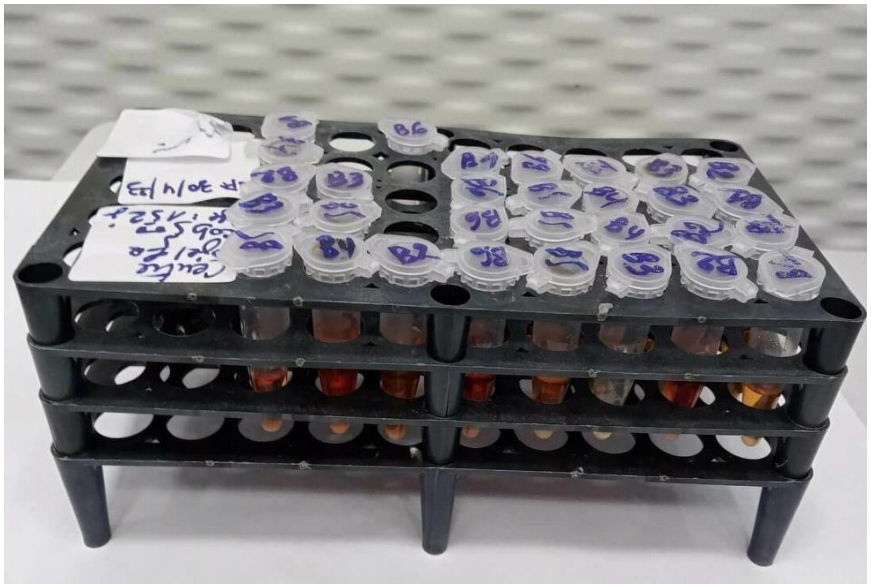


Figure 16: Serum in identified Eppendorfs (Personal photo, 2024)

Laboratory Method:

An indirect ELISA technique was performed using kits from ID.vet Innovative Diagnostics (Montpellier, France): ID Screen® NDV Indirect (for Newcastle disease) (Figure 13).

Figure 17: ID Company kits (Personal photo, 2024)

Samples collected on different dates from various farm buildings were analyzed simultaneously using the same kit to ensure the comparability of test results and proper interpretation of antibody kinetics. The serums were diluted 1:500 and loaded onto ELISA plates to initiate the immuno-absorbent reaction as per the manufacturer's instructions.

ELISA plate readings were conducted using an ELx800 spectrophotometer (DIALAB GmbH, Wiener Neudorf, Austria) with a 450 nm filter. The optical density (OD) obtained was converted into antibody titers.

Figure 18: reading ELISA plates (Personal photo, 2024)

The transformation of ODs, validity tests, average titers, and coefficient of variation (CV) were automatically calculated per strip and per sampling series using software provided by the laboratory (IDSoftTM, Montpellier, France).

Figure 19: Automatic calculation using software. (Personal photo, 2024)

This diagnostic kit is intended for the detection of antibodies directed against Newcastle disease virus (NDV).

It allows for the assessment of the quantity of specific antibodies present in chicken serums.

- Wells are sensitized with purified NDantigen.
- Test samples and controls are distributed into the wells. If ND-specific antibodies are present, they form an antigen-antibody complex.
- A peroxidase-conjugated anti-chicken antibody (HRP) is added to the wells. It binds to the anti-ND antibodies, forming an antigen-antibody-conjugate-HRP complex.
- After removal of excess conjugate by washing, the reaction is revealed using a substrate solution (TMB).
- The resulting coloration is proportional to the amount of specific antibodies present in the test sample:
- In the presence of antibodies in the sample, a blue color develops, which turns yellow after stopping the reaction.
- In the absence of antibodies in the sample, no color develops.
- Readings are taken at 450 nm.

Sample preparation:

To reduce the variation in incubation times between samples, you can prepare a 96-well microplate containing the test samples and control samples. Then, transfer them into the ELISA plate using a multichannel pipette.

Preparation of Wash Solution:

If necessary, bring the concentrated wash solution (20X) to room temperature (21°C ± 5°C) and agitate thoroughly to ensure dissolution of any crystals.

Prepare the wash solution (1X) by diluting the concentrated wash solution (20X) in distilled or deionized water.

Operating Procedure:

Ensure all reagents are brought to room temperature (21°C ± 5°C) before use and homogenize them by gentle inversion or vortexing.

1. Dilute samples 1/500 in Dilution Buffer 14. In a pre-dilution plate, add

1. 245 µl of Dilution Buffer 14 to each well.
   - 5 µl of Negative Control in wells A1 and B1.
   - 5 µl of Positive Control in wells C1 and D1.

- 5 µl of test samples in the remaining wells.

2. In the ELISA plate, add:
   - 90 µl of Dilution Buffer 14.
   - 10 µl of the pre-diluted samples from above
3. Cover the plate and incubate for 30 minutes (±3 minutes) at room temperature (21°C ± 5°C).

Figure 20: plate cover (Personal photo, 2024)

4. Prepare the Conjugate 1X by diluting concentrated conjugate (10X) at 1/10 in Dilution Buffer 3.
5. Wash each well three times with approximately 300 µl of Wash Solution 1X. Avoid allowing the wells to dry out between washes.

Figure 21: plate washer. (Personal photo, 2024)

6. Dispense 100 µl of Conjugate anti-chicken-HRP 1X into each well.
7. Cover the plate and incubate for 30 minutes (±3 min) at room temperature (21°C± 5°C).
8. Wash each well three times with approximately 300 µl of Wash Solution 1X. Avoid allowing the wells to dry out between washes.
9. Dispense 100 µl of Substrate Solution into eachwell.
10. Incubate for 15 minutes (±2 min) at room temperature (21°C ± 5°C) in the dark.
11. Dispense 100 µl of Stop Solution into each well to terminate the reaction. Add the stop solution in the same order as step #9.
12. Measure and record optical densities at 450 nm.

Validation :

The test is valid if:

* The average optical density value of the positive controls (DOCP) is greater than 0.250: DOCP >0.250
* The ratio of the average of Positive Controls (DOCP) to the average of Negative Controls (DOCN) is greater than 3: DOCP / DOCN > 3

Interpretation:

For each sample, calculate the S/P (Signal/Background) and the antibody titer;

Calculation of the S/P ratio:

$S/P = DO_{Sample} - DO_{CN}$

$DO_{CP} - DO_{CN}$

Calculation of antibodytiter :

Log10 (titre) = 0.97x log10 (s/p) + 3.449 titre = 10log10 (titre)

The results are interpreted as follows (Table No. 03):

Table 3: Criteria for interpreting antibody titers obtained from ELISA

S/P value	Antibody titer in ELISA	NDV immune status
S/P < 0.3	Titer< 993	Negative
S/P > 0.3	Titer> 993	Positive

Risk factors:

During each sampling, zootechnical and health data are recorded by interviewing the farmer, the veterinarian responsible for farm monitoring, or through direct observation.

The collected information results in an identification sheet for the farm and a monitoring sheet detailing the overall evolution of the farm's condition.

In addition to the aforementioned data, farmers indicate whether the disease has manifested in current flocks or in previous ones. This information serves as an indicator of the specific wild viral pressure on the farm.

During our investigation, the parameters considered include the region, climate, season, age of onset, density, strain, hygiene, and the vaccination protocol noted (vaccination age, type of vaccine, and vaccination method).

5. Histopathological Study:

The anatomo-pathological service (Anapath ENSV) plays an essential role in the diagnosis, prognosis, and monitoring of tumors and other pathological conditions. The histopathology laboratory (histo-path) is dedicated to preparing, studying, and monitoring healthy and diseased animal tissues on glass slides for microscopic examination aimed at diagnosis, research, and educational purposes.

The purpose of this chapter is to provide our trainees with a methodical and practical approach to the handling of tissue samples for microscopic examination (histology). This includes all stages from sampling to reading, including fixation, embedding, sectioning, staining, mounting, and finally, microscopic examination.

Sampling:

This can be done on both living and deceased subjects, as follows:

* On a cadaver, meaning after autopsy as soon as possible.
* On surgical specimens, such as excised examples like a mammary gland.
* On biopsies

In this study, we have chosen to take 15 tracheal samples from the suspected animals.

For incising sections, sharp instruments like scalpels and bistouries are used to avoid crushing or damaging tissues and thereby prevent artifact formation. Samples would then be properly labeled and promptly placed in a fixing agent to prevent decay.

Figure 22: Samples and materials used (personal photo, 2024)

All received and collected samples are recorded in a laboratory register and identified.

After a time lapse of 6 to 12 hours, the samples are macroscopically cut and incised into pieces approximately one centimeter in dimension and about 0.5 mm in thickness. Each piece is identified with a number and placed in plastic cassettes.

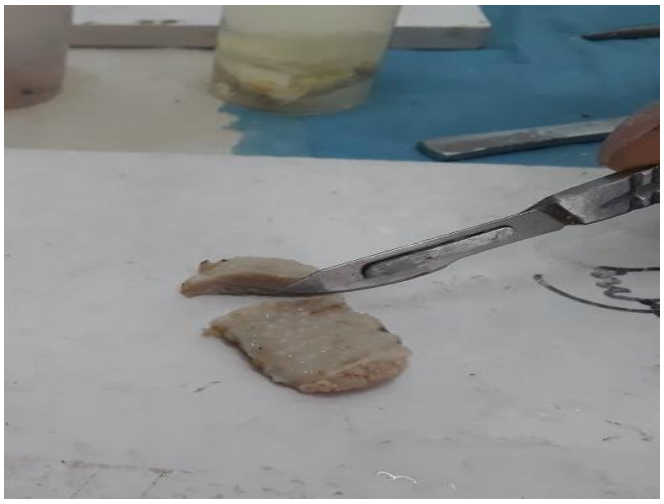


Figure 23: sample cutting

Figure 24: Samples placed in cassettes

A. Fixation of samples:

The fixation aims to preserve morphological structures. The fixative used is 10% formalin (i.e., 1 volume of commercial formalin to 9 volumes of distilled water). It is also referred to as 4% formaldehyde formalin. The recommended fixation duration is 24 to 48 hours and can extend for several days, depending on the size of the sample (e.g., bone biopsy 4 to 8 hours; nerves 12 to 24 hours). The rate of fixative penetration depends on temperature (lower temperature decreases penetration rate) and pH.

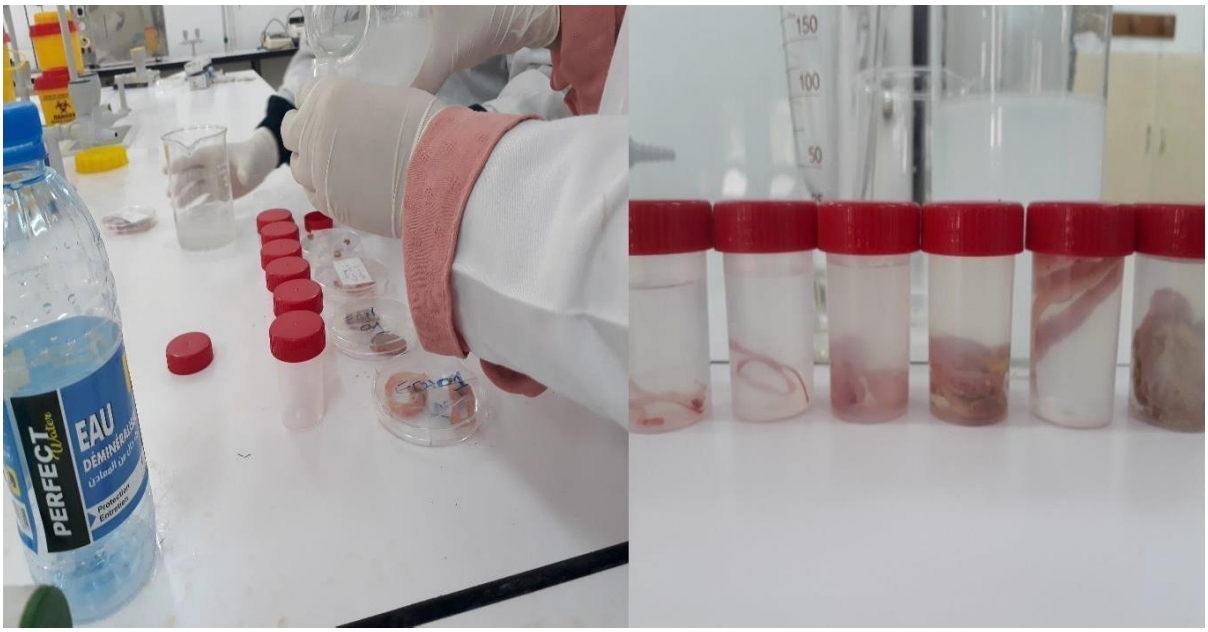


Figure 25: Fixation of samples. (Personal Photo, 2024)

B. Circulation:

This process involves dehydration, clearing, infiltration with paraffin wax, and embedding (blocking).

Purpose: To achieve cellular examination of soft tissue using histological techniques, allowing light to pass through from a microscope source.

Dehydratation:

This process allows for the complete removal of water from tissue. Ethyl alcohol (ethanol) is the most commonly used dehydrating agent. It is miscible in water and in clearing agents (such as toluene, xylene, benzene, etc.) and ensures good preservation of cellular structures.

Dehydration begins with baths of alcohol at a low concentration, typically ranging from A 50 to A 70°, because high concentrations of alcohol can cause tissue deformation, shrinkage, and hardening. The tissue should pass through 6 baths of alcohol, starting from the lowest concentration and ending at A 100, which ensures thorough dehydration and allows the clearing agent to penetrate the tissue effectively. This stage typicallylastsapproximately 5 to 6 hours.

Figure 26: Dehydration of samples (Personal photo 2024)

Clarification:

This step involves replacing the dehydrating agent with a solvent that is miscible with the paraffin embedding solution, which is an anhydrous solvent (does not contain water). Among these solvents are:

* Xylene: Excellent agent but should not exceed 3 hours.
* Toluene: Can remain for up to 12 hours.
* Benzene.

Purpose: The solvent is used to displace alcohol through 3 successive baths over 2 to 3 hours.

Figure 27: Dehydration of samples (Personal photo 2024)

C. Infiltration:

This is the final step of the process, known as embedding, which aims to replace the clearing solvent with the embedding solution, which is melted paraffin (liquid). The melting point temperature of paraffin ranges between 54°C and 58°C in the oven. The goal is to thoroughly fill all tissue cavities, ensuring uniform consistency and providing internal support for sectioning. The tissue staysin 2 baths for 12 hours each.

Figure 28: the impregnation oven. (Personal photo 2024)

D. Embedding:

Embedding involves preparing a paraffin block into which a tissue specimen, processed through the previous steps, is introduced. This embedding process provides external support to the tissue for sectioning with a microtome and ensures better tissue preservation thereafter.

The paraffin block (embedding) is prepared using small stainless steel cups (molds) into which the tissue is placed at the center of the mold, after a thin layer of paraffin has been applied inside. Here are some key steps:

* Ensure the tissue does not touch the sides of themold.
* Ensure tissues do not touch each other if multiple tissues are in the same mold.
* Ensure all tissues are positioned at the same level within the block to avoid any loss.

An embedding cassette is placed over the sample in the mold, then covered with melted paraffin using an automatic paraffin dispenser onto the cassette.

Figure 29: An automatic paraffin dispenser on the cassette (Personal photo 2024)

After complete cooling, the paraffin block is demolded. The embedding medium should be the same as that used for tissue infiltration.

Figure 30: Cooling the paraffin block (Personal photo 2024)

E.Microtomy:

Purpose: This procedure aims to obtain slices with a thickness of 3 to 5 micrometers and mount them on transparent glass slides (slides). The production of high-quality sections largely depends on the tissue preparation steps (fixation, embedding).

A. Procedure:

* Trimming (Equarrissage): Remove excess paraffin around the sample using a scalpel, leaving approximately 5 mm of paraffin.
* Cooling of the Paraffin Block: Cooling the paraffin block facilitates sectioning.
* Orientation of the Paraffin Block: Place the paraffin block on the microtome stage so that it is parallel to the cutting edge (bevel) of the blade.
* Setting the Microtome Blade: Ensure the microtome blade and holder are securely fixed at an angle of 30 degrees to the block, allowing it to contact the back of the bevel.
* Initial Sectioning (Grossing or Trimming): Remove the paraffin in front of the sample to obtain a complete tissue section for staining. Care must be taken not to lose the sample due to excessive trimming.
* Stabilization: Hold the slide as close as possible to the starting point to improve the stability of the paraffin block.
* Sectioning Proper: Make regular passes of the sample in front of the microtome blade. Each pass removes a slice of the selected thickness, either as a single section or in a ribbon series. The movement should be consistent, neither too slow nor too fast, to obtain uniform and representative sections of the selected thickness.
* Blade Maintenance: To avoid damaging the sample, ensure the blade is changed or sharpened when it becomes dull or worn.
* Cleaning: Regularly clean the microtome blade after each paraffin block change to prevent contamination from one section to the next.

Figure 31: Microtomy (Personal photo 2024)

B. Spreading:

Spreading is used to restore the tissue to its original Figure (without folds) before placing it on a slide for examination under a microscope. The most commonly used method of spreading involves placing the tissue on a warm waterbath.

Figure 32: spreading bath (Personal photo 2024)

Spreading bath:

* The temperature should be approximately 10°C below the melting point of paraffin. A higher temperature can melt the paraffin, leading to tissue disintegration during sectioning, while a lower temperature may cause the tissue to retain folds.
* Add a gelatin adhesive at 0.2% concentration or an albumin solution at 10% concentration to the spreading bath water. Also, add a few crystals of thymol or 1 mL of formalin to prevent mold and fungal growth.
* Ensure that sections or ribbons are not left in the bath for too long to prevent tissue swelling.

Sections are transferred using a labeled blade (bearing the block number) and should be free from folds, streaks, holes, bubbles, compression, or any other defects (artifacts). After spreading, they should be drained and dried (either on a warm plate at approximately 37°C or in an oven).

Figure 33: Hotplate used for heating (Personal photo 2024)

Before staining tissue on a slide, the following steps must be taken:

C. Deparaffinization: to remove the internal support paraffin:

Figure 34: Deparaffinization of samples (Personal photo 2024)

Hydration : by ethanol tissue already dehydrated:

Figure 35: hydration of a sample (Personal photo 2024)

Staining:

* Hematoxylin for 30 seconds
* Rinse for 3 minutes under running water (multiple baths).
* Stain with eosin for 4 minutes.
* Quick rinses with ethanol

Figure 36: Eosin stain (Personal photo 2024)

Deshydration:

Is used for preservation and mounting involves removing all traces of water using increasing concentrations of ethanol.

70° …. 60s

90° … 60s

100° …60s

Eclaircissements:

Serves for preservation and mounting Involves removing the traces of alcohol (dehydration agent) using a product that is miscible with both alcohol and non-aqueous synthetic mounting resin.

Xylene …… 05min

Xylene …… 05min

Figure 37: Clarification of the samples. (Personal photo 2024)

Mounting and Reading:

* **Procedure:**

Apply 1 to 2 drops of mounting medium (Eukitt resin) onto the stained section of the slide. Cover it with a coverslip. To prevent air bubbles that could obstruct reading, gently press down on the edges of the coverslip using forceps. Allowit to dry beforereading.

Figure 38: mounting of stained samples (Personal photo 2024)

Figure 39: Observation of stained samples under microscope (Personal photo 2024)

6. Statistical Analysis:

Firstly, descriptive statistics were used to characterize the herds according to different factors. Subsequently, statistical analyses were performed using SAS software (Version 9.1.3, SAS Institute Inc., Cary, NC). Before conducting statistical analysis, the distribution of antibody titers was assessed using PROC UNIVARIATE and the Shapiro-Wilk test.

Antibody titers over time were analyzed by fitting a general linear mixed model using the MIXED procedure in SAS to evaluate seroconversion between the first and second serum collection.

Chapter II :
Results

II. Chapter II : Results :

1. Clinical study :

Clinical signs : The most common clinical signs are respiratory (sneezing and rales), digestive (greenish diarrhea) and nervous (torticollis and motor incoordination).

Lesions : The most frequently observed post-mortem lesions are: petechiae in the proventriculus, tracheitis and enteritis.

Torticollis Tracheitis Petechiae in the proventriculus

Figure 40: Clinical signs and lesions observed

2. Serology :

Table 4 presents the antibody titers results for ND. Out of a total of 12 farms, 8 (60%) tested positive for ND. For all mentioned diseases, a low coefficient of variation (CV) was demonstrated, and there was a significant difference ($p < 0.0001$) in antibody titers between the first and second samples: (LSM ± SE, 2057.00 vs 4765.00 ± 275.00, CV (27-49%).

Table 4: Serological results

Farms	Moy 1	SE1	CV1	Moy 2	SE2	CV2	P
1	721,800	611,592	85	319,267	480,929	151	0,055
2	1823,133	812,357	45	5281,867	2233,698	42	**<0.0001**
3	1418,000	652,525	46	4345,733	2049,057	47	**<0.0001**
4	1324,667	569,733	43	1490,400	620,503	42	0,452

5	136,600	212,256	155	676,533	931,218	138	0,037
6	1979,400	878,160	44	4718,533	1935,036	41	< **0,0001**
7	1571,867	670,755	39	4986,733	1257,844	25	< **0,0001**
8	174,400	110,093	63	284,800	148,017	52	0,028
9	1165,200	963,379	44	3242,533	1422,874	44	**0,022**
10	1361,333	618,638	45	1548,933	759,283	49	0,464
11	2374,133	1541,374	45	5447,067	390,728	7	< **0,0001**
12	3389,867	1907,394	46	4007,533	2225,630	46	**0,042**
13	1648,933	784,479	48	4107,133	2400,459	48	**0,001**
14	2891,200	1341,349	36	6401,200	1328,435	21	< **0,0001**
15	1369,533	1019,351	74	1256,000	804,567	64	0,737
16	1671,133	1398,056	44	3952,733	1213,042	41	**0,012**
17	3202,467	1056,054	33	6373,200	999,876	16	< **0,0001**
18	1270,067	543,956	43	1362,000	521,066	38	0,640
19	1669,800	581,856	35	3729,533	1558,075	42	< **0,0001**
20	1624,467	524,596	32	3175,000	918,955	29	< **0,0001**

CV : Coefficient of Variation, **SE :** Standard Error **P :** Probability

Pathology	**Antibody titers**		**CV**	**SE**	**P**	**Seropositivity**
	Mean 1	**Mean 2**	**(%)**			**(%)**
ND	2057.00	4765.00	27-49	275.00	<0.0001	60

CV : Coefficient of Variation, **SE :** Standard Error, **P :** Probability.

3. Risk factors :

With regard to risk factors: farms with good hygiene were significantly less seropositive to ND by 26% (p = 0.022). However, farms with a higher density or older than 30 days were more seropositive by 47% (p = 0.041) and 45% (p = 0.019) respectively. Finally, when the broilers were not vaccinated against ND, the farms appeared to be 48% more seropositive (p = 0.047).

Table 5: Effect of risk factors of ND.

Factors	**Class**	**Prevalence**	**Estimation**	**SE**	**OR**	**95% CI**	**P**
Protocol of vaccination*	1	21.0	-0.39	0.25	0.67	0.41-1.10	**0.04**
	2	47.3	-0.08	0.20	0.92	0.61-1.39	0.70
	3	31.5	Réf				

Season	Automn	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	Réf				
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	0.22
	ISA	42.1	Réf				
Climate	**Dry**	52.6	-0.19	0.17	0.82	0.58-1.17	0.28
	Wet	47.3	Réf				
Hygiene	Good	15.7	-0.29	0.24	0.74	0.46-1.19	**0.02**
	Moy	26.3	0.12	0.19	1.13	0.77-1.67	0.51
	Bad	57.8	Réf				
Density	>10	57.8	0.06	0.19	1.07	0.73-1.56	**0.04**
	≤10	42.2	Réf				
Age	>30	73.6	-0.01	0.15	0.98	0.71-1.34	**0.02**
	≤30	26.316	Réf				

* vaccination protocol, 1: primary vaccination without booster;2: primary vaccination with one booster; 3: primary vaccination with two boosters.

SE : Standard Error, **OR :** Old Ration

1. **Histopathology :**

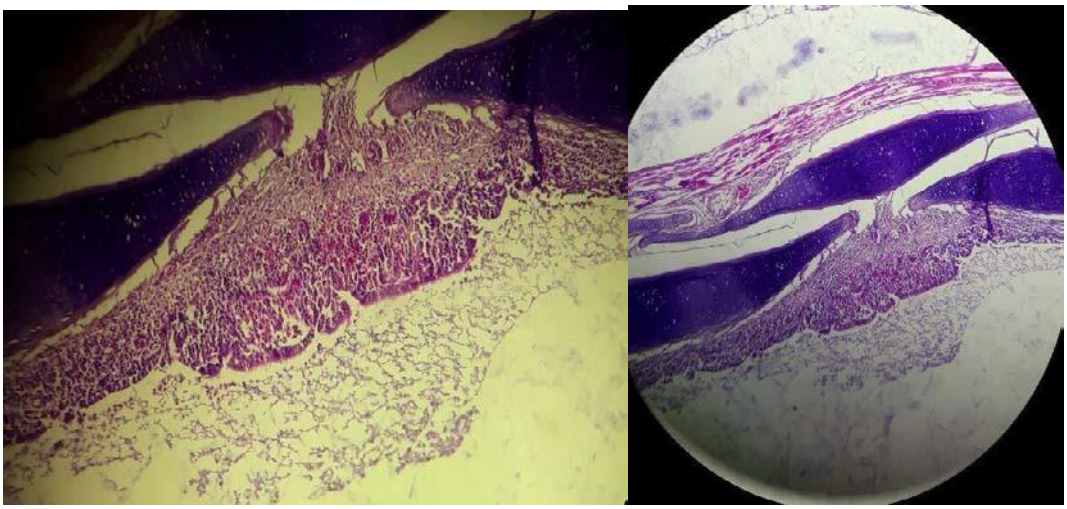


Figure 41u: histopathological sections of broiler chicken trachea (Personal photo 2024)

* **Interpretation:**

We observe thickening of the tracheal mucosa due to intense lymphocyte infiltration. The mucosalepithelium shows areas of necrosis.

The cells lose their connections with adjacent cells and lose their Figure, with eosinophilic cytoplasm and a pyknotic nucleus, indicating cellular death within the glands.

Chapter III :
Discussion

III. Chapter III : Discussion :

In this study, we focused on a sero-epidemiological and histopathological study of the main avian viral infections through a survey and analysis of laboratory samples using the ELISA method for the purpose of assessing immune status by analyzing the serological prevalence of ND in broiler farms in northern Algeria.

The results of the present study largely confirmed our predictions. The farms sampled are suspected of being infected with ND, which expresses typical clinical symptoms and lesions with high morbidity and mortality. All farms were vaccinated with a live vaccine. Our serological results showed that the sampled farms were 63.33% seropositive for ND.

The aim of our study was to assess the immune status of ND in broiler chickens. In fact, the immune status in response to viral diseases is estimated by measuring the serological response, as determined by the detection of specific antibodies produced in response to infection or vaccination (Picaultetal.1993;Brigitte et al. 1997). Finally, protected farms must have an average titre above the protection threshold for all dates analysed, without being very high compared with those resulting from vaccination, albeit in the absence of specific clinical signs (Gardin et al., 2002).

In contrast, our sampled farms were suspected of being 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 high antibody titres. Indeed, epidemics or outbreaks have been reported in vaccinated populations despite the fact that vaccination is widely applied.

Thus, the clinical and lesional manifestations of affected subjects can help diagnose a viral disease, but laboratory analysis (laboratory diagnosis) is required to confirm it. (Rekouche, Hadjerci, 2019).

We observed different prevalences for Newcastle disease virus, with high prevalences for ELISA tests but low for RT-PCR. This has already been reported in studies of avian influenza virus. An initial study carried out in Egypt in October 2007 on 200 birds from the lowlands showed a prevalence of 4.9%, but no RT-PCR-positive swabs (Claudine, Barategui.2018).

In the present study, we took paired samples to determine the serological status of a viral disease such as ND, the first sample was taken at the onset of infection (the appearance of clinical signs), the second two to three weeks later. Indeed, the appearance of antibodies between two successive sera (generally sampled 10 to 21 days apart) indicates that the first contact with the virus took place around the time the first sample was taken (De Wit, 2000; Lopez, 2006).

Indeed, if antibody concentration increases between 02 sera collected, this indicates that we have had a stimulation of the immune system which could be due to a recent infection or symptomatic viral reactivation, in the absence of vaccination. The presence of specific antibodies against a virus indicates that the virus infected the chicken at a given time (Alexander et al, 2004).

However, interpretation of the results of these serological tests is complicated by the fact that infectious and vaccine-induced antibodies cannot be differentiated, and there are few data available on their performance and how to interpret the results. (Auvigne et al, 2013).

We opted for a histopathological study of the main lesions caused by Newcastle disease in different organs. To this end, we performed autopsies on subjects from 2 confirmed Newcastle disease outbreaks. We then took samples from the trachea and proventriculus.

Histological slides were prepared using Haematoxylin-Eosin staining. We found that, in addition to a multitude of macroscopic lesions, the main histopathological lesions observed were: cell inclusions in the trachea, degeneration and hemorrhagic necrosis in the trachea; necrotizing lesions in the proventriculus.

We conclude that the majority of lesions observed are characteristic of NDV passage.

However, interpretation of the results of these serological tests is complicated by the fact that infectious and vaccine-induced antibodies cannot be differentiated, and there are few data available on their performance and how to interpret the results. (Auvigne et al, 2013).

We opted for a histopathological study of the main lesions caused by Newcastle disease in different organs. To this end, we performed autopsies on subjects from 2

confirmed Newcastle disease outbreaks. Samples were taken from the trachea and proventriculus.

Although the prevention of Newcastle disease is based on hygiene and medical prophylaxis.

To this end, it is important to stress that no vaccine can solve the problem of Newcastle disease if the required hygiene precautions are not taken, such as respect for cleaning and disinfecting methods in livestock buildings, and a sanitary vacuum (Orsi et al, 2010).

Newcastle disease induces the most characteristic tracheal lesions.

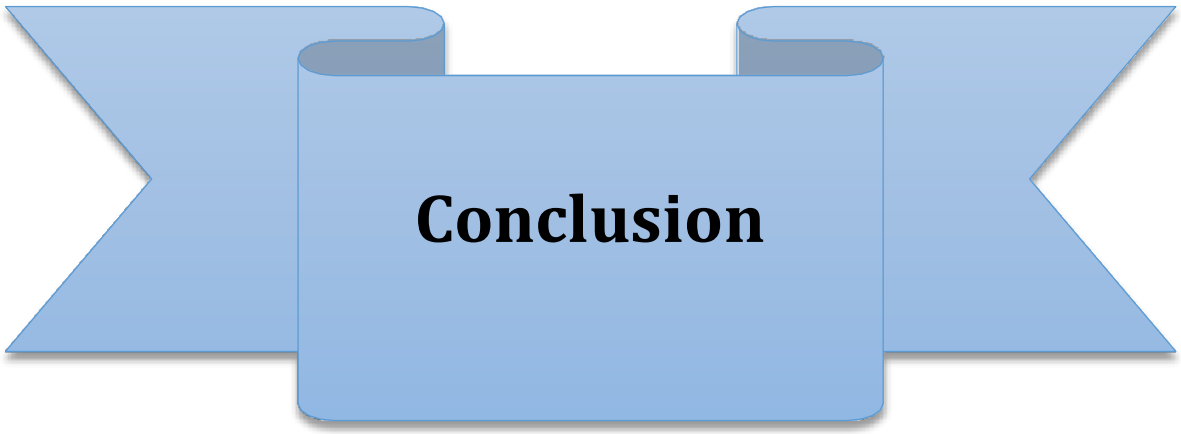
Inoculation of velogenic strains into unvaccinated chickens induces violent inflammatory reactions that evolve into acute necrotizing and hemorrhagic tracheitis in four to five days.

These observations concur with those of Beard and Easterday (1967) and Cheville et al (1972), but disagree with those of Jungherr (1960), who considers epithelial hyperplasia to be a characteristic lesion of Newcastle disease.

In fact, this discrepancy could be explained by the difference in pathogenicity and tropism of the strains used.

Cheville et al (1972) reported differences in the respiratory pathogenicity of velogenic strains. Garside (1965), after examining 80 natural cases of Newcastle disease, observed hyperplastic and necrotic lesions. He assumes that hyperplasia is observed in previously vaccinated poultry, and that necrotic lesions occur in animals that are fully susceptible to Newcastle disease. Mayor (1968) also reports that velogene strains cause hyperplastic lesions in previously vaccinated poultry (Gouffaux, et al. 1977).

The post-mortem lesions most frequently observed on our farms were: petechiae in the proventriculus, tracheitis and enteritis. These results concur with those of Kotani et al (1987), Crespo et al (1999), Talha et al (1999), Pazhanivel et al (2002), Hasan (2010) and Mohammed (2013).


Conclusion

Conclusion:

This study provided important insight into diseases dominant viral diseases in broilers and revealed that ND is a pathology frequent.

Clinical manifestations and post-mortem findings of affected birds can help diagnose disease, but laboratory diagnosis is necessary to confirm the disease.

Histopathology is a key diagnostic method for Newcastle disease, but it must be used in combination with other diagnostic methods such as serology tests to obtain a precise and reliable diagnosis. It is therefore important to have qualified and experienced professionals in the interpretation of histopathology results to improve the quality of Newcastle disease diagnosis.

Furthermore, the results also suggest that risk factors related to biosecurity and agricultural practices appear to play an important role in the severity of the disease observed on affected farms. If these factors are mitigated, the severity of the Newcastle disease problems on farms will be greatly reduced.

There are many factors that contribute to the worsening of viral infections, however, it would be possible to limit its damage by improving breeding conditions.

The use of inactivated vaccines could also strengthen the defense capacity of sensitive organisms. To prevent poultry farms from constantly being subjected to effects of many viral diseases, efforts in epidemiological surveillance should be undertaken.

Finally, poultry farming plays an important socio-economic role in the economy of countries in development. On the other hand, it is practiced in summary breeding conditions, constituting the bed of infections, this is the origin of low productivity. A better control and better management of this breeding allows optimization of this sector of activity.

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