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**THEME**

# Incidence and Pathological Features of Broiler Chicken Proventriculitis in Northeast Algeria

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# Dedication

I dedicate this thesis to my family, whose unwavering support and encouragement have been my constant source of strength throughout this journey. To my parents, whose love and wisdom have shaped me into who I am today; to my siblings, who have always been by my side, offering their guidance and understanding; and to my beloved mentor, who has not only imparted knowledge but also inspired me to pursue excellence with passion and dedication. This work is a testament to the sacrifices, patience, and belief that each of you has shown in me. I am eternally grateful for your presence in my life.

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# Abstract

A digestive disease known as transmissible viral proventriculitis (TVP) is a viral infection that affects chickens, especially broilers, and has substantial repercussions for the health and production of poultry. It causes economic losses in the chicken industry as a result of decreased digestion, poor growth performance, and lower feed conversion efficiency. These symptoms are hallmarks of the disease. Upon macroscopical examination, the disease may be recognized by the presence of proventricular enlargement, fragility, thickness, and pallor. Additionally, the condition is often accompanied with weakening and dilatation of the gastric isthmus. Nevertheless, while these lesions may indicate TVP, they are not definitive. The most accurate and reliable way to diagnose is still through histopathology. The most common signs of a problem are the necrosis of oxynticopeptic cells, the invasion of lymphocytes, and the replacement of glandular epithelium with hyperplastic ductal structures.

An investigation of the epidemiological, clinical, gross, and histological characteristics of proventriculitis in broiler flocks in the east of Algeria was the purpose of this research. Additionally, the study aimed to evaluate the probable role that infectious agents have in the etiology of the disease. Within the scope of this study, a total of 69 chicken flocks were investigated. These flocks included 62 broiler farms, four laying hen flocks, and three broiler breeder flocks. Data were obtained by field trips, post-mortem exams, and histological analysis of proventricular tissue specimens.

The microscopic examination facilitated the categorization of the proventricular lesions into three unique histological classifications:

- **TVP:** Defined by the coexistence of lymphocytic infiltration and necrosis;
- **LP (Lymphocytic Proventriculitis):** Exhibits lymphocytic infiltration absent of necrosis;
- **WP (Without Proventriculitis):** Characterized by the absence of both lymphocytic infiltration and necrosis.

The prevalence of these categories was documented at 23.6% (TVP), 52.8% (LP), and 23.6% (WP), respectively. It was discovered that the total frequency of proventriculitis among broiler flocks that were between 15 and 40 days old was 20.9%, and the death rates ranged from 0.1 percent to 0.5 percent at the time of discovery.

Particularly noteworthy is the fact that both TVP and LP were linked to a significant amount of proventricular wall hypertrophy and a significant amount of lymphocyte infiltration, both of which point to an infectious mechanism of action. On the other hand, instances of WP did not exhibit similar lesions, which suggests that the infection was not infectious or that the reasons were complex and unrelated to viral agents. Infectious agents may function as aggravating rather than main causing elements in the development of proventriculitis, according to the results of this investigation, which provide credence to the concept that infectious agents operate in this manner under the particular circumstances that were seen in this study.

This thesis offers novel insights into the occurrence, categorization, and pathological characterization of proventriculitis in broiler flocks. The persistent association of lymphocytic inflammation, tissue necrosis, and proventricular hypertrophy with TVP and LP patients highlights the significance of infectious processes. These findings provide a significant basis for further virological research and for the development of enhanced diagnostic and preventative measures against this economically critical avian disease.

**Keywords:** Transmissible viral proventriculitis; Lymphocytic proventriculitis; Without proventriculitis; Histopathology; Proventriculus.

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# List of acronyms

**N** flocks number

**n** proventriculus number

**TVP** transmissible viral proventriculitis

**LP** lymphocytic proventriculitis

**WP** without proventriculitis

**GALT** Gut-Associated Lymphoid Tissue

**GIT** Gastrointestinal Tract

**HCl** Hydrochloric Acid

**RSS** Runting Stunting Syndrome

**CPNV** Chicken Proventricular Necrosis Virus

**IHC** Immunohistochemistry

**RT-PCR** Reverse Transcription-Polymerase Chain Reaction

**dpi** Days Post-Infection

**IBDV** Infectious Bursal Disease Virus

**FAdV** Fowl Adenoviruses

**ELISA** Enzyme-Linked Immunosorbent Assay

**FFPE** Formalin-Fixed Paraffin-Embedded

**IBV** Infectious Bronchitis Virus

**H/E** Hematoxylin and Eosin

**PCR** Polymerase Chain Reaction

**RNA** Ribonucleic Acid

**RdRp** RNA-dependent RNA Polymerase

**VP** Viral Protein

**RT-PCR (Real-time)** Real-Time Polymerase Chain Reaction

**IPNV** Infectious Pancreatic Necrosis Virus

**JTT** Jones-Taylor-Thornton

**CD** Cluster of Differentiation 3

**T cells** Thymus-Derived Cells

**IBM** International Business Machines Corporation

**SPSS** Statistical Package for the Social Sciences

**FC** Feed Conversion

**D** Day

# Introduction

Transmissible viral proventriculitis (TVP) is a chicken disease responsible for significant losses in mortality, livestock heterogeneity rates, and chicken weight gain. Bayyari, Huff, Balog, et al. (1995) noted that TVP typically affects broiler chickens from 3 to 6 weeks of age and, to a lesser extent, laying hens and broiler breeders. It was first described in 1978 by Kouwenhoven, who suggested that an infectious agent was the cause (Kouwenhoven et al., 1978). Since then, the disease has been reported in several countries such as Australia (1996), USA (1996), China (2001), Spain (2007), France (2011), South Korea (2015), Iraq (2017), and recently in Poland (2020), Great Britain (2020), and Brazil (2021). However, its geographical distribution is believed to be much wider (Śmiałek et al., 2021). To date, the etiology of TVP has not been explicitly defined since it has been associated with numerous viruses including the infectious bursal disease virus (IBDV) (Dormitorio et al., 2007; Pantin-Jackwood & Brown, 2003), chicken proventricular necrosis virus (CPNV) (Allawe et al., 2017; Leão et al., 2021), infectious bronchitis virus (Li et al., 2020), picornavirus (Yu et al., 2001), gyrovirus (Kim et al., 2015), reovirus (Li et al., 2018), adenovirus (Yan et al., 2020), and cyclovirus (Opengart, 2003), as well as other agents like *Clostridium* (Guy, West, & Fuller, 2011), mycotoxins (Dorner et al., 1983), biogenic amines (Huff et al., 2001), or copper sulfate (Barnes et al., 2001). Dormitorio et al. (2007) reported that proventriculitis can be caused by various factors, including infectious (viruses, bacteria, fungi, or parasites), mycotoxins, and nutritional factors. In this regard, histopathological diagnosis becomes essential for disease confirmation based on specific microscopic lesions of the proventriculus such as necrosis of epithelial glandular cells, lymphocytic infiltrates of the mucous membrane and among the proventricular glands, and ductal epithelial hyperplasia with metaplasia of glandular epithelium to ductal epithelium (Wideman Jr et al., 1996).

The thickening of the proventricular wall has intrigued avian pathology researchers for years. Distinct lesions such as lymphoid infiltration, hyperplasia, and necrosis have been identified as

potential contributors exercising the most pronounced impact on the proventricular wall but also on how age interacts with these lesions, thereby influencing the thickening dynamics. Since the understanding of the disease remains ambiguous and none of the etiological agents have been conclusively proven to cause TVP, this thesis aims to describe histopathological, macroscopic, and epidemiological data that could be associated with possible new etiological agents. Here, we report on the first study of TVP cases in North of Africa.

# Bibliographical Part

# CHAPTER I

## The Anatomy and Physiology of the Avian Proventriculus and Ventriculus

## 1 Introduction to the Avian Digestive System

To satisfy the dietary requirements, the avian digestive system has been specially designed to facilitate the absorption of nutrients, the functioning of the immune system, and general health, instead of a single gut, birds have a categorized stomach consisting of the glandular proventriculus and the muscular ventriculus (or gizzard). This allows for both chemical and mechanical digestions. It is necessary to break down a wide variety of food sources, including fish, insects, and fibrous seeds, in order to release their nutrients (Hristov, 2021). During digestion, the gastrointestinal tract (GIT) plays a crucial part in the process of breaking down complex molecules that are consumed into simpler compounds.

These nutrients are then absorbed via the intestinal mucosa and enter circulation, this mechanism enhances immunological function According to Kleyn and Chrystal's research from 2020. According to Kleyn and Chrystal (2020), this process not only helps the immune system function, but it also has an effect on the use of nutrients, the well-being of birds, and the profitability of commercial broiler rearing. The gastrointestinal tract, as the principal entry route for antigens, infections, and toxic substances, must equilibrate digestion and immunity to maintain avian health (Hristov, 2021). The gut-associated lymphoid tissue (GALT), the body's biggest lymphoid organ, exhibits this equilibrium by integrating digestion with pathogen defense, (Klasing, 1999).

The proventriculus and ventriculus are essential to bird digestion. The proventriculus is responsible for the chemical digestion of food by secreting hydrochloric acid (HCl) and pepsin, whereas

the ventriculus is responsible for the grinding of food into finer particles for intestinal absorption (Kleyn & Chrystal, 2020; Langlois, 2003).

Compared to mammals, birds have a shorter digestive tract, (Table 1). which is made up for by consuming more feed and having better nutritional absorption (McWhorter et al., 2009). Moreover, birds lack teeth and powerful jaw muscles, replacing them with a lightweight bill or beak Food is swallowed whole and thereafter subjected to mechanical processing in the ventriculus or gizzard (Scanes & Dridi, 2021).

Table 1: Dimensions of the digestive tract of various species of birds

| Species | BW (Kg) | Esophagus | | | Proventriculus and gizzard | | | Small intestine | | | Cecum | | Rectum | | | Total | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | L (mm) | Total % | WT (g) | L (mm) | Total % | WT (g) | L (mm) | Total % | WT (g) | L (mm) | WT (g) | L (mm) | Total % | WT (g) | L (mm) | L/BW |
| Chicken | | | | | | | | | | | | | | | | | |
| Leghorn | 1.2 | 136 | 9.9 | 8.2 | 86 | 6.3 | 27 | 1082 | 78.9 | 30 | 127 | 5.2 | 68 | 5 | 2.3 | 1372 | 1.1 |
| Broiler | 3 | 140 | 6.4 | 16.8 | 101 | 4.7 | 44 | 1796 | 82.7 | 74 | 188 | 11 | 134 | 6.2 | 5.1 | 2172 | 0.7 |
| Turkey | 3 | 123 | 5.7 | 8.5 | 110 | 5.1 | 53 | 1853 | 85.7 | 85 | 278 | 20 | 75 | 3.5 | 4.4 | 2161 | 0.7 |
| Japanese quail | - | 75 | 11.5 | - | 38 | 5.8 | - | 510 | 78.1 | - | 100 | - | 30 | 4.6 | - | 653 | - |
| Domestic duck | 2.2 | 310 | 11.7 | - | 130 | 4.9 | - | 2110 | 79.9 | - | 140 | - | 90 | 3.4 | - | 2640 | 1.2 |
| Emu | 53 | 790 | 12.1 | - | 260 | 4 | - | 5200 | 79.4 | - | 120 | - | 300 | 4.6 | - | 6550 | 0.1 |
| Rhea | 25 | - | - | - | 310 | - | - | 1400 | - | - | 480 | - | - | 400 | - | - | - |
| Ostrich | 122 | - | - | - | 480 | - | - | 6400 | - | - | 940 | - | 8000 | - | - | - | - |
| Cedar Waxwing | - | 51 | 16.2 | - | 36 | 11.4 | - | 171 | 54.3 | - | 0 | - | 57 | 18.1 | - | 315 | - |

*The length (L), body weight (BW), and weight (WT) of the gastrointestinal tract can change depending on the environment in which the birds are raised. Reproduced from D. M. Denbow (2015)*

The necessity of keeping a healthy gastrointestinal tract (GIT) for immune control, nutritional absorption, and overall well-being are brought to light by this fundamental knowledge of avian digestion. As a result of the direct influence that efficient digestion and absorption have on field performance and profitability, the gastrointestinal tract (GIT) is an essential area of concentration in broiler nutrition.

# 2 Detailed Anatomy of the Proventriculus

Within the region that lies between the esophagus and the ventriculus, there is a glandular organ known as the proventriculus, In comparison to the digestive tracts of mammals, the digestive tracts

of birds are substantially shorter, with an average digesta transit time of less than three and a half hours (D. Denbow, 2000). This organ is formed like a fusiform and is located between the distal end of the esophagus and the gizzard. It is also located next to the liver. The proventriculus of an adult chicken is normally 4–6 cm long and around 2 cm in diameter.

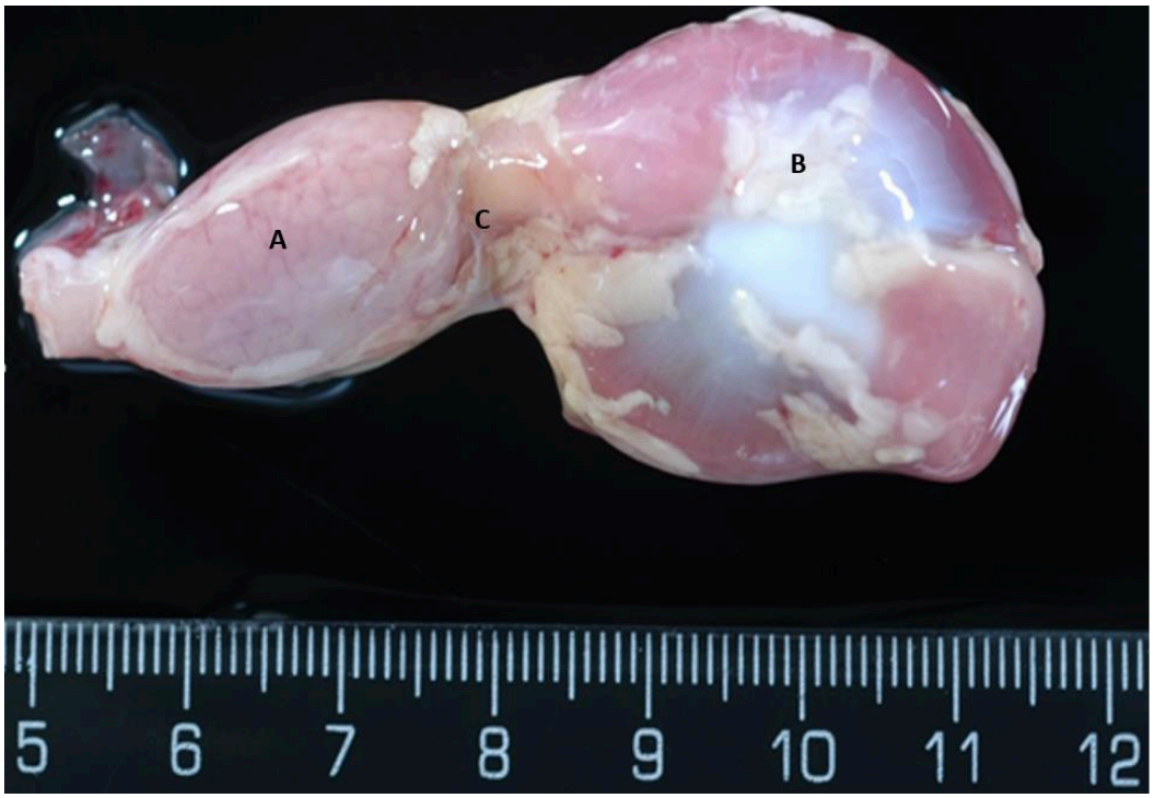


Figure 1: Anatomy of the chicken stomach

*A: Glandular proventriculus; B: Muscular ventriculus or gizzard; C: Connecting isthmus (Wali, 2021)*

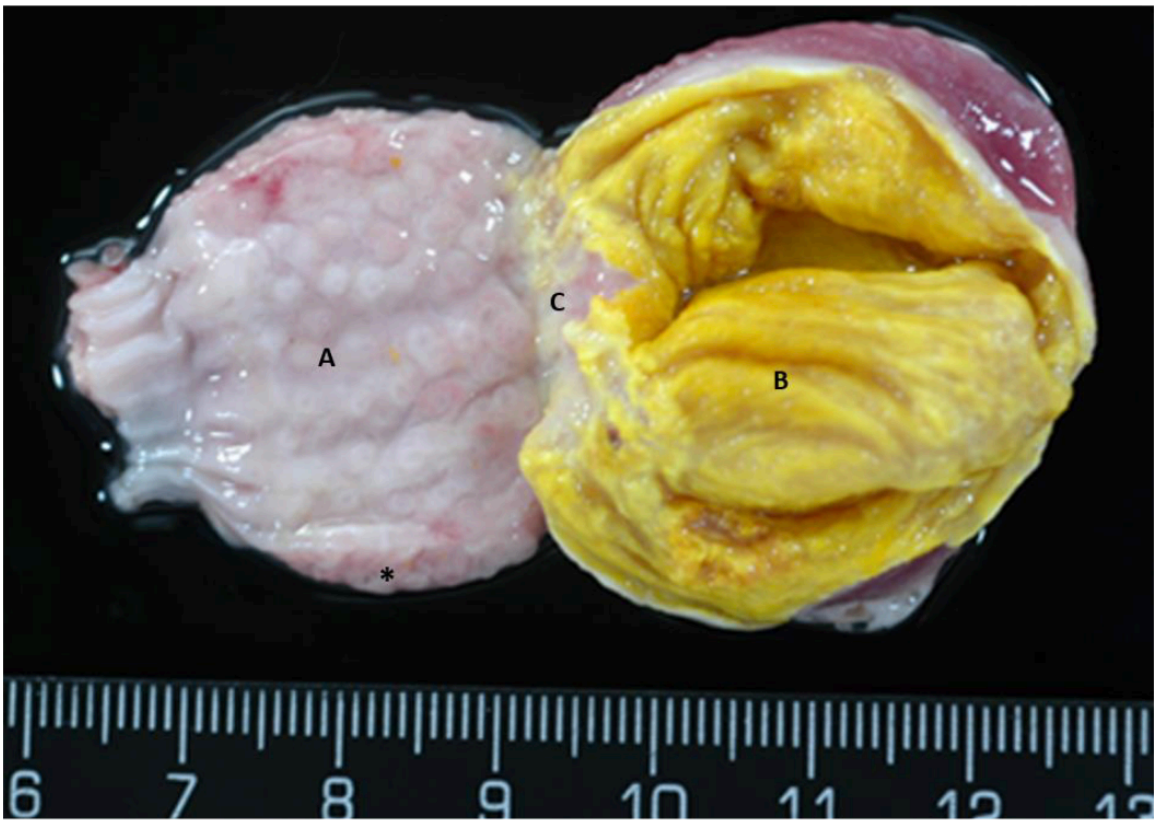


Figure 2: Mucosal lining of the chicken proventriculus, isthmus, and gizzard

*A: Proventriculus; B: Gizzard; C: Isthmus. In the proventricular mucosa, the papillae of the proventricular glands are clearly visible. Reproduced from Wali (2021).*

The intermediate zone, also known as the isthmus, is a relatively short region that connects the proventriculus to the gizzard. It measures approximately 0.75 centimeters in length. An examination of the histological characteristics of the isthmus reveals characteristics that are transitional between the proventriculus and the ventriculus. (Figures 1 and 2) Its primary function is that of a contractile barrier, which regulates the passage of ingesta between the glandular and muscular sections of the stomach. The isthmus works to accomplish this (Wali, 2021).

This structure contains proventricular glands that release hydrochloric acid and pepsinogen, the precursor of pepsin, which is vital for protein digestion (D. Denbow, 2000; Langlois, 2003; McLelland, 1991). Chicken stomachs consisting of a glandular stomach or proventriculus (A) and a muscular stomach, named also ventriculus or gizzard (B). Between both, there is the isthmus

(C) (Wali, 2021). The mucosa, submucosa, muscularis, and serosa are the four separate layers that make up the proventriculus when viewed under a microscope (Figure 3). The proventriculus's mucosal surface is uneven, with several papillae or projections connected to the gastric glands that release gastric secretions (Hodges, 1974).

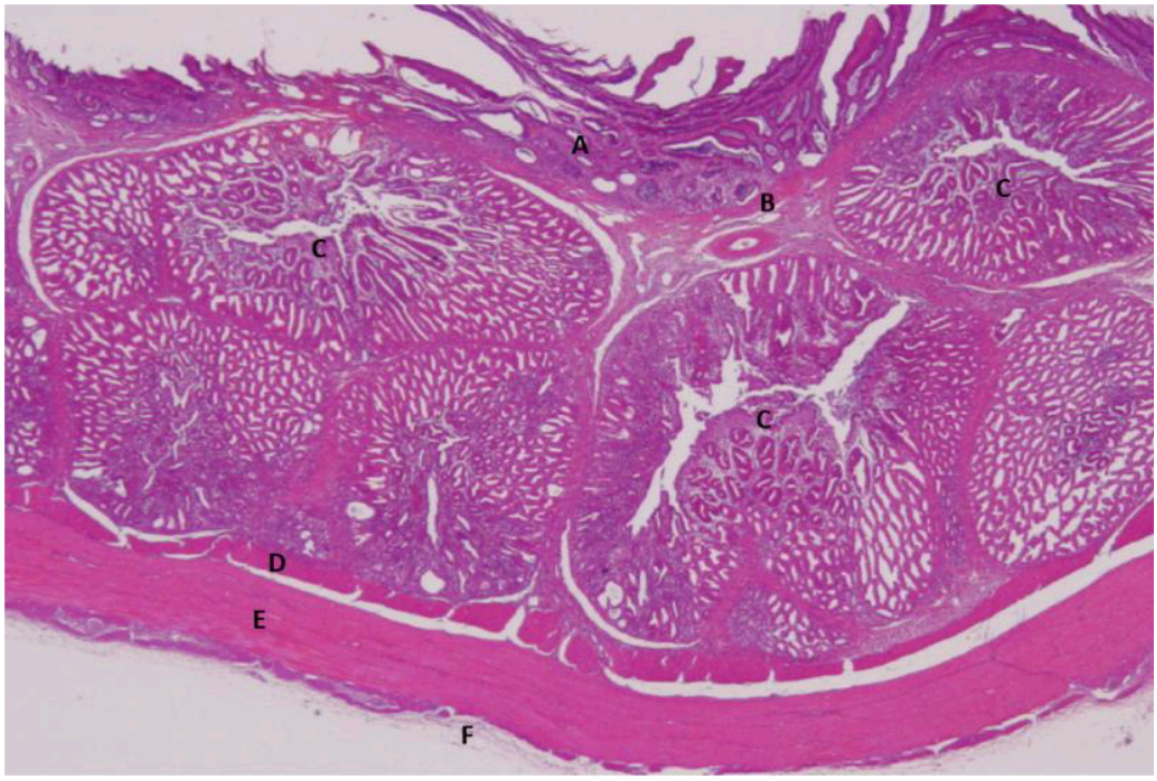


Figure 3: Histological structure of the chicken proventriculus

*A: Columnar epithelium (mucous secretion); B: Lamina propria; C: Multilobular glands with oxynticopeptic cells (secreting pepsin and hydrochloric acid); D: Inner circular muscle layer; E: Outer longitudinal muscle layer; F: Serosa. Reproduced from Wali (2021).*

The mucosal layer is made up of simple villi that are lined by columnar ductal mucous secreting cells. The nucleus of these cells is located in the basal cytoplasmic portion of the cells (Fig. 3). There are many proventriculus glands in the submucosa. According to Reece and Frazier (1990) the substantial portion of the thickness of the proventriculus wall is comprised of the mass of glands. The glands are made up of a large number of lobules that are either spherical or polygonal. These

lobules are grouped in small groups and are divided by connective tissue septa. The glands are made up of collagenous and elastic fibers, along with a few muscle fibers, blood vessels, and nerves. Alveoli 8 that radiate out from the central cavity make up the majority of the lobules. There is a secondary duct that drains the central cavity, and all of the secondary ducts are coming together to form the major ducts that open up to the opening in the lumen of the proventriculus (Hodges, 1974).

Birds don't have a single-chambered stomach like humans do. Instead, the proventriculus does all the chemical processing and sends the food to the ventriculus for mechanical breakdown. (D. Denbow, 2000; Hristov, 2021; Kleyn & Chrystal, 2020). The proventriculus is especially well-developed in birds that eat grains, like chickens, so they can handle meals high in grains. The mucosa contains mucus-secreting cells, whereas the gastric glands, organized in papillae, extend into the lumen to promote the secretion of digesting enzymes (D. Denbow, 2000; Hristov, 2021; Kleyn & Chrystal, 2020). These adaptations signify evolutionary specialization, shown by a developed proventriculus and ventriculus that enhance nutrient release and absorption for grain-centric diets (D. Denbow, 2000; Kleyn & Chrystal, 2020).

# 3 Physiology of the Proventriculus

Through the release of hydrochloric acid, which denatures food proteins, the proventriculus starts the chemical digestion process. Pepsinogen is activated by this acidity to produce pepsin, which breaks down proteins into smaller peptides for further digestion in the ventriculus (D. Denbow, 2000; Langlois, 2003).

Phases of the proventriculus's digestion process include a cephalic phase brought on by the sight and smell of food, a stomach phase where gastrin is released, and an intestine phase where digestive hormones such cholecystokinin have an impact (Kleyn & Chrystal, 2020). The process of reverse peristalsis, also known as gastric reflux, improves the efficiency of nutritional breakdown by allowing partly digested food to return from the ventriculus to the proventriculus for additional

enzymatic treatment (D. Denbow, 2000; Hristov, 2021; Kleyn & Chrystal, 2020).

Pepsin, hydrochloric acid, and mucus are some of the gastric components that are produced and secreted by the proventriculus, which is the principal function of this structural component. The ventriculus is the primary location for mechanical and enzymatic digestion, but it also starts chemical digestion. Strong digestion is facilitated by a pH range of 2.2 to 3.2.

In order to do this, the abrasive action of the cuticle and the strong contractions of the asymmetrical ventricular muscle groups are used. According to D. Denbow (2000), Gastric acid not only helps with the digestion of proteins, but it also improves the absorption of iron, calcium, and vitamin B12. Furthermore, the high acidity of gastric acid contributes to the destruction of bacteria that are consumed and protects against enteric infections (Schubert, 2015). Nevertheless, the proventriculus and ventriculus are not the primary places where nutrients are absorbed by the body. The large intestine has a less significant function in the absorption of carbohydrates, amino acids, peptides, fatty acids, electrolytes, and vitamins than the small intestine (D. Denbow, 2000).

# 4 Comparative Aspects and Functional Significance

Evolutionary adaptations to diverse diets are reflected in the very variable proventriculus and ventriculus structures and functions among bird species. Granivorous species, like chickens, possess a strong ventriculus that allows them to grind tough, fibrous material, but piscivorous and carnivorous birds, which eat softer foods, have a less muscular gizzard (Hristov, 2021; Langlois, 2003).

These changes enable every animal to digest and absorb nutrients in their food the best way possible. Granivores and insectivores possess a highly developed ventriculus that facilitates the grinding of hard substances, whereas piscivores and carnivores depend more on enzymatic digestion in the proventriculus owing to their softer diets (Hristov, 2021; Kleyn & Chrystal, 2020; Langlois, 2003). This variation highlights the resilience of the avian digestive system to different feeding techniques across species (Langlois, 2003).

# CHAPTER II

## Proventriculitis

## 1 Definition and History

A new infectious disease called transmissible viral proventriculitis (TVP) affects broiler chickens, commercial layers, and broiler breeders. The disease significantly impacts the global economy. Stunting and runting are common symptoms of the condition in birds, which is marked by poor digestion, low feed conversion, and diminished production performance. TVP is mostly defined by the proventriculus becoming bigger and more fragile. This can cause it to break during mechanical evisceration in the slaughtering process, which can contaminate the body and lead to its rejection (Dormitorio et al., 2007).

In 1978, Kouwenhoven et al. (1978) conducted an examination at a big broiler farm in the Netherlands that was having runting and poor feed conversion. During this study, they discovered the first incidence of TVP from the country. An extensive enlargement of the proventriculus was seen in the birds that were impacted, and in some instances, Under a microscope, proventricular tissue fibrosis, leukocyte infiltration, glandular necrosis, and bleeding were seen.

Based on the fact that the virus was able to pass through both 450 and 100 nm filters, the clinical picture was effectively replicated by experimental inoculation of filtered homogenates. This led to the conclusion that a virus was the culprit responsible for the disease.

Adenovirus was found in some samples, but how it contributed to the disease's origin was not yet determined (Kouwenhoven et al., 1978). This research was the first to describe an infectious proventriculitis that caused hens to have runting symptoms.

Proventriculitis was also replicated experimentally in subsequent research, such as the ones

conducted by Bayyari, Huff, Beasley, et al. (1995), by making use of filtered homogenates obtained from field cases. It is interesting to note that while proventriculitis was regularly detected, it did not result in stunting or reduced development. This finding suggests that proventriculitis and runting stunting syndrome (RSS) may have separate causes.

By identifying intralesional viral particles in instances of TVP for the first time in 1996, Goodwin et al. (1996) suggested that there is a causal connection between the virus and proventricular lesions. Hexagonal virus particles (65–70 nm in size) were found in the cytoplasm and nucleus of oxynticopeptic cells using transmission electron microscopy; these particles are often linked to chromatin condensation.

Even with these results, the virus could not be fully described because in situ hybridization for adenovirus or polyomavirus nucleic acid did not work. These discoveries established the foundation for recognizing the condition as Transmissible Viral Proventriculitis (TVP).

According to many sources, including those cited by (Dormitorio et al., 2007), (Grau-Roma et al., 2017), and (Leão et al., 2021) TVP has been documented worldwide since its original description. This includes nations like the USA, China, South Korea, the UK, France, Brazil, and Poland. There have been observations of TVP in older birds, including broiler breeders (9–20 weeks) and commercial layers (Marusak et al., 2012; Noiva et al., 2015), however it mostly affects broiler chickens (3–8 weeks).

For decades, the etiology of TVP remained uncertain, with various viruses being implicated, including:

- Infectious bursal disease virus (IBDV)
- Infectious bronchitis virus (IBV)
- Reovirus
- Adenovirus (Guy, West, & Fuller, 2011).

More recently, chicken proventricular necrosis virus (CPNV) has emerged as the primary viral agent associated with TVP. CPNV, a novel birnavirus, has been shown to differ significantly from IBDV,

despite belonging to the same viral family. Molecular studies and experimental inoculations have demonstrated that CPNV can induce TVP-associated lesions, solidifying its role in the disease's pathogenesis (Grau-Roma et al., 2017; Guy, West, & Fuller, 2011).

TVP significantly reduces body weight gain and feed efficiency in affected broiler flocks. Experimental studies showed a 30% reduction in body weight gain in CPNV-infected birds compared to controls over a 14-day period post-infection (Wali, 2021). Flock non-uniformity is a common clinical sign, with some birds stunted to 25% of normal body weight (Noiva et al., 2015). The fragility of the proventriculus, especially at the gastric isthmus, often leads to rupture during evisceration, contaminating the carcass with digestive contents and resulting in condemnation (Dormitorio et al., 2007).

# 2 Geographic Distribution and Epidemiological aspects

In 1978, the Netherlands were the location where the first instance of infectious proventriculitis was recorded (Kouwenhoven et al., 1978). Several other countries have found the disease since then, such as the US (Guy, West, & Fuller, 2011; Guy et al., 2005; Marusak et al., 2012; Noiva et al., 2015; Page et al., 1982) Spain, (Grau-Roma et al., 2010) France (Marguerie et al., 2011) Egypt (Kutkat et al., 2010), South Korea (Kim et al., 2015), Poland (Smilek et al., 2017), China (Li et al., 2018), and the UK (Grau-Roma et al., 2017, 2020).

The vast majority of TVP outbreaks have been documented in broilers that are between three and eight weeks old, with the illness being most often seen in chickens that are between four and five weeks old (Hafner et al., 2013). As of now, there is a singular documented instance of TVP impacting broiler breeders (9–20 weeks old) and layer hens (14–16 weeks old) in the United States (Marusak et al., 2012).

Figure 7 illustrates the frequency of outbreaks and viral identification by PCR based on samples collected from all over the globe. This is despite the fact that full data on the global distribution and prevalence of TVP are still available, and it is still immature to estimate the real prevalence of

infection. Leading to strain variety (Guy, West, Fuller, et al., 2011).


Figure 4: Geographical distribution of TVP cases

# 3 Etiology

Since its first description in 1978 by Kouwenhoven et al. in the Netherlands, the etiology of transmissible viral proventriculitis (TVP) has been the subject of a significant amount of research. Microscopical evidence of glandular epithelial necrosis, lymphoid infiltration, and ductal epithelial hyperplasia, together with proventriculus inflammation, enlargement, and fragility, describe transmissible viral proventriculitis (TVP). TVP was thought to have a variety of infectious and non-infectious causes at first, but new research has shown that the main cause is the Chicken Proventricular Necrosis Virus (CPNV).

## 3.1 Early Investigations into the Etiology of TVP

Through the use of microscopic and gross lesions, along with limited isolation tries, early research suggested the following viral agents as possible causes of TVP:

**Adenoviruses:** Adenoviruses were identified from the proventriculi of infected chickens by Kouwenhoven et al. (1978), Researchers concluded that a tiny, cell-free agent, most likely a virus, was responsible for the sickness after successfully reproducing it experimentally by inoculating healthy hens with filtered proventricular homogenates. The involvement of adenovirus in the pathological process of TVP remains unverified.

**Reoviruses and Infectious Bronchitis Virus (IBV):** The link between reoviruses and infectious bovine viral disease (IVBV) and runting-stunting syndrome (RSS) in hens led to their suggestion as possible causative agents. Apart from these results, the particular TVP lesions could not be well reproduced in experimental infections (Bayyari, Huff, Beasley, et al., 1995; Guy et al., 2005; Huff et al., 2001; Page et al., 1982).

**The birnavirus Infectious Bursal Disease Virus (IBDV):** It was first thought to be responsible for the histological findings seen in tumors caused by transmissible viral proventriculitis (TVP), such as lymphocytic infiltration and glandular necrosis. On the other hand, further research (Guy, West, & Fuller, 2011; Huff et al., 2001) demonstrated that occurrences of TVP were caused by a different virus than IBDV. Infected birds' proventricular glandular cells contained hexagonal virus particles (65-70 nm) in their nuclei and cytoplasm, as noted by Goodwin et al. (1996). This research set the groundwork for recognizing TVP as a viral disease, despite the fact that the virus could not be isolated.

## 3.2 Identification of Chicken Proventricular Necrosis Virus (CPNV)

According to findings from more recent research, the Chicken Proventricular Necrosis Virus (CPNV) has been identified as the major culprit responsible for the development of TVP.

1. Virus Classification and Genomic Structure: Birnaviridae is a family of viruses that have double-stranded RNA genomes that are bisegmented, and CPNV is a member of this family,The RNA-dependent RNA polymerase (RdRp) is encoded by Segment B, whereas structural proteins like as VP2 (major capsid protein), VP3, and VP4 are encoded by Segment A.

Segment A is responsible for encoding structural proteins.

CPNV is genetically different from other birnaviruses, such as Infectious Bursal Disease Virus (IBDV), and has distinctive phylogenetic traits to distinguish itself from other birnaviruses. The VP1 protein has a permuted RdRp sequence motif, distinguishing CPNV from all other known birnaviruses (Guy, West, & Fuller, 2011).

2. Experimental Replication of TVP: TVP has been experimentally replicated by inoculating chickens with proventricular homogenates that contain CPNV. This has allowed for the illness to be reproduced under controlled conditions. By means of these experimental infections, the key characteristics of transmissible viral proventriculitis (TVP) have been successfully replicated. These characteristics include proventricular enlargement and fragility, necrosis of oxynticopeptic cells, and ductal epithelial hyperplasia and severe lymphocytic infiltration. These findings have been documented by Guy et al. (2007), Noiva et al. (2015), and Grau-Roma et al. (2017).

3. CPNV Detection in Clinical Cases: The detection of CPNV has been accomplished by the use of immunohistochemistry (IHC) and reverse transcription-polymerase chain reaction (RT-PCR) targeting the VP1 gene. CPNV has been identified in both experimental and naturally occurring instances of TVP:

    - A positive RT-PCR result for CPNV was seen in 22%-47% of chicks that had their TVP validated (Grau-Roma et al., 2017; Leão et al., 2021).
    - The presence of CPNV, RNA in hens that only had minor lymphocytic proventriculitis is further evidence that the virus may be responsible for subclinical infections or may continue to be present in the proventriculus (Grau-Roma et al., 2017).

4. The Diverse Nature of TVP: It seems that TVP has a complex etiology impacted by several infectious agents and environmental variables, while CPNV is known to be the main cause:

    (a) Concurrent Infections: It is common to see CPNV infection in chickens who already

have a condition that weakens their immune system, such the infectious bursal disease virus (IBDV). This condition might make it easier for CPNV to infect the chickens (Guy, West, & Fuller, 2011; Noiva et al., 2015).

There have been reports of co-infections with fowl adenoviruses (FAdV), which suggests that latent infections may become triggered throughout the course of CPNV infection Transmissible Viral Proventriculitis.

(b) Factors Related to the Environment and Management: Inadequate sanitation, contaminated feed, and poor biosecurity are probable causes of CPNV and TVP epidemics (Leão et al., 2021).

(c) Toxic Causes of Proventriculitis: Mycotoxins, biogenic amines, and excessive copper sulfate are non-infectious entities that may resemble TVP lesions (Bayyari, Huff, Balog, et al., 1995; Dorner et al., 1983; Huff et al., 2001).

# 4 Virus Taxonomy and Classification

## 4.1 Overview of the Birnaviridae Family

Recently identified as Chicken Proventricular Necrosis Virus (CPNV), it has substantial associations with Transmissible Viral Proventriculitis (TVP). This virus infects both vertebrate and invertebrate hosts; it is a member of the Birnaviridae family of double-stranded RNA viruses. There are still some questions about how CPNV interacts with other viruses and external factors, although we possess a better understanding of the evolution of TVP in accordance with the CPNV categorization and its distinctive characteristics.

The Birnaviridae family contains viruses with icosahedral, non-enveloped virion and bisegmented, double-stranded RNA genomes. This family has four recognized genera (figure 5):

- Avibirnavirus: is a virus that causes infections in birds, such as the Infectious Bursal Disease Virus (IBDV).

- Aquabirnavirus: Infects fish and other aquatic animals (as an example, Infectious Pancreatic Necrosis Virus, known as IPNV).
- Blosnavirus: is a virus that is found in snakes.
- Entomobirnavirus: This virus kills insects.

According to genomic divergence and phylogenetic research, CPNV, an unclassified birnavirus, is so different from other known members of the family that it may constitute a new genus within the family (Grau-Roma et al., 2017; Guy, West, & Fuller, 2011).

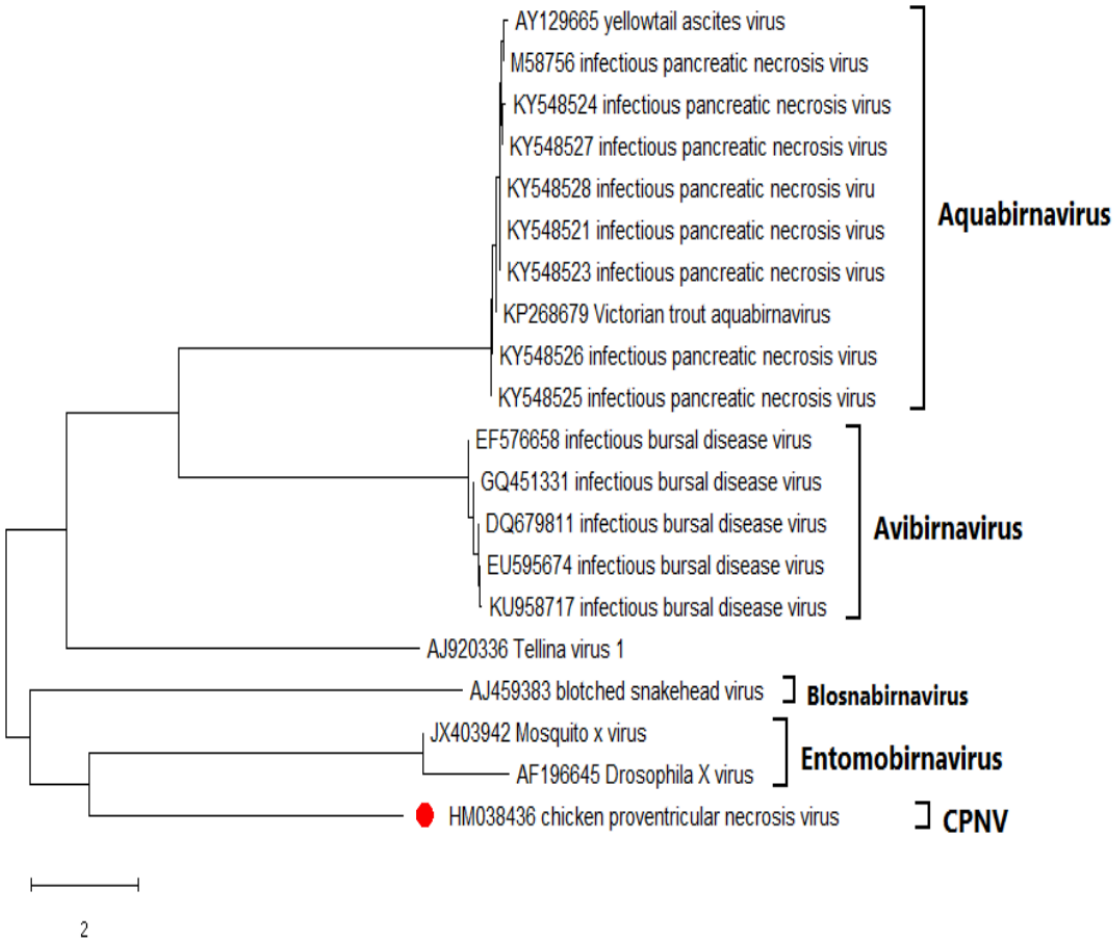


Figure 5: Phylogenetic tree of Birnaviridae VP1 sequences

*Constructed using MUSCLE-aligned sequences and refined manually. Phylogenetic analysis was performed in MEGA X with the JTT model and a gamma distribution. The tree includes multiple Birnaviridae genera, newly identified birnaviruses, and the only available CPNV sequence. (Kumar et al., 2018)*

## 4.2 Characteristics of Chicken Proventricular Necrosis Virus (CPNV)

- The structure of the genome The genome of CPNV is made up of two linear double-stranded RNA segments, which are as follows:
  - Segment A has about 3.6 kb. structural proteins such as VP2, VP3, and VP4 are encoded

by this gene.

- Segment B, which is about 2.9 kb in size, is responsible for encoding VP1, which is an RNA-dependent RNA polymerase (RdRp) that plays a crucial role in viral replication (Guy, West, & Fuller, 2011).

- Viruses' Proteins Viral

As is the case with all birnaviruses, CPNV encodes five main proteins, which are as follows:

VP1: Acts as the viral polymerase, essential for replication.

VP2: The capsid protein that governs the virus's antigenic characteristics and host-virus interactions.

VP3: A structural protein implicated in genome encapsidation.

VP4: A protease that facilitates the digestion of viral polyproteins.

VP5: A non-structural protein thought to contribute to pathogenesis (Pantin-Jackwood & Brown, 2003).

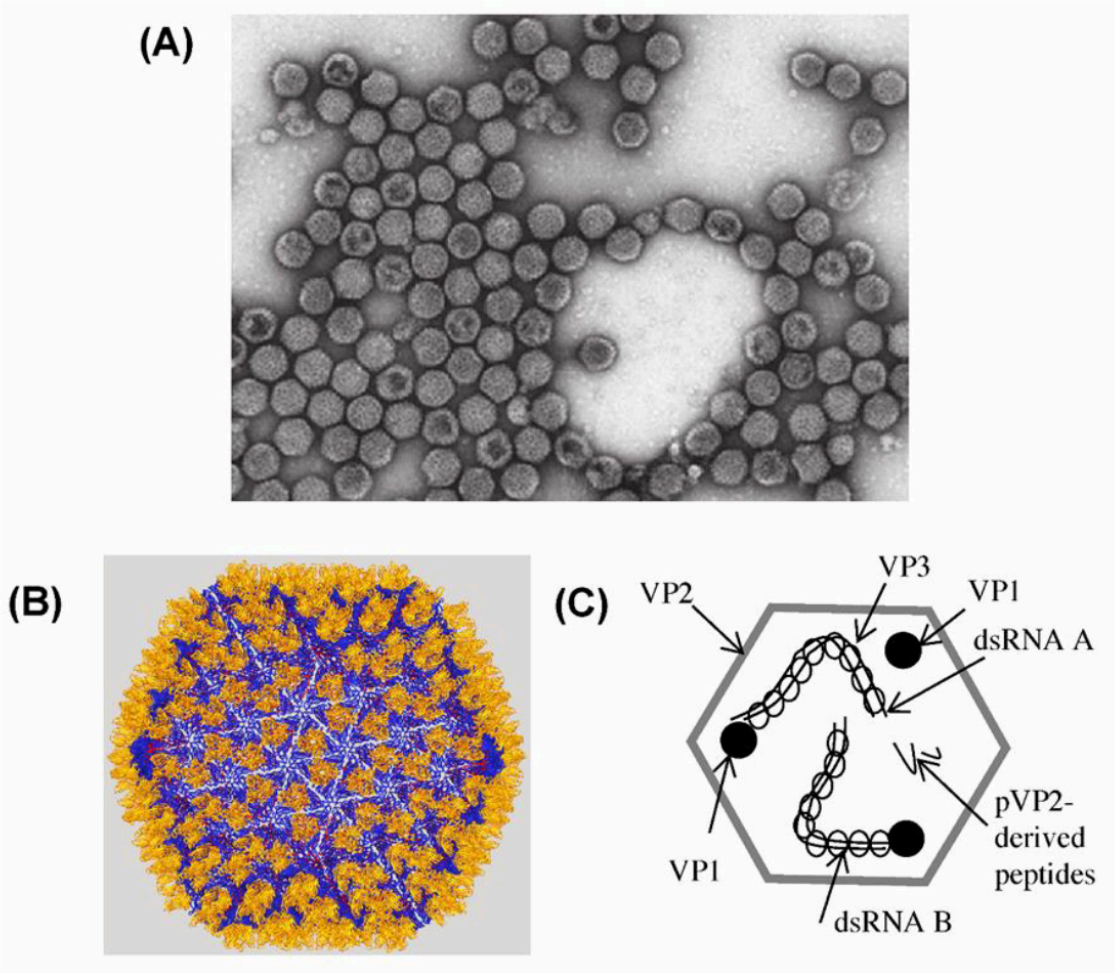


Figure 6: Structure of Birnavirus (infectious bursal disease virus) particle

*(A) Negative-contrast electron micrograph of particles; (B) a three-dimensional model of the virion; (C) diagrammatic representation showing the distribution of the polypeptides and the genome in the virus particle (Delmas et al., 2019).*

- Phylogenetic Divergence:

  Comparing CPNV to other birnaviruses like IBDV or IPNV, phylogenetic studies of VP1 show that there is only around 16% sequence agreement. This shows the significant distinction that exists between CPNV and other members of the Birnaviridae family, which suggests that it may be a member of a new genus (Guy, West, Fuller, et al., 2011).

- Morphology and Structure:

  A non-enveloped icosahedral virus with a diameter of around 75 nm is known as CPNV. Under electron microscopy, its shape resembles other birnaviruses; nonetheless, it has distinct genomic and antigenic characteristics (Goodwin et al., 1996; Guy, West, & Fuller, 2011).

- Serological Cross-Reactivity:

  CPNV demonstrates serological cross-reactivity with Infectious Bursal Disease Virus (IBDV) in some diagnostic procedures. Molecular investigations, however, affirm that CPNV is a unique virus, different from IBDV (Grau-Roma et al., 2017; Wali, 2021).

## 4.3 Historical Context and Viral Identification

As of its first 1978 description by Kouwenhoven et al., the precise etiology of TVP was unknown, but it was thought to involve a viral agent. Adenovirus and reovirus were among the viruses that were implicated in the illness in early studies, but their functions were unclear (Kouwenhoven et al., 1978).

When it came to the nuclei and cytoplasm of proventricular cells in hens infected by TVP, Goodwin et al. were the first to report hexagonal viral particles in 1996.

Electron microscopy revealed these particles, which were 65-70 nm in diameter, and they were highly indicative of a viral cause. Attempts to isolate the virus were unsuccessful (Goodwin et al., 1996). A major step forward was made in 2007 with the isolation and characterization of CPNV by Guy et al., which confirmed its role as the principal agent responsible for TVP.

In order to reliably identify CPNV in clinical cases, researchers used genomic analysis to validate the virus's birnavirus origin and developed RT-PCR tests targeting the VP1 gene (Guy, West, Fuller, et al., 2011).

## 4.4 Experimental Studies and Pathogenesis

### Experimental Infections

Proventricular enlargement, glandular necrosis, and ductal epithelial hyperplasia are some of the clinical and histological features of TVP, which may be reproduced in experimental inoculation with CPNV. Microscopically small lesions may be seen as early as 5 days after infection (Grau-Roma et al., 2017; Guy, West, & Fuller, 2011), however larger lesions don't show up until 7–14 days after infection (dpi).

### Replication and Persistence

Destroying cells that secrete pepsinogen and hydrochloric acid, CPNV mostly multiplies in the proventricular epithelium and hinders digestion (Goodwin et al., 1996) . As soon as acute damages recover, the virus is no longer identifiable in the proventriculus, but it may stay there for up to 14 days (Leão et al., 2021).

### Potential Co-Infections

Research has shown that some viruses including adenoviruses or immunosuppressive agents, like IBDV, might enhance the severity of the condition when combined with CPNV (Wali, 2021).

# 5 Clinical Signs and Gross Lesions

## 5.1 Clinical Signs

- When compared to healthy birds, the TVP results in a reduction of up to thirty percent in the amount of body weight gained. Variable flock size and elevated feed conversion ratio (Guy, West, Fuller, et al., 2011).
- Decreased homogeneity, ruffled feathers, and poor development (Dormitorio et al., 2007).

- Runting Stunting Syndrome (RSS): Characterized by stunted development, diarrhea, and elevated death rates (Noiva et al., 2015).

## 5.2 Gross and Microscopic Lesions

- **Macroscopic Lesions**

  The predominant gross lesion in TVP is proventricular enlargement and wall thickening, often associated with pallor and sporadic hemorrhages in the mucosa (Grau-Roma et al., 2017).

- Wall thickening, wietch of the mucosa, and enlargement on the Proventriculus.

- Flattened mucosal papillae and glandular dilatation were seen in the study conducted by Grau-Roma et al. (2017).

- Worst case scenario: the stomach isthmus is weak and bursts during processing, which causes carcass contamination and financial losses) (Dormitorio et al., 2007).

- **The triad Microscopic Lesions of TVP includes:**

  – Necrosis of glandular epithelium: Affects up to 80% of cells in severe cases;

  – Lymphocytic infiltration: Multifocal to diffuse infiltration in the lamina propria and proventricular glands (Noiva et al., 2015).

  – Ductal hyperplasia and metaplasia: Glandular cells replaced by hyperplastic ductal cells (Dormitorio et al., 2007).

  – Inclusion Bodies: Occasionally observed in oxynticopeptic cells, though not consistently reported in all studies (Goodwin et al., 1996; Grau-Roma et al., 2017).

  Other lesions:

  – Glandular ectasia, fibrosis, and reactive lymphoid follicles (Leão et al., 2021).

# 6 Pathogenesis and Experimental Findings

## 6.1 Pathogenesis

Chicken proventricular necrosis virus (CPNV), a birnavirus, is the principal culprit responsible for the development of transmissible viral proventriculitis (TVP). In TVP, the proventriculus shows signs of ductal epithelial hyperplasia, lymphocytic infiltration, and glandular epithelial necrosis. A crucial organ for secreting digestive enzymes including pepsinogen and hydrochloric acid, the proventriculus, becomes less effective due to these pathological alterations. Broiler chicks' development is stunted, feed conversion is poor, and flock uniformity is affected when CPNV destroys these cells (Goodwin et al., 1996; Guy, West, & Fuller, 2011).

## 6.2 Stages of Infection

- **Initial Infection and Viral Proliferation:**
  - The oxynticopeptic (glandular) cells of the proventriculus are the main targets of CPNV. Viral replication starts rapidly upon infection, according to experimental investigations, mainly in the proventricular glands' epithelial lining (Guy, West, & Fuller, 2011).
  - inflammation in the glandular interstitium, ductal epithelium degradation, and necrosis of the glandular epithelium are all visible within three to five days after inoculation (dpi) (Goodwin et al., 1996; Grau-Roma et al., 2017).
- **Acute Lesions (5–14 days post-inoculation):**
  - During the acute phase, the proventriculus undergoes glandular necrosis, which indicates the death of cells that secrete pepsinogen and hydrochloric acid (Guy, West, & Fuller, 2011; Wali, 2021).
  - The condition known as ductal epithelial hyperplasia occurs when the cells lining the ducts multiply and eventually replace the cells lining the glands. A kind of infiltra-

tion known as lymphocyte infiltration occurs when lymphocytes primarily invade the afflicted glands and form nodular aggregates.

  - Dispersed in a zonal fashion, these lesions impact deeper glandular tissues as well as the mucosa (Noiva et al., 2015).

- **Persistent Lesions and Resolution:**

  - Chronic inflammatory alterations show up by 14 dpi. Among them are: Particularly in the glandular stroma, there is a severe infiltration of lymphocytes.
  - After the glandular epithelium dies, ductal-like epithelium grows in its place (Grau-Roma et al., 2017).
  - CPNV is often no longer detected in tissues at this point, indicating that the virus has been cleared, but the pathological alterations continue and hinder the proventricular function (Guy, West, & Fuller, 2011; Leão et al., 2021).

## 6.3 Pathophysiological Mechanisms

1. **Targeting and Destruction of Cells:** The oxynticopeptic cells of the proventriculus are the principal locations for CPNV replication. Digestive enzymes and acids are produced by these cells, and their involvement is crucial in breaking down food (Goodwin et al., 1996). As shown in early electron microscope investigations (Goodwin et al., 1996), cytopathic consequences, including cell lysis, vacuolization, and the creation of intranuclear inclusion bodies, are caused by viral replication.

2. **Inflammatory Response:** The lymphocytic infiltration that occurs in the glandular interstitium is the primary component of the immunological response to CPNV infection. The majority of these infiltrates are CD3+ T cells, with an excessive number of CD8+ cytotoxic T cells, according to clinical trials. Chronic lesions also exhibit plasma cells and macrophages, indicating a sustained inflammatory response (Pantin-Jackwood et al., 2004).

3. **Digestive Dysfunction:** The loss of glandular epithelium significantly reduces the output of pepsinogen and hydrochloric acid. This makes it harder for the animals to digest protein and absorb all the nutrients they need, which can cause stunting, poor feed conversion, and differences in the flock (Guy, West, & Fuller, 2011; Wali, 2021).

4. **Hyperplasia and Metaplasia of the Ducts:** One characteristic of TVP is the metaplastic substitution of glandular epithelium with hyperplastic ductal epithelium. Persistent inflammation and fibrosis, associated with this, further impair proventricular function (Grau-Roma et al., 2017).

## 6.4 Experimental Reproduction of TVP

1. **Oral Inoculation:** The infection has been effectively replicated in birds by experimental inoculation with homogenized proventriculi from hens infected by TVP. The lesions that are typical of TVP were seen as early as 5 days post infection (dpi), and by 14 days post infection (dpi), there was extensive glandular necrosis and ductal metaplasia (Grau-Roma et al., 2017; Guy, West, & Fuller, 2011).

2. **Co-Infections and Synergistic Effects:** Immunosuppressive viruses, such as Infectious Bursal Disease Virus (IBDV), may make TVP worse, according to some research. Studies have shown that when CPNV and IBDV are both present, the amount of glandular damage and lymphocytic infiltration is higher (Wali, 2021). However, it is not possible to rule out the possibility that additional pathogens, such as adenoviruses and reoviruses, may be involved in the exacerbation of TVP lesions (Noiva et al., 2015). CPNV is regarded to be the principal etiological agent.

## 6.5 Multifactorial Pathogenesis

Although CPNV is strongly implicated, other factors may contribute to TVP:

- Co-infections with IBDV, FAdV, or other viruses.
- Immunosuppressive conditions facilitating secondary infections (Noiva et al., 2015).

# 7 Diagnosis

## 7.1 Clinical Diagnosis

When it comes to clinical applications, TVP is suspected in poultry flocks that have poor development, flock non-uniformity, reduced feed conversion, and in certain instances, birds that are stunted and have feathers that are ruffled. The occurrence of TVP in broiler chickens is commonly seen between the ages of three and eight weeks. However, it has also been shown in older birds, such as broiler breeders and commercial layers (Leão et al., 2021; Noiva et al., 2015).

According to Grau-Roma et al. (2017), birds may exhibit symptoms of poor digestion, including a pale comb, lethargy, and the presence of undigested meal in their feces. An rise in culling rates owing to poor performance and unequal weight distribution is typical (Dormitorio et al., 2007; Noiva et al., 2015). This is the case even when death rates are not considerably raised in flocks that have been afflicted by the disease. The ambiguous character of these signals requires validation by necropsy and laboratory examination.

## 7.2 Necropsy and Gross Lesions

Necropsy is an essential step in the process of diagnosing TVP because it enables the detection of macroscopic lesions that are typical of the proventriculus. Here are some important findings:

1. **Enlargement of the Proventriculus**: The proventriculus is obviously enlarged and may have a pallid wall that has thickened at the same time.
2. **Fragility of the Gastric Isthmus**: This often results in ruptures during the process of evisceration, which in turn leads to contamination of the corpse (Dormitorio et al., 2007).

3. **Occasional hemorrhagic alterations**: Hemorrhagic alterations in the mucosa are noticed, coupled with lobular patterns of discoloration on the serosal surface (Grau-Roma et al., 2017; Leão et al., 2021). Spotty discoloration accompanied with hemorrhages are also noted.

This may be an indication of decreased digestion related with proventricular dysfunction (Noiva et al., 2015). Another symptom is the presence of undigested feed in the crop and the gizzard. Nevertheless, macroscopic lesions are not always enough for diagnosis, since same features may manifest in non-infectious proventriculitis or other infected conditions.

## 7.3 Histopathological Examination

TVP may still be diagnosed via histopathology since the microscopic lesions are quite unique to the condition (Goodwin et al., 1996; Guy, West, Fuller, et al., 2011). The following three findings are included in the distinctive trinity of histological findings:

- **Oxynticopeptic Cell Necrosis**: Hypereosinophilia, nuclear fragmentation, and cell sloughing into the glandular lumen are signs of necrotic glandular epithelium of the proventriculus (Goodwin et al., 1996).
- **Lymphocytic Infiltration**: The glandular interstitium is characterized by the presence of lymphocytic infiltrates that range from multifocal to severe. The majority of these lymphocytes are CD3+ T cells, and there is a significant amount of CD8+ cytotoxic T cells involved (Pantin-Jackwood et al., 2004). These lymphocytes often cluster in nodular patterns.
- **Ductal Epithelial Hyperplasia and Metaplasia**: The glandular epithelium is replaced by hyperplastic ductal epithelium, which is a characteristic of chronic TVP (Grau-Roma et al., 2017).

Additionally, glandular ectasia and fibrosis may be seen in chronic instances, however, these are secondary alterations that may be observed. Microscopic analysis yields a conclusive diagnosis when coupled with molecular identification of CPNV (Leão et al., 2021).

## 7.4 Molecular Diagnosis

Real-time polymerase chain reaction (RT-PCR) is very sensitive and specific because it targets the VP1 gene, which is essential for CPNV identification, It is possible to detect viral antigens in proventricular tissues using immunohistochemistry (IHC), especially in the early stages of infection, While electron microscopy may show viral particles in infected tissues, it is not a very useful diagnostic tool (Grau-Roma et al., 2017; Guy, West, Fuller, et al., 2011).

RT-PCR is the most dependable molecular method for detecting CPNV. On segment B of the virus's bisegmented RNA genome, it primarily targets the VP1 gene. In both experimental and naturally occurring TVP instances, RT-PCR has been routinely used to confirm CPNV (Grau-Roma et al., 2017; Guy, West, & Fuller, 2011; Leão et al., 2021).

Although real-time polymerase chain reaction (RT-PCR) detection of CPNV is extremely specific, it may provide false negative results in chronic cases due to the fact that viral clearance often takes place before lesions improve. It is also possible to use RT-PCR to analyze formalin-fixed paraffin-embedded (FFPE) tissues, however, the sensitivity of this technique is somewhat diminished when used to such samples (Lewis et al., 2001).

When examining fresh proventricular tissue, RT-PCR has been shown to have a sensitivity of 88% and a specificity of 83% (Guy, West, & Fuller, 2011). In the process of evaluating FFPE proventriculi, these values are significantly increased.

Samples that have been shown to be positive by RT-PCR might be subjected to phylogenetic analysis in order to compare the nucleotide sequences of the VP1 gene with those of known CPNV isolates. CPNV has been shown to have unique genetic lineages in both Europe and the Americas, according to research conducted by Grau-Roma et al. (2017). This finding suggests that the virus may exhibit regional heterogeneity across different regions.

## 7.5 Serological Diagnosis

**ELISA (Enzyme-Linked Immunosorbent Assay)**

For the purpose of detecting seroconversion to viral agents that may be related with TVP, such as infectious bursal disease virus (IBDV), infectious bronchitis virus (IBV), and fowl adenoviruses (FAdV), as stated in the article Wali (2021) ELISA tests are used. According to Noiva et al. (2015) there is evidence that CPNV is the major etiological agent since there is a large rise in the amount of anti-CPNV antibodies in birds that have been infected with the virus.

Since CPNV and other birnaviruses, especially IBDV, may cause a broad variety of serological cross-reactivity, serology alone is not enough to provide a diagnosis (Wali, 2021).

## 7.6 Differential Diagnosis

The need of a differential diagnosis cannot be overstated since the symptoms of TVP might be similar to those of other proventricular diseases. Conditions to consider include:

- Dietary variables such as mycotoxins, biogenic amines, or excessive copper sulfate levels may be the cause of non-infectious proventriculitis (Bayyari, Huff, Balog, et al., 1995; Dormitorio et al., 2007).
- Infections caused by bacteria and fungi: Proventricular lesions may be brought on by some types of bacteria, such as *Clostridium* spp., as well as fungal and yeast infections (Goodwin et al., 1996).
- Other Viral Infections: Diseases caused by adenoviruses, reoviruses, infectious bronchitis virus (IBV), or infectious bursal disease virus (IBDV) may also create comparable lesions (Guy, West, & Fuller, 2011).

In order to separate TVP from these situations, it is necessary to perform molecular detection of CPNV in addition to histological documentation.

## 7.7 Limitations of Diagnostic Methods

- **False Negatives in Chronic Patients**: Chronic TVP patients often provide negative RT-PCR findings because of the clearance of CPNV, despite the fact that lesions continue to be present (Guy, West, & Fuller, 2011).
- **Subclinical Infections**: The presence of CPNV, RNA in proventriculi that are histologically normal raises doubts about the function of the virus in cases that are asymptomatic or subclinical, as shown by Leão et al. (2021).
- **Co-Infections**: The interpretation of diagnostic results is made more difficult by the presence of co-infecting pathogens, such as viruses that weaken the immune system (Wali, 2021).

# 8 Treatment and Prevention

## 8.1 Treatment

There is currently no particular antiviral medication available for the condition known as Transmissible Viral Proventriculitis (TVP), which is brought on by the Chicken Proventricular Necrosis Virus (CPNV). The disease's recent appearance, complexity, and multifaceted character, as well as the lack of a thorough knowledge of its pathophysiology, all contribute to the lack of focused therapies. It is common practice to manage infected flocks by providing supportive care and taking steps with the goal of reducing production losses.

**Supportive Measures**

When providing supportive care, the primary goals are to improve nutrition, manage stress, and reduce the number of secondary illnesses. Although the use of feed that is contaminated with mycotoxins or biogenic amines might make the condition worse (Barnes et al., 2001; Dormitorio et al., 2007) it is essential to use feed that is of excellent quality and does not include any contaminants.

Similar to this, ensuring that there is clean water and treating concomitant infections like coccidiosis will help lessen the clinical indications that are present.

**No Effective Antiviral Therapy**

A reduction in immunosuppression in birds may help attenuate the effects of TVP, according to research, despite the fact that antiviral medication is not yet accessible. There have been other instances in which the immunosuppressive effects of concurrent infections, such as the Infectious Bursal Disease Virus (IBDV), have been ((Grau-Roma et al., 2017; Guy et al., 2007) It is possible to indirectly improve the prognosis of flocks that have been impacted by TVP by responding to these co-infections via vaccination and biosecurity measures.

## 8.2 Prevention

Due to the fact that there are presently no commercial vaccinations or particular prophylactic measures available against CPNV, the prevention of TVP is largely dependent on biosecurity measures and hygiene habits.

**Biosecurity Protocols**

In poultry farms, the implementation of stringent biosecurity policies is very necessary in order to stop the introduction and spread of CPNV. These comprise:

- **Hygiene on the farm**: It is vital to perform routine cleaning and disinfection of chicken housing facilities in order to reduce the amount of viral persistence in the environment.
- **Controlling Access:**: Strict control over farm access for trucks, staff, and equipment may help limit the danger of the virus being introduced into the farm.
- **Isolation and Quarantine**: In order to limit the epidemic, those who are infected or suspected of being infected should be separated from healthy flocks.

- **Control of Rodents and Wild Birds**: It is suggested that you avoid coming into touch with wild birds and rodents, since these animals have the potential to function as carriers of infections (Velkers et al., 2017).

**Feed Quality and Storage**

The severity of TVP lesions may be made worse by contaminated feed, which is why feed quality and storage are important considerations. It is essential to make certain that the feed is of a good quality and does not include any biogenic amines, mycotoxins, or other toxins. In addition it is essential to store feed in an appropriate manner in order to avoid the formation of mold (Barnes et al., 2001; Dorner et al., 1983).

**Vaccination Against Concomitant Illnesses**

According to (Leão et al., 2021) vaccination against immunosuppressive diseases like as Infectious Bursal Disease virus (IBDV) and Marek's Disease might assist in the preservation of the flock's general immunity, hence minimizing the likelihood of transmission of tetravalent vesicle-borne pathogens (TVP). In a study that was conducted by Guy et al. (2007), it was discovered that immunosuppression brought on by IBDV may have a role in the development of TVP.

**Environmental Controls**

It is possible that environmental stresses, such as overcrowding, inadequate ventilation, and temperatures that are below ideal levels, might increase clinical indications that are associated with TVP. According to Hafner et al. (2008) in order to avoid the emergence of illness, it is vital to have proper flock management and housing conditions.

# 9 Prognosis

There are a number of factors that influence the prognosis of TVP, including the severity of the lesions, the age of the birds, and the management measures that are used by the farm.

## 9.1 Impact on Production Performance

TVP significantly impacts productivity, primarily due to decreased feed conversion efficiency, poor weight gain, and non-uniformity in flock size (Dormitorio et al., 2007; Grau-Roma et al., 2017). Birds with severe glandular necrosis and inflammation may show growth retardation of up to 30% lower body weight gain compared to unaffected flocks (Guy et al., 2007).

## 9.2 Mortality Rates

Contrary to the majority of other viral infections, viral hepatitis (TVP) does not often result in an increase in fatality rates. On the other hand, birds may need to be culled because of severe stunting and poor physical condition, which might indirectly lead to an increase in economic losses (Noiva et al., 2015). The prognosis of TVP varies depending on the severity of the lesions, the age of the birds, and the management practices adopted by the farm.

## 9.3 Long-Term Flock Effects

Chronic occurrences of TVP might result in flocks that are permanently stunted and have a worse overall performance. During the process of evisceration in processing factories, proventricular ruptures may lead to the condemnation of the carcass due to contamination, which further contributes to the financial losses that are incurred (Leão et al., 2021).

### 9.4 Disease Recovery

even if CPNV is no longer detectable in RT-PCR tests, lesions in the proventriculus tend to continue for many weeks. This is the case even after the disease has recovered. There is a possibility that birds will recover from the acute stage of the illness; nevertheless, research conducted by Guy, West, and Fuller (2011) found that birds often do not reach their full development potential.

### 9.5 Economic Prognosis

Economic losses associated with TVP include increased feed conversion ratios, uneven flock weights, and condemnation of carcasses. The disease has been reported to affect up to 50% of a flock, with cumulative mortality rates averaging 5.4% in some cases (Leão et al., 2021).

## 10 Research Gaps

A number of elements of the illness are still not well understood, despite the fact that great progress has been made in determining that CPNV is the principal etiological agent of TVP.

- One of the most important topics for study in the future is the creation of vaccinations that are successful in preventing CPNV infection.
- Investigating the possibility of the presence of co-factors, such as environmental stresses or other infectious agents, that may play a role in the development of TVP.
- Improving diagnostic methods in order to increase the quality of early diagnosis of CPNV in situations when symptoms are absent.
- Antiviral treatments and immune modulators are being investigated as potential means of mitigating the severity of the condition.

# Practical Part

# CHAPTER I

# Materials and Methods

## 1 Ethical statement

No ethical committee approval was necessary because no birds were sacrificed for this study. The samples were collected from birds that died naturally or from diseases within the flocks in the study area.

## 2 Study design and sample collection

### 2.1 Study Area, Duration, and Flock Characteristics

Necropsy procedures followed national and international standards according to the guidelines established by the European Union Council (2010/63/EU). This study was conducted from 2020 to 2022 in various regions of northern Algeria, specifically within the geographic coordinates of approximately 34.8 °N to 36.8 °N latitude and 4.2 °E to 7.8 °E longitude, covering the provinces of Bejaia, Setif, Bordj Bou Arréridj, M'sila, Batna, Constantine, and Guelma. (Figure 7) The samples were from 62 broiler farms with capacities ranging from 5 000 to 20 000 broilers, four laying hen flocks each containing 60 000 individuals, and three broiler breeders each containing 10 000 individuals. The feed composition, either in mash or crumbled form, varied among the farms, and bedding predominantly used straw. All broiler flocks including strains such as Cobb 500, Hubbard, and Arbor acres, (table 2) underwent vaccination against bronchitis, Newcastle disease, and Gumboro disease. Additional vaccinations against Marek's disease, egg drop syndrome, encephalomyelitis, avian influenza, infectious laryngotracheitis, and fowl pox were administered

for laying hens and broiler breeders.

## 2.2 Veterinary Collaboration, Disease Detection, and Sampling Procedures

In field practice, we collaborated with local veterinarians who were part of an epidemiological surveillance network. Their role was to identify instances of the disease throughout the entire rearing cycle, primarily through observing proventricular enlargement in dead birds. In farms where the disease was suspected, proventriculus showing enlargement was har-vested post-necropsy from specimens that had passed away on that day.

In this detailed prospective study, we systematically collected proventriculus specimens from diverse flocks, each batch typically included 3 to 5 proventriculi, leading to a total of 55 proventriculi across 13 farm samples. These samples were rigorously cataloged based on their flock origin, geographical setting, and rearing practices, then preserved in a 10 % neutral buffered formaldehyde solution and stored under sterile conditions. Following preservation, the samples underwent a comprehensive preparation process: rinsing in running tap water, dehydration through a graded ethyl alcohol and xylene series, embedding in paraffin, sectioning at 5−6 μm for optimal histological evaluation, and staining with hematoxylin and eosin. This preparatory work culminated in a detailed examination under a light microscope to meticulously assess cellular alterations, ensuring a thorough analysis of the proventriculus tissues' pathological features. After the histopathological diagnosis of the disease, clinical signs, gross lesions in the necropsied carcasses, as well as primary epidemiological features, were documented. We computed various metrics, including the disease's prevalence, flock homogeneity rates, age of disease onset, progression of the disease, mortality rates, and the average live weight at the slaughter age of broilers (typically between the 50th−60th d). Moreover, prominent health disturbances that emerged during the rearing processes across different production types were meticulously recorded.

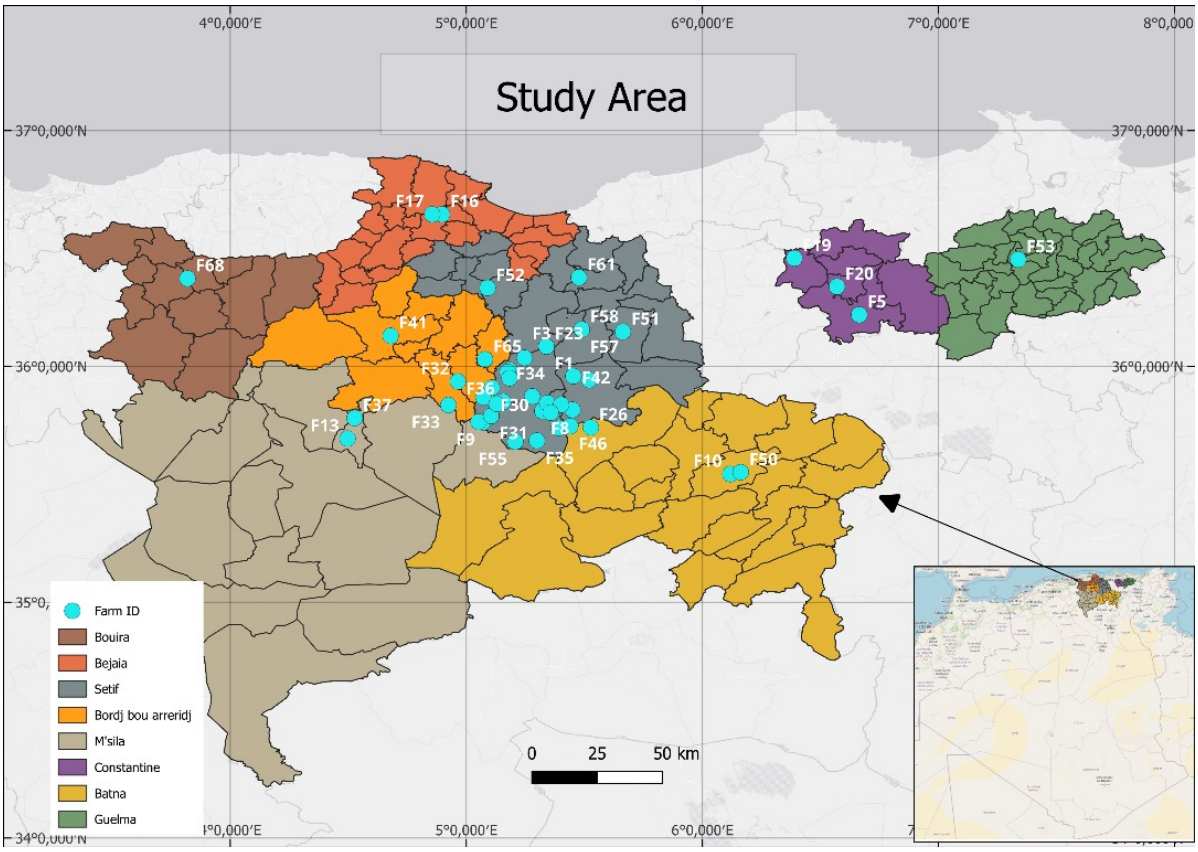


Figure 7: Regional Study of TVP

# 3 Histopathological investigation

To date, histopathological examination has been regarded as the most reliable means of diagnosing TVP. The histopathological lesions of the proventriculus are characterized by a triad of lesions related to the necrosis of glandular epithelial cells, lymphocytic infiltration, and hypertrophy of the excretory duct epithelial cells accompanied by a replacement of the glandular epithelium. Based on the histological findings, chickens were categorized into three case statuses: TVP characterized by the presence of lymphocytic infiltration coupled with necrosis, LP marked by lymphocytic infiltration but without necrosis and, WP where neither lymphocytic infiltration nor necrosis is observed in the proventriculus indicating the absence of proventriculitis (Śmiałek et al., 2020). Inflammatory infiltration within the lamina propria was disregarded, as it is recognized to be a common occurrence in healthy birds and therefore not a reliable indicator of disease (Kadhim et al., 2011). This

exclusion was necessary to avoid misclassification or false-positive results that could arise from interpreting normal physiological findings as pathological changes. A scoring system similar to that presented by Grau-Roma et al. (2017) was employed for each of the aforementioned parameters: negative (none of the glands affected), mild + (> 0 to 10 % of the glands affected), moderate ++ (> 10 to 50 % of the glands affected), and severe +++ (> 50 % of the glands affected).

# 4 Statistical analysis

To garner an in-depth understanding of proventriculus enlargement patterns in broilers and the nuanced interplay of age with this process, we deployed a comprehensive suite of statistical methodologies facilitated by IBM SPSS version 26.0. Data from a total of 55 samples was meticulously analyzed, each undergoing stringent measurements to determine the proventricular wall's thickness. These samples were subsequently classified based on the presence and typology of the lesions, with a keen focus on the three pivotal ones: lymphoid Infiltration, hyperplasia, and necrosis. Through the application of both ANOVA and Post Hoc tests, we aimed to unravel the statistical significance and complex interrelationships between age, the identified lesions, and the resultant proventricular wall thickening. This study used IBM SPSS version 26.0 for statistical analyses, and the histological evaluations had values ranging from - to +++ which were converted to 0, 1, 2, and 3, respectively. The dependent variable was found to be normally distributed according to the Shapiro-Wilk test (P=0.132), hence parametric tests of ANOVA and Student t were deemed appropriate. A significant level of $P < 0.05$ was considered for this analysis.

# CHAPTER II

## Resultats et Discussions

# 1 Results

## 1.1 Histopathological findings

Out of the 62 broiler flocks visited, proventriculus enlargement was identified in 13 flocks, equating to a prevalence of 20.9 %. A total of 55 thickened proventriculus samples, taken from 3 to 5 dead birds per affected flock, representing 13 samples, were subjected to histopathological examination. Microscopic observations revealed lymphocyte and macrophage infiltration, necrosis of the glandular epithelium, as well as hyperplasia of the glandular epithelium (Figure 8). The lymphocytic infiltration was predominantly multifocal and situated within the interstitium of the proventriculus glands. At times, the lymphocytic cells would cluster to form nodular aggregates.

In certain instances, inflammation was so pronounced that it extended into the muscular layers. Necrotic cells were typically swollen, comprising an accumulation of necrotic debris and shed cells. The glandular epithelium was often replaced by hyperplastic ductal epithelium, termed ductal epithelial metaplasia (Figure 9). Other lesions identified included hemorrhages, eosinophilic material, cysts, squamous metaplasia, epithelial ulcerations, dilated crypts in the mucosa, and loss of the muscular layer. Histological changes recorded in the enlarged proventriculus, based on the presence of lymphocytic infiltration and/or glandular epithelial necrosis and replacement of glandular epithelium by hyperplastic ductal epithelium, were characteristic and aligned with TVP, LP, and WP diagnoses. A comprehensive review of the histopathological analysis for each chicken (Table 4) revealed that out of the 55 proventriculus samples, 13 were affected by TVP, 29 showed signs

of LP without necrosis, and 13 demonstrated WP attributes without lymphocytic infiltration and necrosis. Among the 13 TVP-affected cases, severe lymphocytic infiltration was present in 6 (46 %), while the remainder displayed moderate lesions. The median score for lymphocytic infiltration in the TVP-affected cases was significantly higher than in the LP-affected group, where only 5 out of 29 proventriculus samples manifested severe lymphocytic infiltration. Necrosis was graded mild (+) in 10 TVP-affected cases (78 %), while the other 3 cases (22 %) displayed a moderate (++) score. Six out of the 13 proventriculus samples diagnosed with TVP showcased significant, widespread occurrences of severe hyperplasia and metaplasia of ductal epithelial cells. Contrarily, this histopathological feature was absent in the LP-affected and WP groups, which presented mild to moderate scores. Regarding laying hens and broiler breeders, the mortality rate during the rearing cycle did not surpass 7 % and 10 %, respectively. Furthermore, no proventriculus lesions were detected in deceased specimens, highlighting the relative absence of TVP in these two types of rearing.

The statistical analysis provided a more detailed quantification of these observations. Most of the samples (61.8 %) showed proventricular enlargement, with an average enlargement score of 4.7294. Among these, 12.7 % had concomitant hemorrhagic/nodules, with a higher mean enlargement score of 5.7000. In comparison, 25.5 % of samples with whitish characteristics had an average score of 5.1571, lymphoid infiltration, an essential marker of TVP, varied among the samples. About 30.9 % had an infiltration score of $\leq 1$, 49.1 % had a score of 2, and 20 % had the highest score of 3. The mean scores associated with these gradations were 4.0294, 5.1444, and 5.9545, respectively, indicating a significant increase in lymphocytic infiltration with higher scores. Notably, 76.4 % of the samples showed no necrosis.

The remaining 23.6 % that did present necrosis had a mean score of 5.9538, signaling the presence and severity of necrotic changes. Hyperplasia was observed in a broad spectrum of cases with 47.3 % having a score of $\leq 1$, 41.8 % a score of 2, and a smaller subset of 10.9 % showing the most pronounced hyperplasia with a score of 3. Considering the case states, LP was the predominant condition, affecting over half of the samples. The average scores for LP, TVP, and WP were 5.0517,

5.9538, and 3.7692, respectively, illustrating the differential severity of the conditions. (Tables 4 and 5).

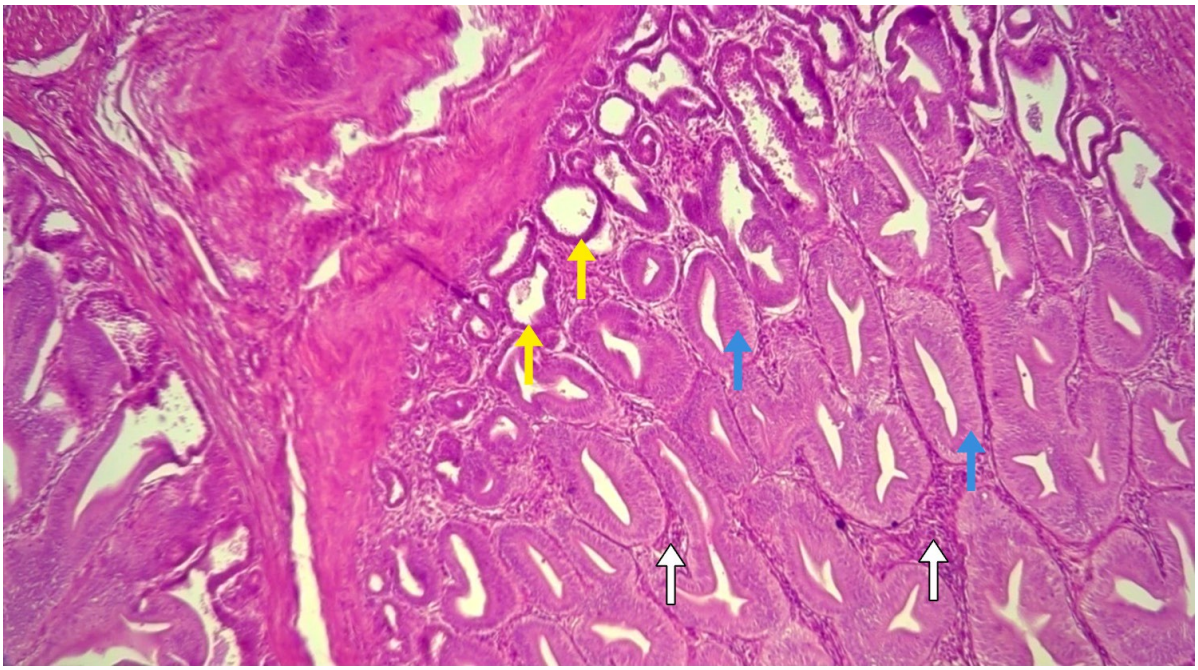

Figure 8: Histological indicators of tissue remodeling in glandular epithelium

*Degeneration and necrosis of glandular epithelium; epithelial replacement by ductal hyperplasia; and lymphoid cell infiltration (H&E, ×100; yellow, blue, and white arrows respectively).*

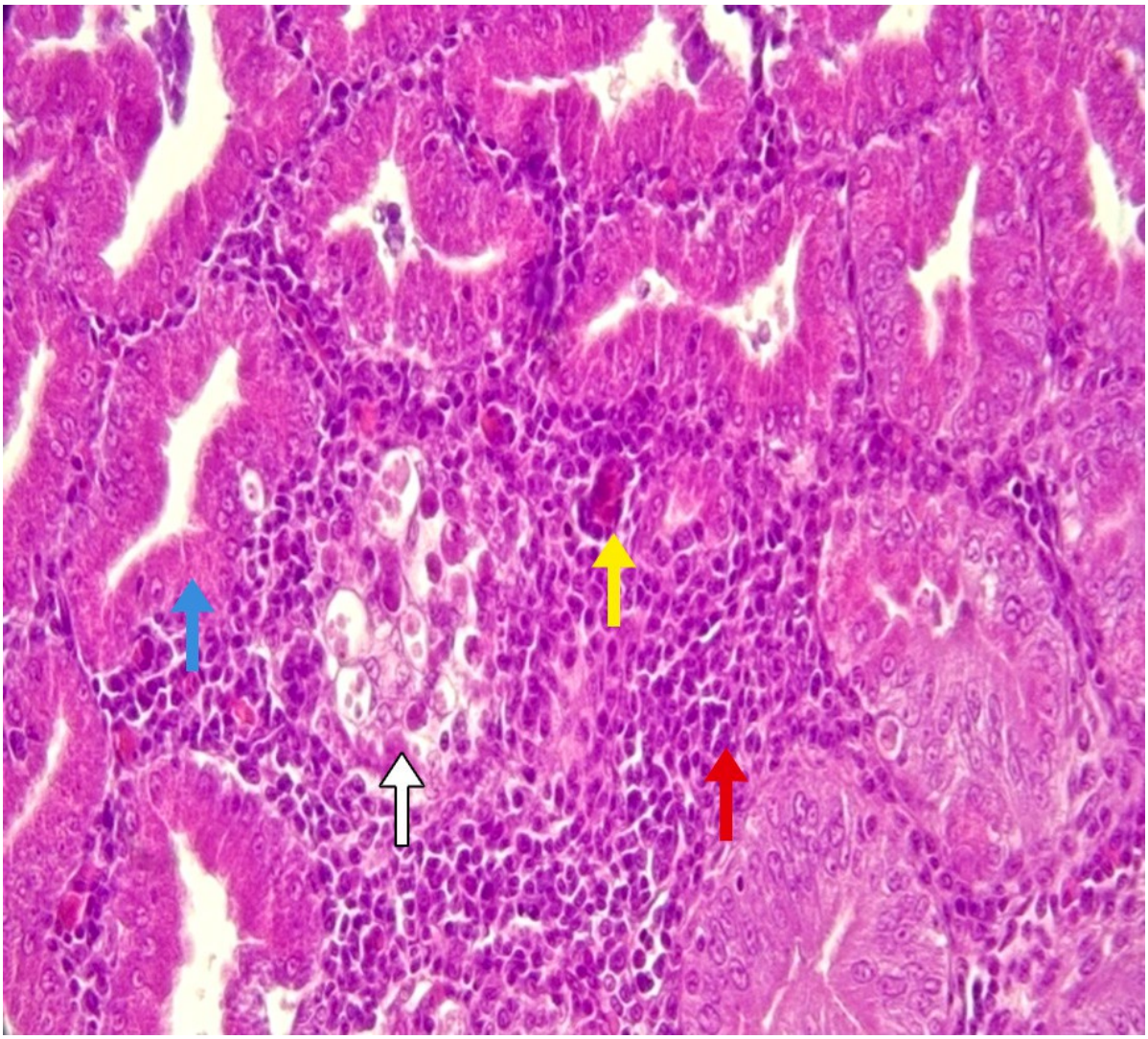

Figure 9: Histopathological indicators of epithelial transformation and tissue injury

*Replacement of glandular epithelium by hyperplastic ductal structures; necrotic cell clusters; hemorrhagic lesions; and dense lymphocytic infiltration (H&E, ×400; blue, white, yellow, and red arrows respectively).*

Table 2: Case status of proventriculus samples according to histopathological changes in broiler flocks

| Case number | Proventricular macro-lesions | Thickened wall (mm) | Proventricular micro-lesions | Case status | | |
|---|---|---|---|---|---|---|
| | | | | Interstitial lymphocytic infiltration | Mean necrosis score | Ductal epithelial hyperplasia and metaplasia |
| 1 | Enlargement, whitish | 4.6 | ++ | - | + | LP |
| | Enlargement, whitish | 4.4 | ++ | - | + | LP |
| | Enlargement, mottled pink | 5.4 | +++ | + | ++ | TVP |
| | Enlargement, reddened appearance | 5.6 | ++ | + | ++ | TVP |
| | Enlargement, mottled pink | 7.9 | +++ | + | +++ | TVP |
| 2 | Enlargement, reddened appearance | 5.9 | ++ | + | +++ | TVP |
| | Enlargement, whitish | 5.2 | ++ | - | ++ | LP |
| | Enlargement, whitish | 5.3 | +++ | - | ++ | LP |
| | Enlargement, mottled pink | 6.2 | +++ | + | +++ | TVP |
| | Enlargement, mottled pink | 5.6 | ++ | + | ++ | TVP |
| 3 | Enlargement, reddened appearance | 6.9 | +++ | + | +++ | TVP |
| | Enlargement, whitish | 6.7 | ++ | - | ++ | LP |
| | Enlargement, prominent papillae | 5.5 | ++ | ++ | ++ | TVP |
| | Enlargement | 5.5 | ++ | - | ++ | LP |
| 4 | Enlargement | 5.4 | + | - | ++ | LP |
| | Enlargement, whitish | 4.0 | - | - | + | WP |
| | Enlargement, whitish | 6.0 | +++ | - | ++ | LP |
| | Enlargement | 4.3 | ++ | - | + | LP |
| | Enlargement | 3.9 | - | - | ++ | WP |
| 5 | Enlargement | 4.3 | +++ | - | + | LP |
| | Enlargement | 3.7 | ++ | - | + | LP |
| | Enlargement | 3.1 | - | - | + | WP |
| | Enlargement | 4.0 | + | - | + | LP |
| | Enlargement | 5.9 | +++ | - | ++ | LP |
| 6 | Enlargement | 5.1 | ++ | - | + | LP |
| | Enlargement | 4.8 | ++ | - | + | LP |
| | Enlargement, mottled pink | 5.4 | +++ | + | +++ | TVP |
| | Enlargement | 3.9 | - | - | ++ | WP |
| 7 | Enlargement | 6.5 | ++ | - | ++ | LP |
| | Enlargement, whitish | 3.7 | ++ | - | + | LP |
| | Enlargement, mottled pink | 5.7 | +++ | + | ++ | TVP |
| | Enlargement | 6.5 | ++ | - | ++ | LP |
| 8 | Enlargement, prominent papillae | 5.9 | ++ | ++ | + | TVP |
| | Enlargement | 4.8 | ++ | - | + | LP |
| | Enlargement | 3.5 | ++ | - | + | LP |
| | Enlargement | 4.3 | + | - | + | LP |
| | Enlargement | 3.9 | - | - | + | WP |
| 9 | Enlargement | 5.8 | + | - | + | LP |
| | Enlargement, whitish | 3.4 | - | - | + | WP |
| | Enlargement, whitish | 4.2 | - | - | ++ | WP |
| | Enlargement, whitish | 4.5 | ++ | - | ++ | LP |
| 10 | Enlargement, whitish | 3.6 | - | - | + | WP |
| | Enlargement | 5.1 | ++ | - | + | LP |
| | Enlargement | 5.4 | ++ | - | ++ | LP |
| 11 | Enlargement | 3.3 | - | - | + | WP |
| | Enlargement | 4.1 | ++ | - | - | LP |
| | Enlargement, prominent papillae | 5.8 | ++ | ++ | ++ | TVP |

| Case number | Proventricular macro-lesions | Thickened wall (mm) | Proventricular micro-lesions | Case status | | |
|---|---|---|---|---|---|---|
| | | | | Interstitial lymphocytic infiltration | Mean necrosis score | Ductal epithelial hyperplasia and metaplasia |
| 12 | Enlargement | 5.8 | ++ | - | ++ | LP |
| | Enlargement | 4.2 | - | - | + | WP |
| | Enlargement | 3.9 | - | - | + | WP |
| 13 | Enlargement, mottled pink | 5.6 | ++ | ++ | +++ | TVP |
| | Enlargement | 3.5 | - | - | + | WP |
| | Enlargement | 4.1 | - | - | ++ | WP |
| | Enlargement | 5.1 | ++ | - | + | LP |
| | Enlargement | 6.2 | +++ | - | ++ | LP |

*N: flocks number; n: proventriculus number; TVP: transmissible viral proventricilitis; LP: lymphocytic proventricilitis; WP: without proventricilitis.*

Table 3: Mean Scores, Standard Deviations, and Number of Cases for Histopathological Features

| Histopathological feature | Mean score | Standard deviation | N (Number of cases) |
|---|---|---|---|
| *Proventricular macro-lesions* | | | |
| Enlargement only | 4.7294 | 0.91271 | 34 |
| Enlargement and hemorrhagic/nodules | 5.7000 | 0.58310 | 7 |
| Enlargement with whitish | 5.1571 | 1.40533 | 14 |
| *Lymphoid infiltration* | | | |
| Score $\leq 1$ | 4.0294 | 0.68352 | 17 |
| Score 2 | 5.1444 | 0.84368 | 27 |
| Score 3 | 5.9545 | 0.94272 | 11 |
| *Necrosis* | | | |
| Score 0 | 4.6548 | 0.97034 | 42 |
| Score $\geq 1$ | 5.9538 | 0.70901 | 13 |
| *Hyperplasia* | | | |
| Score $\leq 1$ | 4.2462 | 0.73606 | 26 |
| Score 2 | 5.4174 | 0.80772 | 23 |
| Score 3 | 6.3167 | 0.93684 | 6 |
| *Case states* | | | |
| LP | 5.0517 | 0.89308 | 29 |
| TVP | 5.9538 | 0.70901 | 13 |
| WP | 3.7692 | 0.35446 | 13 |
| **Total average** | 4.9618 | 1.06623 | 55 |

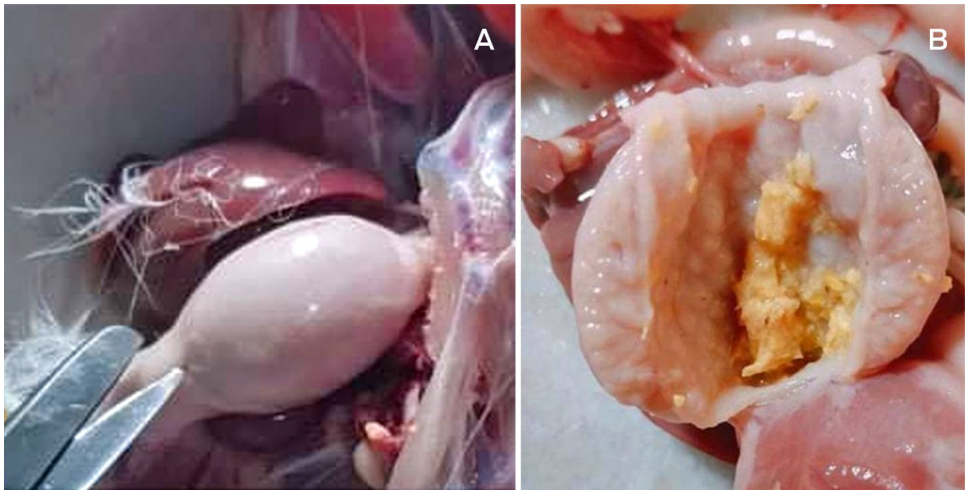


Figure 10: Macroscopic Alteration of the Proventriculus: Marked Enlargement and Wall Thickening

## 1.2 Symptoms and gross lesions

Throughout the progression of the disease, no distinct symptoms were evident, and the birds were often found dead suddenly. The primary lesion discerned during necropsy was the thickening of the proventriculus wall to various extents (Figure 10 A-B). In 13 TVP-positive cases, the most frequent lesions encompassed an enlarged proventriculus and thickened walls measuring more than 5 mm, with a median thickness of 5.9 mm (ranging from 5.4 mm to 7.9 mm). Additionally, 7 cases displayed a discolored proventriculus that appeared patchily pink, 3 cases featured notably prominent papillae, and 3 cases had a reddened appearance. The lesions possessed a mucoid exudate on the surface without any neoplastic presence.

In LP-affected cases, the proventriculus measurements ranged between 3.5 and 6.7 mm, averaging 4.7 mm, while in WP cases, the thickened wall ranged from 3.1 to 4.2 mm, with an average of 3.7 mm. Proventriculus walls from deceased birds aged 7, 21, and 45 d from farms free of TVP were sampled and measured, yielding average values of 2 mm, 3 mm, and 4 mm, respectively. As the disease advanced, sporadic signs of the runting-stunting syndrome (RSS) became apparent, including the characteristic helicopter sign, which refers to uncoordinated, whirling movements of the affected chicks that resemble the rotating blades of a helicopter. This behavior is indicative

of neurological involvement or severe developmental disturbances. Additional clinical manifestations included respiratory complications, nonspecific diarrhea, and neurological symptoms associated with hypovitaminosis B. The most common gross lesions encountered were respiratory issues, nonspecific enteritis, nephritis, hepatitis, and a reactionary bursa of Fabricius (pinkish).

## 1.3 Epidemiological features of TVP

No notable differences were detected in the distribution of the disease across regions or flocks within the studied broiler population. The disease presented itself in flocks aged between 15−40 d. Upon identification of the disease, the mortality rate fluctuated between 0.1 % and 0.5 %. The birds' average weight at 45−50 d of age spanned between 2.6 −2.8 kg. Nevertheless, the average weights at the time of slaughter were substantially lower than those of unaffected birds, being more than 2.8 kg. Calculations for homogeneity yielded an average value of 77.7 %. Birds that remained underdeveloped, weighing less than 1.7 kg, constituted an average of 4.3 % of the total population (Table 3).

## 1.4 Statistical findings

Age-related effects and proventricular thickening on histopathological changes: The interplay between age and proventricular thickening presents a complex picture of their influence on histopathological changes within the avian proventriculus. This section elucidates the patterns and associations uncovered through our statistical analysis, highlighting how these factors contribute to the disease processes observed. Our investigation into age-related changes in the avian proventriculus reveals a complex interplay of histopathological features. The overarching analysis did not demonstrate a significant age-related trend across proventricular macro-lesions, as a whole ($F(2.50) = 0.336$, $P = 0.7162$). This suggests that while individual birds may exhibit specific proventricular changes, these do not collectively increase or decrease significantly with age. Delving deeper, we observed that lymphoid infiltration presents a more nuanced relationship with age. Despite the

ANOVA indicating a significant difference across age groups (F (2.50) = 3.239, P = 0.0476), the post-hoc analysis clarified that the mean differences among infiltration levels were not individually significant.

This finding invites a more detailed look into how age might subtly influence the severity or likelihood of lymphoid infiltration, without manifesting linearly or uniformly across the population. Similarly, the impact of age on necrosis was not statistically significant (F (1.51) = 1.619, P = 0.2089), aligning with the notion that necrotic processes in the proventriculus may be more influenced by factors other than the chronological age of the birds. Hyperplasia, too, showed no significant direct relationship with age (F (2.50) = 1.592, P = 0.2137), although the post-hoc analysis revealed certain differences that, while not reaching significance, suggest a pattern that merits further investigation within the broader context of avian pathology. When considering the overall state of cases, including LP, TVP, and WP, age appears to play a marginal role. The slight differences noted by the ANOVA (F (2.50) = 1.653, P = 0.2018) were not substantiated by significant post hoc mean differences, reinforcing the complexity of disease progression and the multifactorial nature of histological states in birds. Through this integrated lens, wegain insight into the subtle yet potentially meaningful relationships between age and specific histopathological changes. While age alone may not be a definitive factor in the development of proventricular lesions, necrosis, or hyperplasia, its role in lymphoid infiltration could be indicative of a broader, age-related physiological landscape that influences avian health. Such insights pave the way for a refined understanding of avian histopathology and its implications for disease susceptibility and management, In addition to exploring the nuanced relationships between age and various histopathological changes, our analysis extends to examining the significant impact of proventricular thickening on these conditions. The detailed statistical findings, summarized in the table below, illustrate the profound influence of proventricular wall thickness on the histological landscape of the avian proventriculus. Following the comprehensive summary provided by the table, we delve deeper into the significant associations observed, particularly focusing on the effects of proventricular thickening.

Table 4: Summary of statistical analyses on age-related effects and proventricular thickening on histopathological changes

| Factor | Degrees of freedom (df) | F value | P value | Notes |
|---|---|---|---|---|
| *Age-related effects* | | | | |
| Proventricular macrolesions | 2.50 | 0.336 | 0.7162 | No significant age-related difference |
| Lymphoid infiltration | 2.50 | 3.239 | 0.0476 | A significant difference, but post hoc non-significant |
| Necrosis | 1.51 | 1.619 | 0.2090 | No significant effect of age |
| Hyperplasia | 2.50 | 1.592 | 0.2137 | No significant effect of age |
| Case states (LP, TVP, WP) | 2.50 | 1.653 | 0.2018 | Marginal difference, not statistically significant |
| *Proventricular thickening effects* | | | | |
| Macro lesion | 2.52 | 2.913 | 0.0632 | Near-significant relationship |
| Lymphoid infiltration | 2.52 | 19.775 | <0.0001 | Significant effect of thickening |
| Necrosis (Unequal variances) | – | –4.460 | <0.0001 | Significant difference |
| Hyperplasia | 2.52 | 23.437 | <0.0001 | Significant effect of thickening |
| Case state (LP, TVP, WP) | 2.52 | 27.432 | <0.0001 | Significant effect of thickening |

- Post-hoc analyses where applicable are noted for their specific outcomes. The F value for necrosis is replaced by the t statistic due to the use of a t-test for this analysis. P values marked as "< 0.001" indicate a high level of statistical significance.

Exploring the relationship between proventricular thickening and various histological changes further enriches our understanding of avian proventriculus pathology: the nuanced analysis of how these physical alterations correlate with specific histopathological states unveils a series of significant associations. The examination into proventricular thickening revealed its near significant impact on macro-lesions ($F(2.52) = 2.913$, $P = 0.0632$), suggesting a trend where the extent of thickening might influence the development or presence of such lesions, although not conclusively. This trend spans different types of macrolesions, including those characterized by enlargement alone or accompanied by hemorrhagic/nodules and whitish characteristics, where the differences hinted at potential distinctions in their relationship with thickening. A pivotal finding was the significant correlation between proventricular thickening and lymphoid infiltration ($F(2.52) = 19.775$, $P < 0.0001$), exact value 0.00004102 highlighting a robust link that suggests an increase in wall thickness could be associated with or indicative of greater lymphoid activity. This relationship underscores the potential of proventricular thickening as a marker for underlying immunologi-

cal responses or pathological conditions. In contrast, the study's dive into the association between thickening and necrosis through t-test analyses illuminated a significant divergence ($t(53) = -4.460$, $P < 0.00001$exact value 0.00004296 for equal variances; $t(27.265) = -5.256$, $P < 0.0001$ for unequal variances) exact value 0.00001489, emphasizing that the degree of proventricular thickening might vary significantly with the presence of necrotic tissue. Such a finding implies a critical interaction where thickening could either contribute to or result from necrotic processes within the proventriculus. Furthermore, hy- perplasia's significant association with proventricular thickening ($F(2.52)= 23.437$, $P < 0.0001$) exact value 0.0000000555 indicates a compelling link, suggesting that the proliferation of cells within the proventricular lining is closely related to the extent of wall thickening. This finding is particularly noteworthy, as it suggests that thickening may either facilitate the hyperplastic process or serve as a response to it. The analysis also extended to the overall state of cases, including LP, TVP, and WP, where a significant relationship was found ($F(2.52) = 27.432$, $P < 0.001$) exact value 0.0000000074. This indicates that the physical condition of the proventriculus, as reflected in its thickness, plays a crucial role in the histopathological categorization and possibly the clinical manifestation of these conditions. This integrated analysis, by weaving together the impacts of proventricular thickening on various histopathological features, offers a holistic view of the intricate dynamics within the avian proventriculus. It highlights the significance of structural changes and their potential role in influencing or reflecting the pathological state of the organ, providing a foundation for further exploration into the mechanisms driving these associations and their implications for avian healt.

# 2 Discussions

Based on the histopathological findings, the current study highlights the status of TVP, LP, and WP at proportions of 23.6 %, 52.8 %, and 23.6 % respectively. The inflammatory process, as evidenced by the presence of lymphocytes and necroses accompanied by the thickening of the proventriculus wall (TVP), or in the case of LP (without necrosis), is likely due to the introduction of infectious

agents. However, the presence of WP status without inflammatory lesions suggests that this disease cannot be solely attributed to these etiological agents. Moreover, factors such as biogenic amines, copper sulfate, mycotoxins, the inclusion of whole grains in pelleted broiler diets (Taylor & Jones, 2004), or the presence of a toxic factor(s) in the chicken-house litter (Maas & Van De Venne, 1985) have been implicated in proventricular enlargement.

This accounts for Riddel's assertion in 1987 that the proventriculus enlargement, characterized by hyperplasia of the glandular epithelium, fibrosis, and edema observed in North America, contrasts with the swelling of the proventriculus due to significant infiltration of lymphoid cells in the glandular tissue and mucosa as described in Holland (Riddell, 1987). On the other hand, Guy, West, Fuller, et al. (2011) and Yan et al. (2020) reported that in experimentally reproduced TVP-affected cases, moderate-to-severe lymphocytic infiltrates pronounced tubular hyperplasia, and metaplasia are indicative of chronic stages where the virus is undetectable. Pantin-Jackwood and Brown (2003) in a study of severe lymphocytic infiltration in TVP cases, found that the distribution in these infiltrates of both cell-mediated and humoral immune responses is activated during both acute and chronic phases of transmissible proventriculitis. Grau-Roma et al. (2017) and Marusak et al. (2012) suggested that several LP-affected chickens with negative CPNV RT-PCR results may represent chronically affected TVP cases. While CPNV was detected in proventriculus with both TVP and LP-lesions, other viruses besides CPNV might be the culprits for some TVP-affected cases, as pointed out by Grau-Roma et al. According to the literature, more than 13 different infectious or non-infectious agents have been associated with TVP (Hafner et al., 2008). However, none of these agents provide compelling evidence of their role in TVP. Field observations indicate that all of these ubiquitous or opportunistic agents are commonly found in broilers, especially viruses, and the commensal bacterial flora, manifesting either clinically or subclinically (carrier state). Dormitorio et al. (2007) mentioned that diseases involving the proventriculus, such as the malabsorption syndrome (MAS), runting/stunting syndrome, pale-bird syndrome, and feed passage syndrome, have been recognized in broilers since the early 1970s. It can be hypothesized that multiple agents could be responsible for TVP. It is possible that an immunosuppressive viral

agent, such as IBDV, chicken infectious anemia virus, or reoviruses, facilitates the introduction of secondary invaders like bacteria to contribute to the syndrome's development. Laying hens and broiler breeders were not affected by the disease. This refractory state can be explained by the management of these farms in terms of rationed feeding, light programs, and strict adherence to sanitary barriers and technical parameters compared to broiler farming where food is consumed ad libitum and where intensive farming conditions are generally less controlled. Marusak et al. (2012) diagnosed TVP in broilers, breeding hens, and commercial laying hens ranging from 9 to 20 weeks of age. The hen included here is the oldest chicken reported to be affected by TVP, even though it tested negative for CPNV RT-PCR. Hafner et al. (2008) noted that their histological study of TVP became the second report of this disease in laying hens in peer-reviewed literature. The chickens in the study were primarily broiler chickens, with only one case corresponding to a layer hen. The reason is, that TVP predominantly affects broiler chickens, which make up 80 % of the chicken post-mortems conducted by the poultry clinicians who submitted the samples. The disease prevalence in this study was 20.9 %. A retrospective study on TVP between 1998 and 2019 found that the year with the highest case frequency, 26 %, was in 2011 (Wali, 2021). A recent study conducted by Grau-Roma et al. (2020) in the UK revealed a prevalence of 19 %. The enlarged proventriculus was observed in broilers reared from the fifteenth day (beginning of the growth phase) to 40 d old (end of the growth phase), and the mortality rate upon disease detection ranged between 0.1 % and 0.5 %. These findings align with previous studies and suggest that there isn't a significant increase in mortality in flocks affected by TVP (Hafner et al., 2008). On the other hand, Bayyari, Huff, Balog, et al. (1995) reported that broilers affected by TVP were aged between 21−49 d. The average mortality rate of broiler flocks in the UK was previously documented at 4.1 %. Hafner et al. (2013) reported that chickens diagnosed with proventriculitis were aged between 30−45 d, with an average age of 34.0 d and a median age of 35 d (Hauck et al., 2020). Lesions were more frequently observed in 33−36 day-old broilers than in those younger than 30 d (Leão et al., 2021). In the present study, clinical signs were largely unnoticeable, with exceptions being an average weight and a homogeneity rate slightly below the standards, and a significant rate of

culling for underdeveloped chickens. Wali (2021) reported that in terms of clinical signs, 60 % of the cases had poor growth or lack of uniformity, anorexia, and heightened mortality. In 30 % of these cases, there was concurrent bursal atrophy, and in 26 %, hepatitis was diagnosed alongside proventriculitis. The main clinical signs included poor uniformity, increased mortality, and culling rates ranging from 0.50 % to 1.25 % per week for smaller (stunted) birds (Noiva et al., 2015). The average weight gained by TVP-infected birds was 1.1 kg, in contrast to the control group, which gained 1.58 kg (Śmiałek et al., 2020). TVP is characterized by proventriculitis paired with poor weight gain, compromised feed digestion, and heightened feed conversion (FC), resulting in significant economic losses (Bayyari, Huff, Balog, et al., 1995; Kouwenhoven et al., 1978; Marusak et al., 2012; Noiva et al., 2015). Aside from the enlargement of the proventriculus, gross lesions were characterized by nonspecific lesions in various organs. They were also characterized by specific lesions from common diseases in broilers such as infectious bursal disease (often called Gumboro disease), runting/stunting syndrome, mycoplasmosis, clostridiosis, and colibacillosis. The statistical analysis revealed intriguing interactions concerning the proventricular thickening wall and its implications for both lymphoid infiltration and hyperplasia. At its core, the proventricular thickening wall serves as a potential marker for deeper pathological processes, including heightened lymphoid activity and cellular proliferation. Lymphoid Infiltration: as the thickening of the proventricular wall increases, we observed a correlating rise in lymphoid infiltration scores. This suggests that the thicker the wall, the higher the lymphoid activity, indicative of an immune response. It's feasible to infer that this thickening could be a defensive mechanism against a foreign agent, or perhaps an autoimmune reaction, prompting a higher rate of lymphoid infiltration. Hyperplasia: on the hyperplasia front, the thickened proventricular wall again plays a pivotal role. With hyperplasia indicating an increase in the number of cells, possibly due to increased cell production or decreased cell death, it aligns with the notion that a thicker wall might be a product of such a cellular increase. However, whether this proliferation is a cause or an effect of the thickening of the wall remains a subject for deeper exploration. In essence, the prominence of the proventricular thickening wall in our data underscores its importance as a potential focal point for future inves-

tigations. It seems to be at the crossroads of several crucial pathological pathways, making it a keystone in understanding proventricular conditions. This study showed that the disease is quite prevalent, especially during the growth phase of farming. However, it often goes unnoticed due to the lack of detection of thickened proventriculus and sudden deaths. The mortality rate during the disease's progression is low but can be exacerbated by viral infections or commensal bacteria such as Clostridium, Escherichia coli, Pseudomonas, Mycoplasma, and Pasteurella. The most significant losses are mainly represented by the presence of underdeveloped subjects at the end of the cycle. Statistically, future research might focus on understanding the direct cause-and-effect relationship between age and these pathological factors, potentially illuminating any age-related susceptibility or resistance in avian healt.

# Conclusion

In conclusion, the study showed that statuses TVP, LP, and WP were identified on the pathological and histological lesions. Intense lymphocytic proliferation and necrosis were indicative of infectious agents in TVP and LP while the absence of these features in WP suggests non-infectious origins for proventriculitis. Moreover, severe hypertrophy of the proventricular wall in TVP and LP compared to WP suggests, only for the conditions of this study, that the infectious agents as merely exacerbating factors and not the cause of the disease. The cause should be sought at the level of broiler feed intake. The research into the origin of this condition should be related to the various nutrients or other organic materials that trigger the reactivity of the proventriculus as a glandular stomach.

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# Appendix

Table 5: Farm and SampleData

| Farm ID | Geographic Coordinates (Province, Latitude, Longitude) | Type of Production | Farm Capacity | Feed Type | Type of Bedding Used |
|---|---|---|---|---|---|
| 1 | Sétif (36.08470, 5.338800) | broiler Arbor acre | 5000 | crumbled | wood shavings |
| 2 | Sétif (36.08470, 5.338800) | broiler Arbor acre | 8000 | crumbled | straw |
| 3 | Sétif (36.08470, 5.338800) | broiler Arbor acre | 5000 | crumbled | wood shavings |
| 4 | Sétif (35.8108, 5.3219) | broiler Arbor acre | 6600 | crumbled | wood shavings |
| 5 | Constantine (36.2927, 6.3277) | broiler Cobb 500 | 5000 | crumbled | straw |
| 6 | Constantine (36.2927, 6.3277) | broiler Cobb 501 | 5000 | crumbled | straw |
| 7 | Sétif (35.8108, 5.3219) | broiler Cobb 500 | 12000 | crumbled | straw |
| 8 | Sétif (35.8108, 5.3219) | broiler Cobb 500 | 12000 | crumbled | straw |
| 9 | Sétif (35.7897406, 5.1051736) | broiler Cobb 500 | 12000 | crumbled | wood shavings |
| 10 | Batna (35.5430623, 6.1180411) | broiler Cobb 500 | 7500 | crumbled | straw |
| 11 | Sétif (35.7897406, 5.1051736) | broiler Cobb 500 | 8200 | crumbled | straw |
| 12 | Sétif (35.7897406, 5.1051736) | broiler Cobb 500 | 8200 | crumbled | straw |
| 13 | M'sila (35.6936363, 4.4966741) | broiler Cobb 500 | 7500 | crumbled | wood shavings |
| 14 | Setif (36.0364693, 5.2462288) | broiler efficiency plus | 5240 | crumbled | wood shavings |
| 15 | Sétif (35.7897406, 5.1051736) | broiler Cobb 500 | 9260 | crumbled | wood shavings |
| 16 | Bejaia (36.6448707, 4.8964374) | broiler Arbor acre | 9000 | crumbled | straw |
| 17 | Bejaia (36.6448707, 4.8964374) | broiler Cobb 500 | 8000 | crumbled | wood shavings |

*Continued on next page*

| Farm ID | Geographic Coordinates (Province, Latitude, Longitude) | Type of Production | Farm Capacity | Feed Type | Type of Bedding Used |
|---|---|---|---|---|---|
| 18 | Sétif (35.8108, 5.3219) | broiler Cobb 500 | 7000 | crumbled | straw |
| 19 | Constantine (36.2927, 6.3277) | broiler efficiency plus | 6500 | crumbled | straw |
| 20 | Constantine (36.2927, 6.3277) | broiler Cobb 500 | 6500 | crumbled | straw |
| 21 | Sétif (35.8726114, 5.2791717) | broiler Cobb 500 | 5500 | crumbled | straw |
| 22 | Sétif (35.815974, 5.4495789) | broiler Cobb 500 | 10000 | crumbled | straw |
| 23 | Sétif (36.08470, 5.338800) | broiler Artbor acre | 9000 | crumbled | straw |
| 24 | Sétif (35.7897406, 5.1051736) | broiler Artbor acre | 8000 | crumbled | wood shavings |
| 25 | Sétif (35.9837723, 5.182692) | broiler Cobb 500 | 10000 | crumbled | wood shavings |
| 26 | Batna (35.7408831, 5.5269684) | broiler Artbor acre | 12000 | crumbled | straw |
| 27 | Sétif (35.7897406, 5.1051736) | broiler Artbor acre | 20000 | crumbled | straw |
| 28 | Bordj Bou Arreridj (36.0295594, 5.0786974) | broiler Cobb 499 | 70000 | crumbled | wood shavings |
| 29 | Bordj Bou Arreridj (36.0295594, 5.0786975) | broiler Cobb 500 | 70000 | crumbled | wood shavings |
| 30 | Sétif (35.9099615, 5.1080468) | broiler Artbor acre | 75000 | crumbled | wood shavings |
| 31 | Sétif (35.7897406, 5.1051736) | broiler Artbor acre | 75000 | crumbled | straw |
| 32 | Bordj Bou Arreridj (35.9362142, 4.963743) | broiler Cobb 500 | 19000 | crumbled | straw |
| 33 | Bordj Bou Arreridj (35.9362142, 4.963743) | broiler Cobb 500 | 19000 | crumbled | straw |
| 34 | Sétif (35.9759158, 5.1746889) | broiler Cobb 500 | 6500 | crumbled | straw |
| 35 | Sétif (35.6847698, 5.2986704) | broiler Cobb 500 | 5000 | crumbled | wood shavings |
| 36 | Sétif (35.9759158, 5.1746889) | broiler Cobb 500 | 6500 | crumbled | wood shavings |

*Continued on next page*

| Farm ID | Geographic Coordinates (Province, Latitude, Longitude) | Type of Production | Farm Capacity | Feed Type | Type of Bedding Used |
|---|---|---|---|---|---|
| 37 | M'sila (35.6936363, 4.4966741) | broiler efficiency plus | 5500 | crumbled | wood shavings |
| 38 | Sétif (35.9500276, 5.1828029) | broiler efficiency plus | 7000 | crumbled | straw |
| 39 | Sétif (35.8450963, 5.3424151) | broiler Cobb 500 | 5500 | crumbled | straw |
| 40 | Sétif (35.7897406, 5.1051736) | broiler Cobb 500 | 6500 | crumbled | straw |
| 41 | Bordj Bou Arreridj (36.1296842, 4.6791871) | broiler Cobb 500 | 10000 | crumbled | straw |
| 42 | Sétif (35.9585993, 5.4532231) | broiler Cobb 500 | 9000 | crumbled | wood shavings |
| 43 | Sétif (35.9585993, 5.4532231) | broiler Cobb 500 | 5500 | crumbled | straw |
| 44 | Sétif (35.9423583, 5.5208691) | broiler Cobb 500 | 6500 | crumbled | wood shavings |
| 45 | Sétif (35.823521, 5.2505765) | broiler Cobb 500 | 7000 | crumbled | straw |
| 46 | Sétif (35.7460566, 5.4401485) | broiler Cobb 500 | 7000 | crumbled | wood shavings |
| 47 | Sétif (35.9585993, 5.4532231) | broiler Cobb 500 | 5000 | crumbled | straw |
| 48 | Sétif (35.8686123, 5.0726981) | broiler Cobb 500 | 5500 | crumbled | straw |
| 49 | Sétif (35.9585993, 5.4532231) | broiler Cobb 500 | 5500 | crumbled | wood shavings |
| 50 | Batna (35.5508753, 6.1623721) | broiler Cobb 500 | 5000 | crumbled | wood shavings |
| 51 | Sétif (36.1468803, 5.6628481) | broiler Cobb 500 | 6000 | crumbled | wood shavings |
| 52 | Sétif (36.3326912, 5.0899951) | broiler Cobb 500 | 6000 | crumbled | straw |
| 53 | Ghelma (36.4535247, 7.3371899) | broiler Cobb 500 | 8000 | crumbled | straw |
| 54 | Sétif (36.3770835, 5.4767719) | broiler Cobb 500 | 8000 | crumbled | straw |
| 55 | Sétif (35.6808446, 5.2075339) | broiler Cobb 500 | 11000 | crumbled | wood shavings |

*Continued on next page*

| Farm ID | Geographic Coordinates (Province, Latitude, Longitude) | Type of Production | Farm Capacity | Feed Type | Type of Bedding Used |
|---|---|---|---|---|---|
| 56 | Sétif (36.1562882, 5.4885679) | broiler Cobb 500 | 20000 | crumbled | wood shavings |
| 57 | Sétif (36.1562882, 5.4885679) | broiler efficiency plus | 86000 | crumbled | wood shavings |
| 58 | Sétif (36.1562882, 5.4885679) | broiler efficiency plus | 20000 | crumbled | straw |
| 59 | Sétif (36.3770835, 5.4767719) | broiler Cobb 500 | 20000 | crumbled | wood shavings |
| 60 | Sétif (36.3770835, 5.4767719) | broiler Cobb 500 | 5000 | crumbled | wood shavings |
| 61 | Sétif (36.3770835, 5.4767719) | broiler Cobb 500 | 6900 | crumbled | wood shavings |
| 62 | Sétif (36.3770835, 5.4767719) | broiler efficiency plus | 20000 | crumbled | straw |
| 63 | Sétif (36.0364693, 5.2462288) | lohmann brown | 60000 | mash | straw |
| 64 | Sétif (35.9423583, 5.5208691) | lohmann brown | 60000 | mash | straw |
| 65 | Bouira (36.3727969, 3.8185088) | lohmann brown | 60000 | mash | straw |
| 66 | Sétif (35.9423583, 5.5208691) | lohmann brown | 60000 | mash | straw |
| 67 | Bordj Bou Arreridj (36.0295594, 5.0786975) | broiler breeder efficiency plus | 10000 | mash | straw |
| 68 | Bordj Bou Arreridj (36.0295594, 5.0786975) | broiler breeder efficiency plus | 10000 | mash | straw |
| 69 | Bordj Bou Arreridj (36.0295594, 5.0786975) | broiler breeder efficiency plus | 10000 | mash | straw |

Table 6: Epidemiological Data

| Farm ID | Disease Prevalence | Flock Homogeneity (%) | Age of Disease Onset (day) | Disease Progression | Mortality Rate During Disease (%) |
|---|---|---|---|---|---|
| 1 | Presence | 78.1 | 29 | persists until slaughter | 0.34 |
| 2 | Presence | 76.4 | 25 | persists until slaughter | 0.12 |
| 3 | Presence | 79.5 | 31 | persists until slaughter | 0.22 |
| 4 | Presence | 75.7 | 24 | persists until slaughter | 0.15 |
| 5 | Presence | 78.9 | 25 | persists until slaughter | 0.4 |
| 6 | Presence | NA | NA | NA | NA |
| 7 | Presence | 77.2 | 33 | persists until slaughter | 0.26 |
| 8 | Presence | 76.8 | 33 | persists until slaughter | 0.25 |
| 9 | Presence | 79.1 | 38 | persists until slaughter | 0.49 |
| 10 | Presence | 77.5 | 28 | persists until slaughter | 0.26 |
| 11 | Presence | 75.9 | 27 | persists until slaughter | 0.44 |
| 12 | Presence | 78.4 | 37 | persists until slaughter | 0.27 |
| 13 | Presence | 76.2 | 25 | persists until slaughter | 0.18 |
| 14 | Suspect | NA | 38 | NA | NA |
| 15 | Suspect | NA | 22 | NA | NA |
| 16 | Suspect | NA | 25 | NA | NA |
| 17 | Suspect | NA | 29 | NA | NA |
| 18 | Suspect | NA | 32 | NA | NA |
| 19 | Suspect | NA | 25 | NA | NA |
| 20 | Suspect | NA | 32 | NA | NA |
| 21 | Suspect | NA | 25 | NA | NA |
| 22 | Suspect | NA | 18 | NA | NA |
| 23 | Suspect | NA | 27 | NA | NA |
| 24 | Suspect | NA | 25 | NA | NA |
| 25 | Suspect | NA | 25 | NA | NA |
| 26 | Suspect | NA | 21 | NA | NA |
| 27 | Suspect | NA | 39 | NA | NA |
| 28 | Suspect | NA | NA | NA | NA |
| 29 | Suspect | NA | 34 | NA | NA |
| 30 | Suspect | NA | 23 | NA | NA |
| 31 | Suspect | NA | 36 | NA | NA |
| 32 | Suspect | NA | 26 | NA | NA |
| 33 | Suspect | NA | 43 | NA | NA |
| 34 | Suspect | NA | 21 | NA | NA |
| 35 | Suspect | NA | 25 | NA | NA |
| 36 | Suspect | NA | 32 | NA | NA |
| 37 | Suspect | NA | 33 | NA | NA |
| 38 | Suspect | NA | 17 | NA | NA |

*Continued on next page*

| Farm ID | Disease Prevalence | Flock Homogeneity (%) | Age of Disease Onset (day) | Disease Progression | Mortality Rate During Disease (%) |
|---|---|---|---|---|---|
| 39 | Suspect | NA | 28 | NA | NA |
| 40 | Suspect | NA | 31 | NA | NA |
| 41 | Suspect | NA | 37 | NA | NA |
| 42 | Suspect | NA | 26 | NA | NA |
| 43 | Suspect | NA | 35 | NA | NA |
| 44 | Suspect | NA | 33 | NA | NA |
| 45 | Suspect | NA | 27 | NA | NA |
| 46 | Suspect | NA | 42 | NA | NA |
| 47 | Suspect | NA | 31 | NA | NA |
| 48 | Suspect | NA | 39 | NA | NA |
| 49 | Suspect | NA | 31 | NA | NA |
| 50 | Suspect | NA | 28 | NA | NA |
| 51 | Suspect | NA | 27 | NA | NA |
| 52 | Suspect | NA | 27 | NA | NA |
| 53 | Suspect | NA | 24 | NA | NA |
| 54 | Suspect | NA | 27 | NA | NA |
| 55 | Suspect | NA | 31 | NA | NA |
| 56 | Suspect | NA | NA | NA | NA |
| 57 | Suspect | NA | NA | NA | NA |
| 58 | Absence | NA | NA | NA | NA |
| 59 | Absence | NA | NA | NA | NA |
| 60 | Absence | NA | NA | NA | NA |
| 61 | Absence | NA | NA | NA | NA |
| 62 | Absence | NA | NA | NA | NA |
| 63 | Absence | NA | NA | NA | NA |
| 64 | Absence | NA | NA | NA | NA |
| 65 | Absence | NA | NA | NA | NA |
| 66 | Absence | NA | NA | NA | NA |
| 67 | Absence | NA | NA | NA | NA |
| 68 | Absence | NA | NA | NA | NA |
| 69 | Absence | NA | NA | NA | NA |