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Pathological, epidemiological features, and statistical study of histopathological changes in chicken transmissible viral proventriculitis

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Abstract

Transmissible viral proventriculitis (TVP) is a chicken disease whose etiology is not fully understood. This study aims to describe histopathological, macroscopic, and epidemiological data associated with possible new etiological agents. The samples comprised 62 broiler farms, 4 laying hen flocks, and 3 broiler breeders. The disease was identified by proventriculus thickening, confirmed through histopathological examination as the most reliable diagnostic method for TVP. Prevalence, clinical signs, gross lesions, epidemiological features, and statistical analysis were calculated. Microscopic findings confirmed the disease, which was classified into three distinct statuses: TVP characterized by the presence of both lymphocytic infiltration and necrosis; lymphocytic proventriculitis (LP) identified by lymphocytic infiltration alone, without the presence of necrosis (WP) denoting cases devoid of both lymphocytic infiltration and necrosis in the proventriculus. These statuses occurred at 23.6 %, 52.8 %, and 23.6 % rates, respectively. The disease prevalence was 20.9 % in flocks aged 15 to 40 days, with a mortality rate from 0.1 % to 0.5 % upon discovery. TVP and LP are marked by intense lymphocytic proliferation and necrosis, hinting at the involvement of infectious agents. Conversely, the absence of these characteristics in WP points to non-infectious etiologies for proventriculitis. The distinct proventricular wall hypertrophy observed in TVP and LP, as opposed to WP, reinforces the interpretation that, only for the conditions of this study, infectious agents amplify existing conditions rather than serve as primary catalysts for the disease.

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

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Study contribution

Most authors associate TVP with infectious agents, but none has been proven to cause the disease. This study shows that three stages of the disease (TVP, LP, and WP) were identified based on histopathological changes. The presence of intense lymphocytic proliferation and necrosis in TVP and LP suggests the involvement of infectious agents. In contrast, WP's lack of these indicators supports a non-infectious origin for proventriculitis. Furthermore, the observation of severe proventricular wall hypertrophy in TVP and LP, unlike in WP, underscores our conclusion that, within the confines of this study, infectious agents act as exacerbates rather than the primary cause of the disease. The research into the origin of this condition should be related to the responsiveness of proventriculus as a glandular-type stomach to various nutrients or other organic materials ingested including management practices and genetics.

Introduction

Transmissible viral proventriculitis (TVP) is a chicken disease responsible for significant losses in mortality, livestock heterogeneity rates, and chicken weight gain.[1] TVP typically affects broiler chickens from 3 to 6 weeks of age and, to a lesser extent, laying hens and broiler breeders.[2]

It was first described in 1978 by Kouwenhoven who suggested that an infectious agent was the cause.[3] 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.[4]

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),[2, 5] chicken proventricular necrosis virus (CPNV),[6–8] infectious bronchitis virus,[9, 10] picornavirus,[11] gyrovirus,[12] reovirus,[13] adenovirus,[14] cyclovirus,[15] as well as other agents like *Clostridium*,[16] mycotoxins,[17] biogenic amines,[18] or copper sulfate.[19] Dormitorio et al.[2] 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.[20] 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 study 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 Africa.

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Materials and methods

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.

Study design and sample collection

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. 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, 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. 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 harvested 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 50^{th}–60^{th} d). Moreover, prominent health disturbances that emerged during the rearing processes across different production types were meticulously recorded.

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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.[21] Inflammatory infiltration within the lamina propria was disregarded as it is noted to be a common occurrence in healthy birds.[22]

A scoring system similar to that presented by Grau-Roma et al.[23] 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).

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.

Results

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 1). 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.

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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 2). 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 1) 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 ≤ 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 ≤ 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. (Table 2).

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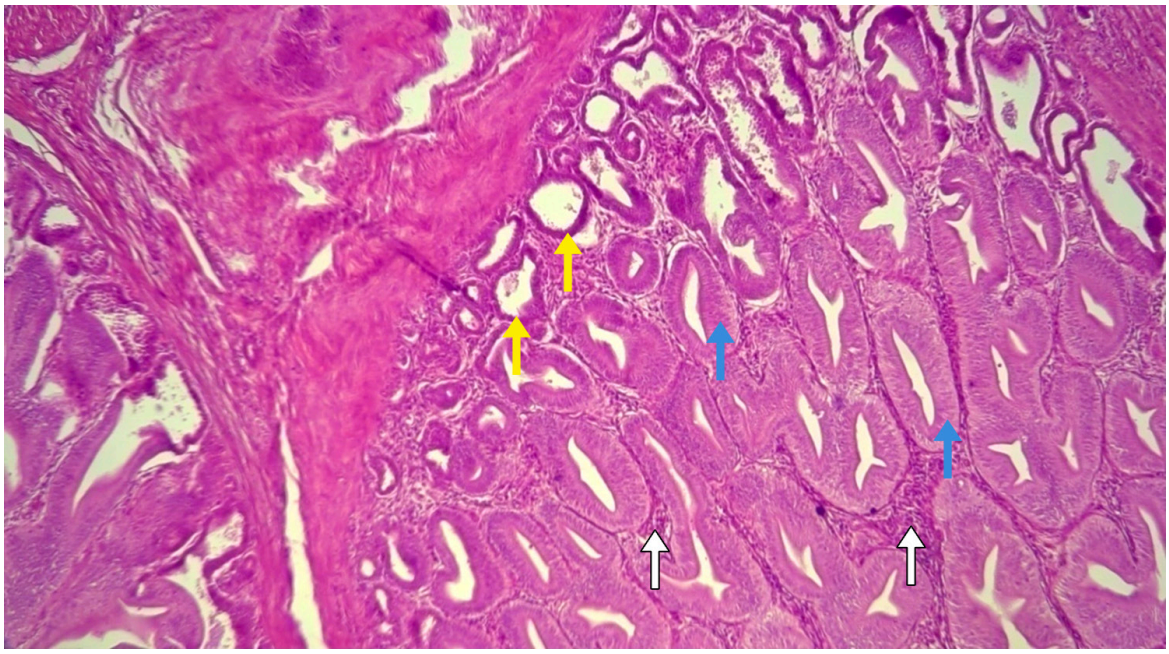

Figure 1. Vacuolar degeneration and necrosis of glandular epithelium (yellow arrows); replacement of glandular epithelium by hyperplastic ductal epithelium (blue arrows); infiltration of lymphoid cells (white arrows). Hematoxylin and Eosin staining (× 100).

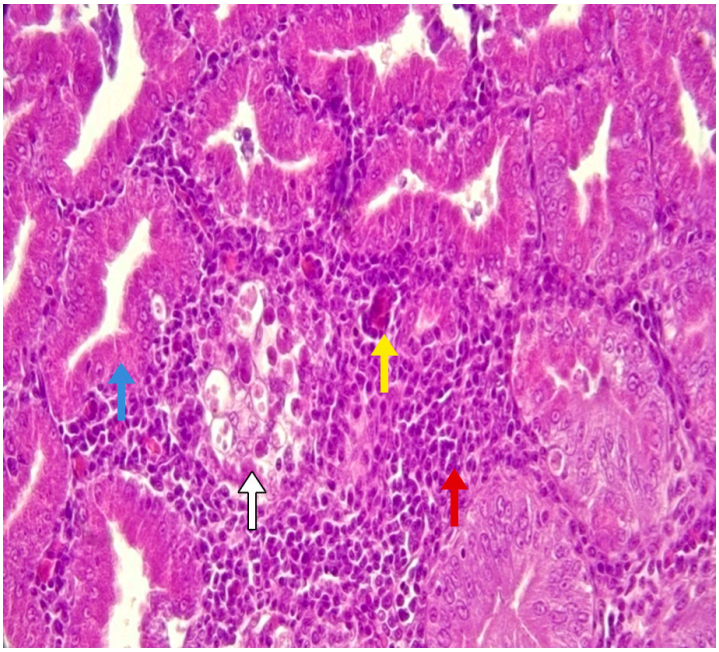

Figure 2. The glandular epithelium is replaced by hyperplastic ductal epithelium (blue arrow). Clusters of dead cells (white arrow), hemorrhage (yellow arrow), and severe lymphocytic infiltration (red arrow). Hematoxylin and Eosin staining (× 400).

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Table 1. 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
N = 13	n = 55			Interstitial lymphocytic Infiltration	Mean necrosis score	Ductal epithelial hyperplasia and metaplasia	
1	1	Enlargement, whitish	4.6	++	-	+	LP
	2	Enlargement, whitish	4.4	++	-	+	LP
	3	Enlargement, mottled pink	5.4	+++	+	++	TVP
	4	Enlargement, reddened appearance	5.6	++	+	++	TVP
	5	Enlargement, mottled pink	7.9	+++	+	+++	TVP
2	6	Enlargement, reddened appearance	5.9	++	+	+++	TVP
	7	Enlargement whitish	5.2	++	-	++	LP
	8	Enlargement whitish	5.3	+++	-	++	LP
	9	Enlargement, mottled pink	6.2	+++	+	+++	TVP
	10	Enlargement, mottled pink	5.6	++	+	++	TVP
3	11	Enlargement, reddened appearance	6.9	+++	+	+++	TVP
	12	Enlargement, whitish	6.7	++	-	++	LP
	13	Enlargement, prominent papillae	5.5	++	++	++	TVP
	14	Enlargement	5.5	++	-	++	LP
4	15	Enlargement	5.4	+	-	++	LP
	16	Enlargement, whitish	4.0	-	-	+	WP
	17	Enlargement, whitish	6.0	+++	-	++	LP
	18	Enlargement	4.3	++	-	+	LP
	19	Enlargement	3.9	-	-	++	WP
5	20	Enlargement	4.3	+++	-	+	LP
	21	Enlargement	3.7	++	-	+	LP
	22	Enlargement	3.1	-	-	+	WP
	23	Enlargement	4.0	+	-	+	LP
	24	Enlargement	5.9	+++	-	++	LP
6	25	Enlargement	5.1	++	-	+	LP
	26	Enlargement	4.8	++	-	+	LP
	27	Enlargement, mottled pink	5.4	+++	+	+++	TVP
	28	Enlargement	3.9	-	-	++	WP
7	29	Enlargement	6.5	++	-	++	LP
	30	Enlargement, whitish	3.7	++	-	+	LP
	31	Enlargement, mottled pink	5.7	+++	+	++	TVP
	32	Enlargement	6.5	++	-	++	LP
8	33	Enlargement, prominent papillae,	5.9	++	++	+	TVP
	34	Enlargement	4.8	++	-	+	LP
	35	Enlargement	3.5	++	-	+	LP
	36	Enlargement	4.3	+	-	+	LP
	37	Enlargement	3.9	-	-	+	WP

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Case number		Proventricular macro-lesions	Thickened wall (mm)	Proventricular micro-lesions			Case status
N = 13	n = 55			Interstitial lymphocytic Infiltration	Mean necrosis score	Ductal epithelial hyperplasia and metaplasia	
9	38	Enlargement	5.8	+	-	+	LP
	39	Enlargement, whitish	3.4	-	-	+	WP
	40	Enlargement, whitish	4.2	-	-	++	WP
	41	Enlargement, whitish	4.5	++	-	++	LP
10	42	Enlargement, whitish	3.6	-	-	+	WP
	43	Enlargement	5.1	++	-	+	LP
	44	Enlargement	5.4	++	-	++	LP
11	45	Enlargement	3.3	-	-	+	WP
	46	Enlargement	4.1	++	-	-	LP
	47	Enlargement, prominent papillae	5.8	++	++	++	TVP
12	48	Enlargement	5.8	++	-	++	LP
	49	Enlargement	4.2	-	-	+	WP
	50	Enlargement	3.9	-	-	+	WP
13	51	Enlargement, mottled pink	5.6	++	++	+++	TVP
	52	Enlargement	3.5	-	-	+	WP
	53	Enlargement	4.1	-	-	++	WP
	54	Enlargement	5.1	++	-	+	LP
	55	Enlargement	6.2	+++	-	++	LP

N: flocks number
n: proventriculus number
TVP: transmissible viral proventriculitis, LP: lymphocytic proventriculitis, WP: without proventriculitis.

Table 2. Mean scores and standard deviation of 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 ≤ 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 ≥ 1	5.9538	0.70901	13
Hyperplasia			
Score ≤ 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

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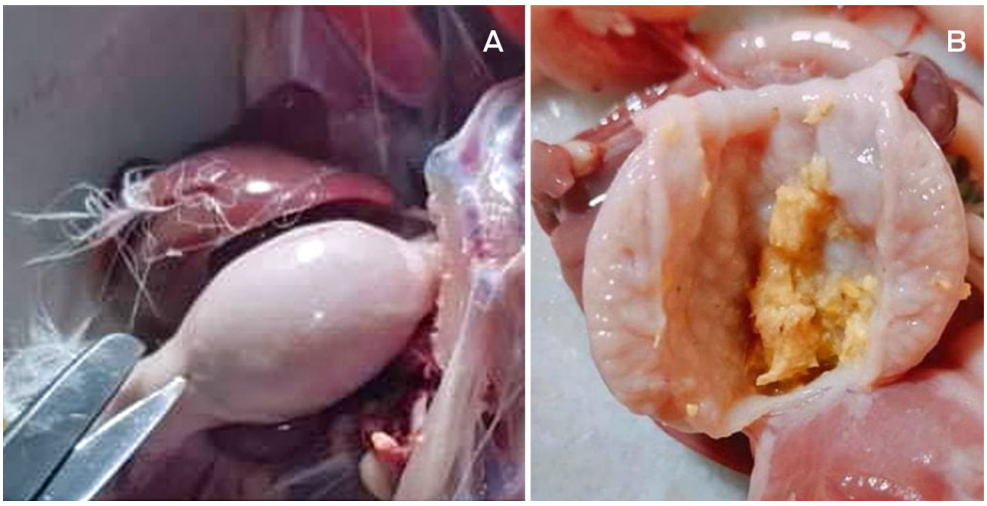

Figure 3A-B. Enlarged affected proventriculus with thickened wall.

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 3A-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 (helicopter sign), respiratory complications, nonspecific diarrhea, and neurological symptoms associated with hypovitaminosis B became apparent. The most common gross lesions encountered were respiratory issues, nonspecific enteritis, nephritis, hepatitis, and a reactionary bursa of Fabricius (pinkish).

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.

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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, we gain 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.

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Table 3. 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				
Macrolesion	2.52	2.913	0.0632	Near-significant relationship
Lymphoid infiltration	2.52	19.775	< 0.0001	Significant effect of thickening
Necrosis (Equal variances)	-	-4.460	< 0.0001	Significant difference
Necrosis (Unequal variances)	-	-5.256	< 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

Notes:
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 immunological 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-

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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 health.

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,[24], or the presence of a toxic factor(s) in the chicken-house litter[25] have been implicated in proventricular enlargement.

This accounts for Riddel's assertion in 1987[26] 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. On the other hand, Guy et al.[14] 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 et al.,[27] 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.[28] 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.[29]

According to the literature, more than 13 different infectious or non-infectious agents have been associated with TVP. However, none of these agents provide

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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.[2] 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.[28] 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.[29] 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.[30] A recent study conducted by Grau-Roma[31] 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.[29] On the other hand, Bayyari et al.[1] 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 %.[32] 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.[33]Lesions were more frequently observed in 33–36 day-old broilers than in those younger than 30 d.[8]

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. Ali Wali[30] 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.[34] The average weight gained by TVP-infected birds was 1.1 kg, in contrast to the control group, which gained 1.58 kg.[21] TVP is characterized by proventriculitis paired with

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poor weight gain, compromised feed digestion, and heightened feed conversion (FC), resulting in significant economic losses.[1, 3, 28, 34, 35]

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 investigations. 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 health.

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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Data availability

The original datasets used in this research and if applicable, supporting information files, are deposited and available for download at the SciELO Dataverse repository doi: 10.48331/scielodata.JNA9UW.

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Conflicts of interest

The authors declared no potential conflicts of interest.

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