Research Article

An Assay to Detect the Impact of Biosecurity Levels of Farms on Broilers Gut Health

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Abstract | During this study, we aspired and attempted to detect a correlation between the biosecurity level of 18 commercial broiler farms scattered in the Doukkala area of the Casablanca-Settat region and the health of the digestive tract of the birds. The 18 farms were firstly categorized into three (3) groups according to their performances on a biosecurity level: P group for "poor" performances, D group for "decent" performances, and G group for "good" performances. Each farm was then sampled twice on day 11 and day 35 of the same production cycle. In order to evaluate the gut health of the broilers, we used a panel of tools and techniques, ranging from numerical tools used to gather data from the farms to bacteriological material geared toward pathogens detection. The microscopic lesions found on most of the farms of the P group on day 35 indicated the ongoing of a regenerative process, contrarily to the farms of the D and G groups, in which the guts still underwent inflammation on day 35. Coccidiosis infection was heavily widespread on birds at day 35. Differences between the farm in D and P groups weren't striking as the disease was detected on the intestinal portions of almost all the broilers of the farms belonging to those categories. Adversely to the G group in which coccidiosis was mostly detected on the caecal portion of the broilers. Bacterial isolation following liver translocation revealed the existence of a number of bacterial entities. Most of them were foodborne pathogens, in fact, all the groups displayed close levels of infection by *Escherichia coli, Staphylococcus aureus, Salmonella spp, and Staphylococcus spp;* And *Yersinia enterocolitica* was also detected on one of the farms. Broiler performance can be affected to varying degrees by biosecurity levels on farms. Higher biosecurity levels can prevent early onset of gut inflammation, while lower levels may cause inflammation and reduce production. This study suggests that high biosecurity and management are not the sole factors influencing animal health and farm production. Other factors, such as breeder efficiency, worker experience, and chick and feed quality, must also be considered.

Keywords | Foodborne pathogens, Coccidiosis infection, Enteric health, Histopathology, Microbiology.

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INTRODUCTION

In poultry, intestinal health generally equals the overall health of the birds, and healthy birds perform the best when their intestinal health is at peak efficiency (Diaz Carrasco et al., 2019). Gut health is commonly described, in the absence of a consensus definition, as the absence of enteric diseases (Shini & Bryden, 2021), such as enteritis caused by bacteria like *Clostridium perfringens (C. perfringens)*, *Clostridium colinum*, parasitic diseases caused by Coccidia, and a panel of viruses (Boulianne et al., 2020; Cunha et al., 2020). Occurrence of enteric diseases in poultry production is heavily widespread considering the rocket-like growth the sector has known these past few decades (Hafez & Attia, 2020). A close sighted look on the enteric health of poultry has become an obvious subject of interest in the researching field, as the primary step to better gut health is its understanding and its assessing (Wickramasuriya et al., 2022). In fact, the present study aimed to demonstrate and also to assess the impact of various factors - primarily related to the biosecurity of poultry farms' environment -on the intestinal health of broilers. A weak biosecurity level is not only responsible for the apparition of respiratory diseases (caused by high levels of ammonia and poor ventilation) but also for the apparition of gut related illnesses since the immunological system of broilers can be adversely affected by environmental stresses, which could potentially lead to the inability to maintain microbes in their GIT lumen and the subsequent translocation of bacteria into the bloodstream (Weiss & Hennet, 2017; Wang et al., 2023).

MATERIALS AND METHODS

Animals and Sampling

Broiler chickens were obtained from 18 commercial farms located in the Doukkala area in the Casablanca-Settat region in Morocco. Two samples consisting of five randomly selected healthy birds were collected from each farm at 11 days old and 35 days old (Apajalahti et al., 2004; Wise & Siragusa, 2006; Crhanova et al., 2011). Animals were transported to the Avian Pathology Unit in Hassan II's Agronomy and Veterinary Institute. Animals were euthanized the same day as they arrived using the cervical dislocation method (Martin, 2015; Leary et al., 2020). Before proceeding to the necropsy examination and the collection of samples, the carcasses were bathed quickly using tap water and soap, and the viscera were exposed by the sectioning of ribs.

Biosecurity evaluation grid system

In order to proceed with the study, the level of biosecurity of the farms was determined by using an evaluation system based on the parameters that appear in the Law n°49-99, relating to the sanitary protection of poultry farms, the control of the production and marketing of poultry products, promulgated by the Dahir n°1-02- 119 of 1 rabii Il 1423 (13 June 2002).

Many criteria were used to evaluate farms, commonly served to check feed, light, litter, air, water, (bio)security, sanitation, space and staff, according to management tips for better poultry performance found on multiple guides (AVIAGEN, 2018; Confédération paysanne, 2018; GIPAG, 2017; Putnam, 2016). Actually, each parameter was assigned an impact factor subjectively by the study group,

Table 1: List of Criteria for biosecurity parameters adapted to the study.

N°	Parameter	Criterion	Impact factor
	Fencings	Presence and quality of fencing	3
	Autoluve	Presence of functional autoluve	3
	SAS	Operational Sanitary SAS	3
	Dress code	Proper Attire (boots or pedibags, gloves)	2
	Surroundings	Clear building vicinity and concrete edges	1
	Footbaths	Presence of operational footbaths (clean water, disinfectant solution)	3
	Atmosphere	Quality of the atmosphere;	3
	Temperature and Ventilation and ventilation	State of the means of control of temperature and ventilation	3
	Cleaning	Easiness to clean floors, walls, and ceilings of buildings	2
	Surface/Density	Surface/density ratio	3
	Chicken's state	General state of chickens (ruffled feather, immobile chicks, state of feet)	3
	Feeders and waterers	Suitability of feeders and waterers (condition, good height, ease of access, presence of food)	2
	Food	Food presentation and quality	3
	Pests	Pest control (rodent and insect control)	
	Presence of an input and output storage zone	Presence of an input and output storage zone	2
	Storage quality	Quality of feed storage (humidity, ventilation, temperature, and cleanliness).	3
	Water Quality	Quality of drinking water (water hardness and use of products)	3
	Record	Presence of maintained records	3
	Corpse pit	Presence of functional and hermetically closed corpse pit.	3

varying from 1 to 3. (Table 1) A weighted score of either 1, 2, or 3 was then attributed to each of these parameters for each farm, 1 being poor, 2 indicating moderate, and 3 indicating good. The data gathered during the visits made to the 18 farms, located in the Douakala area, allowed us, to fill in the evaluation grid system and obtain a general score for each farm. Thus, we could classify these farms, into three groups, in terms of biosecurity performance: group **D** for "decent" performance, group **G** for "good" and group **P** for "poor". To classify the farms, the followings intervals were used (see Table 2).

Table 2: Intervals used to categorize the farms into three groups.

Score X	50≤ X ≤80	80< X ≤120	120< X ≤150
Group	P	D	G

The extreme numbers were calculated using the worse and best-case scenarios, by attributing scores of "1" to all the parameters to get the lowest score, and scores of "2" and "3" to determine the average and highest scores respectively. The intervals were then subjectively agreed upon by the workgroup.

The link between biosecurity levels and gut health of the birds was made using stacked bar graphs for each group, comparing the evolution of macroscopic and microscopic lesions on 11 days old and 35 days old chicks.

Necropsy

The examination of multiple apparatus was carried on the following apparatus and organs: the locomotor apparatus, the respiratory tract, the liver, the heart, the digestive tract and annexed glands, and the urogenital tract. The lesions observed were then scored using a macroscopic lesion scoring system ranging from 0 to 3 (Kraieski et al., 2017). Pieces the intestinal portions (duodenum, jejunum, ileum and caeca) and the liver were collected for histopathology. The samples were sent to the anatomical pathology laboratory of Hassan II's Agronomic and Veterinary Institute for the preparation of histology slides using the hematoxylin and eosin dye, and lesions were scored using the ISI (I See Inside) method (Belote et al., 2019).

During the carrying of the autopsies, the scoring of macroscopic lesions was recorded on an excel sheet containing different sections for organs and the description of some lesions. The processing of microscopic lesions was done after receiving the histopathology slides from the IAV's anatomical pathology laboratory which took on average from a week to a couple of weeks after the realization of the autopsies, the microscopic lesions were then scored in the same manner as the macroscopic ones.

Bacterial screening

The bacterial translocation of liver was performed, MacConkey plates and Columbia agar enriched with 5% sheep blood plates were streaked and incubated at 37°C for 24 h. Bacterial identification was done using the API 2O E Enteric identification system (bioMérieux).

In order to test for *Clostridium perfringens* presence, the fecal samples from the caeca and content samples from the ileum were diluted to 10^{-1} with a saline solution and inoculated on a Tryptose Sulfite Cycloserine (TSC).

The plates were put in an anaerobic jar system and incubated at 37°C for 24h. The growth was tested for *Clostridium perfringens* using Columbia agar enriched with 5% sheep blood (Nazki et al., 2017), streaked with colonies from the TSC plates to demonstrate double hemolysis. Buffered Peptone Water was inoculated with colonies from TSC medium, sealed with wax, and incubated at 37°C for 48h to show gas production.

The growths observed on the TSC plates were scored in order to quantify the clostridial population present on the ileum and caeca at 11 days (D11) and 35 days (D35). The scoring was carried out as stated below (Table 3).

Table 3: Scores attributed to TSC agar plates' growths

Score X	Signification
3	Saturation of the plate
2	Up to 70% of the plate presents growth
1	Less than 20% of the plate presents growth

Coccidiosis oocyst detection

Intestinal scrapings from duodenal, jejunal, ileal, and caecal portions were spread on microscope slides and viewed under an X40 lens (Garton, 2014).

Slide preparation for histological observations

Fixation of the samples was carried out using a 10% formalin solution for at least 5 days. The samples were then dehydrated using a series of ethanol baths and cleared with two baths of toluene. The samples were carefully positioned in a mold filled with molten paraffin and oriented according to their plane of section. After that, the resulting block was cooled. A JUNG microtome was used to cross-section paraffin-embedded samples into thin slices of 5 micrometers. The sections were then attached to slides washed in advance using an albumin-water solution and dried overnight at 50 °C in an incubator. The samples were deparaffinized, rehydrated, and then stained using hematoxylin and eosin stains (Slaoui & Fiette, 2011).

RESULTS

Farm evaluation and biosecurity levels

The general scores obtained using the evaluation grid system allowed for the categorizing of the 18 farms, and the results were as the following (see Table 4):

Table 4: Number of farms of each group.

Score X	50≤ X ≤80	80< X ≤120	120< X ≤150
Group	P	D	G
Number of farms	5	7	6

An average of the general scores obtained was then calculated for each group (see Figure 1).

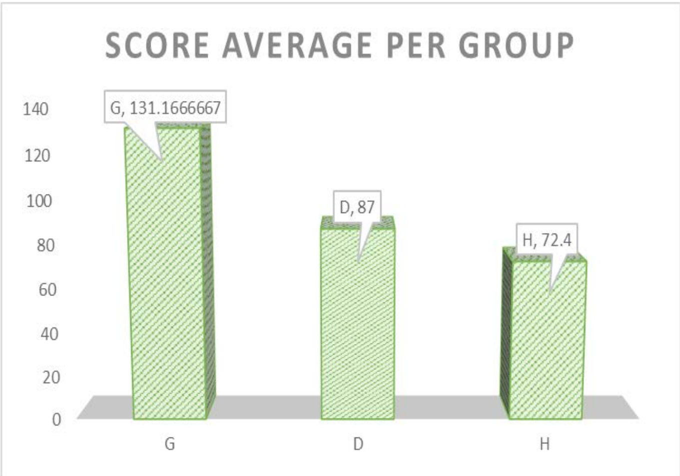

Figure 1: A chart displaying the difference in the average score between the three groups.

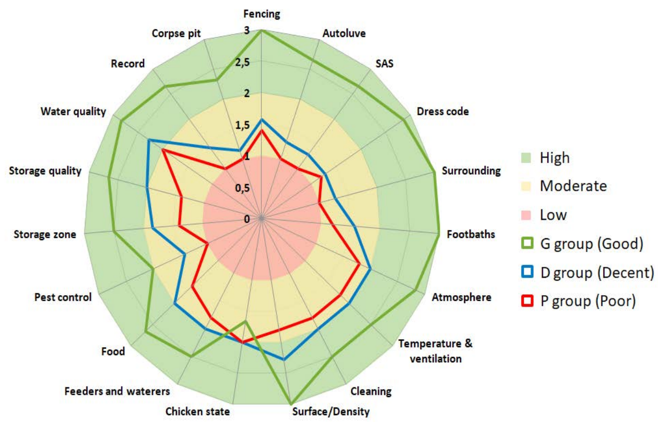

Figure 2: Radar charts exhibiting the average scores of the farms in the 3 groups regarding 19 parameters of biosecurity.

The evaluation grid system also permitted the construction of radar charts for each group containing the biosecurity parameters (see Figure 2): The radar charts exhibit a general overview of the characteristic of each group. The charts were built based on the average scores of the farms of each group tallied on each of the 19 parameters included in the evaluation grid system.

The figures reveal that both the P group and the D group seem to be lacking in terms of parameters with an impact factor of "3" such as the fencing quality, the presence of a functioning autoluve and a sanitary room, pest control, and the presence and maintenance of both a record and a corpse pit. Farms of the D group tend to score better than those of the P group regarding parameters such as the surface/density ratio, quality of food, presence of footbaths, and the presence and quality of a storage zone. On the other hand, farms in the G group exhibit a low average score as to the general state of the chickens and moderate scores concerning pest control and the presence of a corpse pit.

Necropsy results

The macroscopic lesions that stood out in 11 days old chicks are notably those of friability of the liver (Image 1), congestion of the duodenum and the jejunum and peteachiae in the caecum (see Image 2).

Image 1: Friability of the liver (score 3).

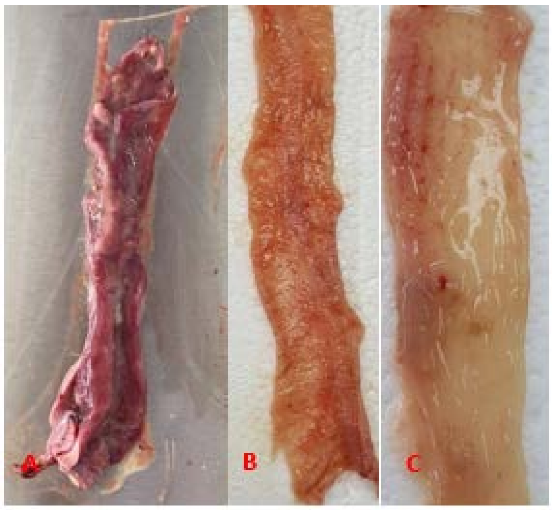

Image 2: (A) Portion of the duodenum with severe congestion (score 3). (B) Congestion of the jejunum (score2) with thinning of the walls (score 1). (C) Petechia on the caeca (score 1).

At day 35, the most striking lesions were those of bilateral pododermatitis (Image 3) and liver necrosis paired with congestion and desquamation of intestinal walls (Image 4). The macroscopic and microscopic lesions were then presented side by side for each farm on day 11 and day 35, using stacked bar graphs. A graph was constructed for each group as the following: The same color was used on the stacked bars to depict the macroscopic and microscopic lesions of the duodenum, the ileum, the caeca, the liver. (Figures 3, 4 and 5).

Image 3: Bilateral pododermatitis on a 35 day old broiler chicken.

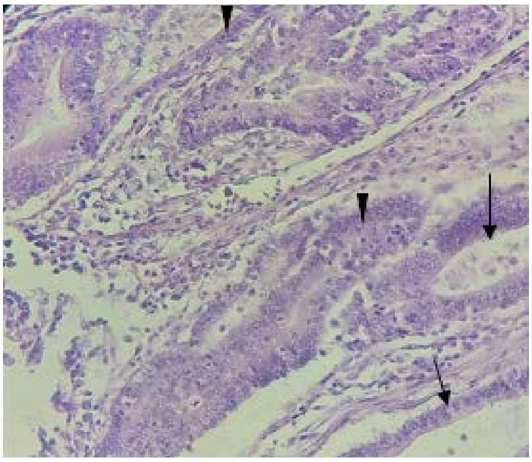

Image 4: Histopathology slide of the mucosa of the ileum portion of a broiler chicken at 35 days presenting deformed villi and enlarged crypts containing inflammatory cells (arrow), and *Eimeria* in the mucosa (arrowhead). Hematoxylin and eosin coloration, x40.

A general augmentation of macroscopic and microscopic lesions on day 35 (D35) compared to day 11 (D11) was observed in the D group (Figure 4) and the G group (Figure 3), where 85% and 83.3% of farms observed an increase in terms of macroscopic lesions, and 71.4% and 83% of farms reveals a rise in microscopic lesions, in the D group and G group respectively. Whereas the P group (Figure 5) exhibited an increase in macroscopic lesions in all the farms, but a fall in microscopic lesions in 60% of the farms. The Figures 6 and 8 both depict point cloud graphs of the distribution of macroscopic lesions on 11 days and

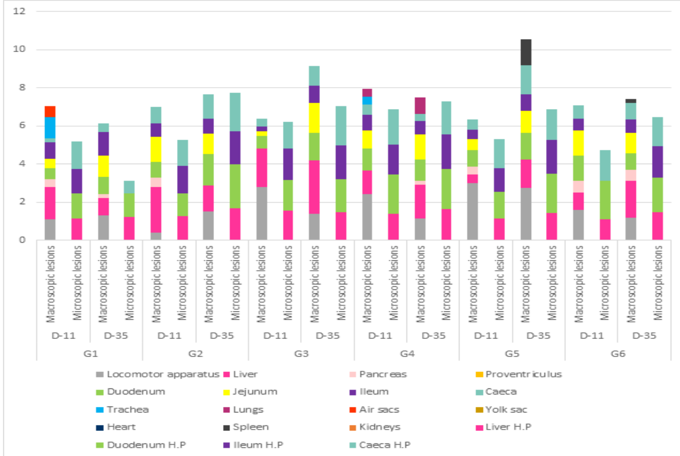

Figure 3: Stacked bar graphs displaying macroscopic and microscopic lesions found on day 11 and day 35 of broiler chickens of farms of the G group.

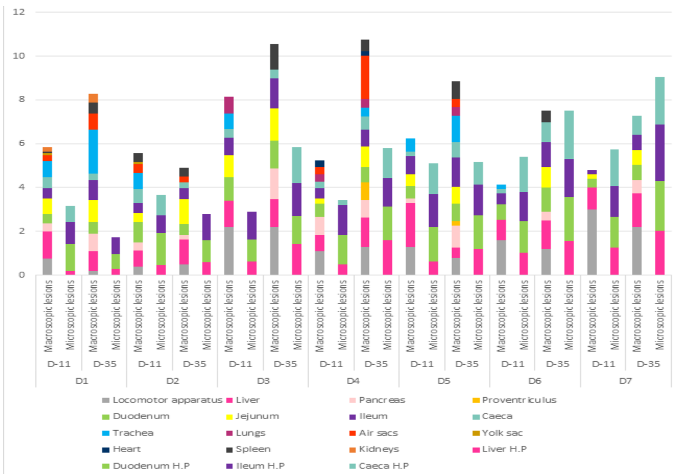

Figure 4: Stacked bar graphs displaying macroscopic and microscopic lesions found on day 11 and day 35 of broiler chickens of farms of the D group

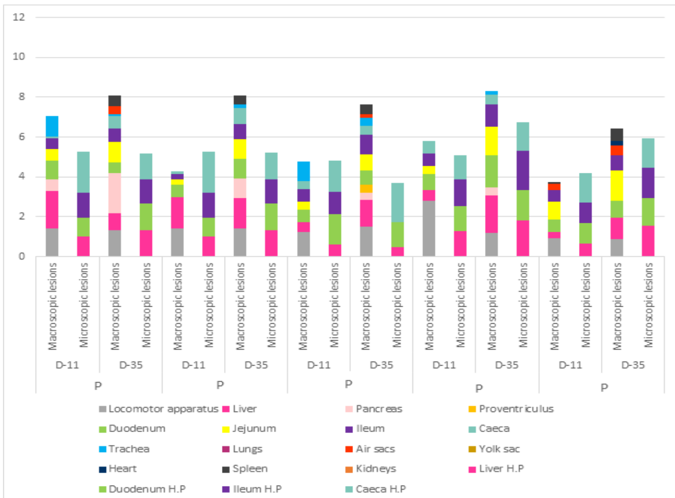

Figure 5: Stacked bar graphs displaying macroscopic and microscopic lesions found on day 11 and day 35 of broiler chickens of farms of the P group.

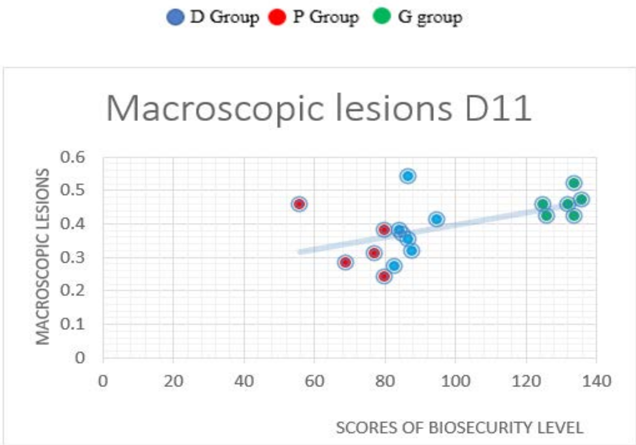

Figure 6: Point cloud representing the distribution of macroscopic lesions throughout the farms groups on day 11.

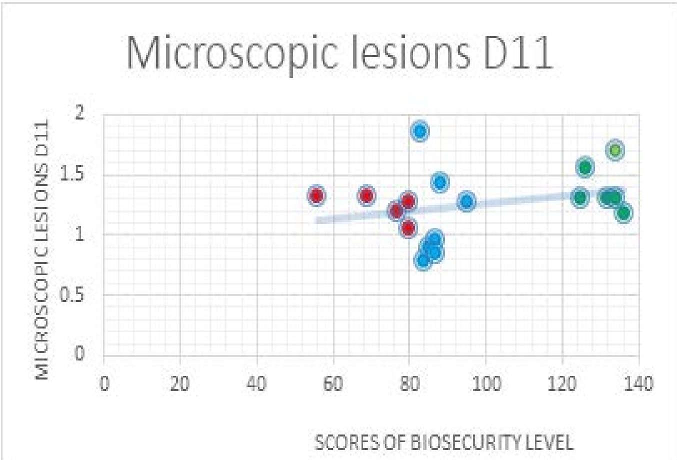

Figure 7: Point cloud representing the distribution of microscopic lesions throughout the farms groups on day 11.

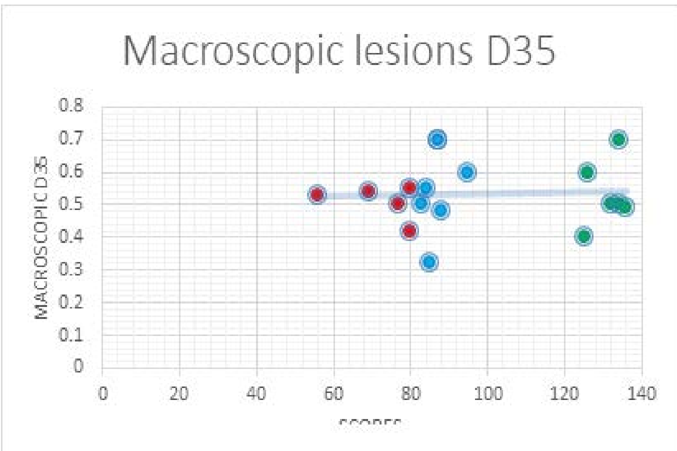

Figure 8: Point cloud representing the distribution of macroscopic lesions throughout the farms groups on day 35.

35 days old broilers on the 3 farm groups. On one hand, there is a significant increase of macroscopic lesions on D11 throughout the farms with the increase of biosecurity scores. On the other hand, there was only a slight increase noted for macroscopic lesions on day 35.

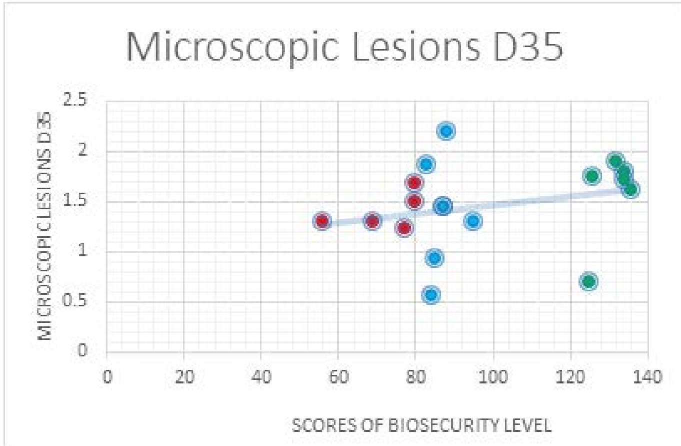

Figure 9: Point cloud representing the distribution of microscopic lesions throughout the farms groups on day 35

The Figures 7 and 9 both depict point cloud graphs of the distribution of microscopic lesions on 11 days and 35 days old broilers on the 3 farm groups. What was observed is that there is a significant increase of microscopic lesions both on D11 and D35 throughout the farms with the increase of biosecurity scores.

Eimeria oocyst detection

No oocysts were detected at 11 days old on any gut portion on any farms. However, on the 35 days old chicks, different levels of oocyst detection depending on the intestinal portions and the farm groups were noted (Figure 10). Oocysts were also visualized on the histopathology slides, embedded in the mucosa of different gut portions, image 3 shows Eimeria oocysts implanted in the mucosa of the ileum.

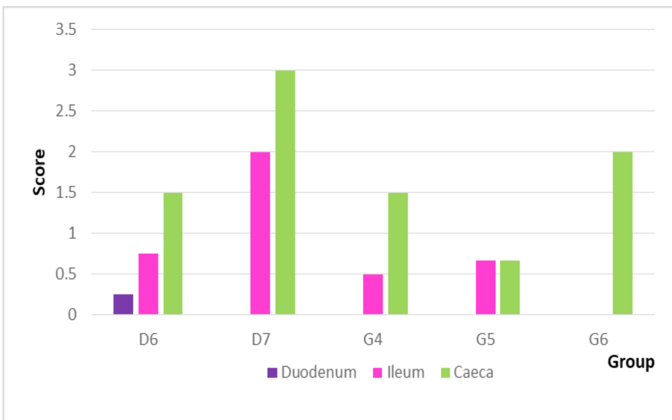

Figure 10: Eimeria oocysts detected microscopically at day 11.

The point cloud graph shows the decline in the distribution of coccidiosis infections throughout the farms, where it is the highest in the farms of P group (red dots), and the lowest in the G group (green dots) (Figure 12).

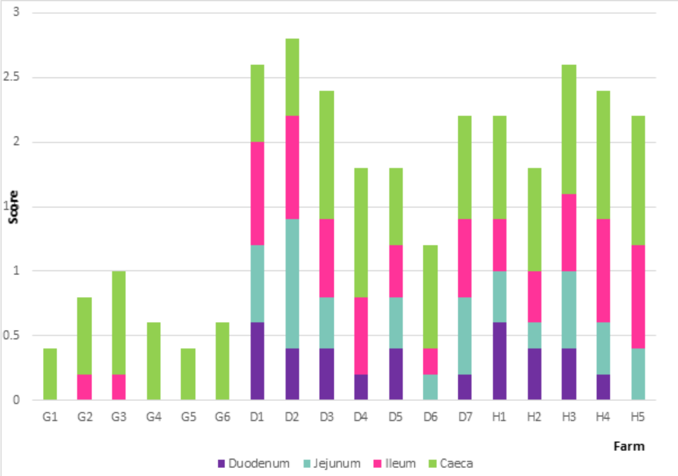

Figure 11: Average of *Eimeria* oocysts detection rate in every farm at day 35 from intestinal scrapings.

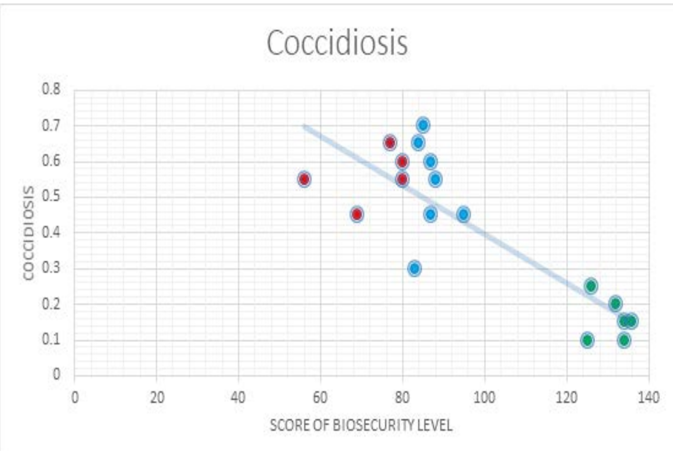

Figure 12: Point cloud distribution of Coccidiosis throughout the farm groups at day 35.

LIVER BACTERIAL TRANSLOCATION

In terms of diversity, the P group showcased the highest number of bacteria species detected and five (5) out of the six (6) species are foodborne pathogens. *E. Coli* was found on 38% of the farms, *Staphylococcus aureus* on 31% of the farms, *Salmonella* spp. on 15 % of the farms, and both *Staphylococcus* spp. and *Yersinia enterocolitica* on 8% of the farms (Figure 13).

D group presented five (5) bacteria species in total, whereas the G group only displayed four(4) bacteria species, four(4) bacteria species out of the five (5) species exhibited in the D group, and all the bacteria found in the G group were foodborne pathogens. *E. coli* was found on 44% and 40% of the farms, *Salmonella* spp. was found in 12% and 20%, *Staphylococcus aureus* was found on 19% and 20% of the farms, and *Staphylococcus* spp. was found in 25% and 20% of the farms, in D group and G group respectively (Figure 13).

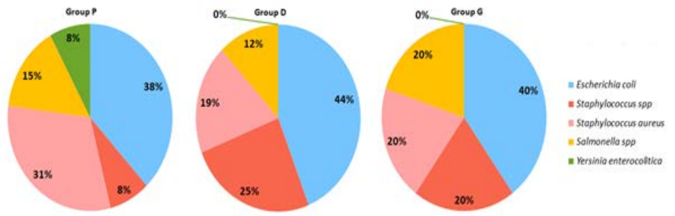

Figure 13: Percentage of the bacteria present on livers of broiler chickens from the 3 groups.

CLOSTRIDIUM PERFRINGENS DETECTION

The clostridial population in all the farms has risen, since 80% of the farms of the P group, 66.66% of the farms of the G group, and 57.14% of the farms of the D group have encountered an increase on day 35 compared to day 11 on the caecal level. As for the ileum, a clostridial population was detected on all the farms of the P group, and on 50% of the farms of the G group, and on 28% of the farms of the D group.

The results obtained from these tests showed that from the 18 farms tested, *Clostridium perfringens* was only detected on the farms listed below (see Table 5). Considering the results exhibited in the table above, it appears that within the farms where a clostridial population was detected on the ileum, *Clostridium perfringens* presence was only detected on one farm: P5.

Table 5: Detection of *Clostridium perfringens* on some farms.

Farm	D11		D35	
	Caeca	Ileum	Ileum	Caeca
P4	+			
P5	+	+	+	+
D4				+
G3	+			+

DISCUSSION

Gut health is capital and vital in every animal production, seeing its consequential impact on the production performances. In poultry, intestinal health is equivalent to animal health. Although there isn't a clear-cut definition of gut health that encompasses all its physiological functions - including nutrient digestion and absorption, a stable microbiota, intestinal barrier functions, and mucosal immune response - a direct connection between possessing a healthy gut and animal performance does exist. Actually, there are no defined means by which one can state what gut health is, nor how to assess it or more importantly how to measure it. It is in the current of trying to evaluate the health of the birds' guts that this study was partially aiming for, among other goals.

Upon working on the 18 farms, it has been noted that broilers from the farms all around suffered a certain level of intestinal discomfort. The intestines suffered mild to severe gut lesions, on both chicks aged 11 and 35 days old. The dominant lesions being the congestion, and the desquamation of the mucosa, and changes in the thickness of the intestinal walls. One can easily speculate that the gut damages observed are directly connected to the enormous stress that the guts are under, which is an immediate aftereffect of the rapid growth that the broiler production industry has seen soar these past decades. Indeed, the weight of a 42-day old broiler has reached 2,900 g whereas a bird of the same age weighted approximatively 600 g in 1957 (Zuidhof et al., 2014).

These damages suggest that an inflammatory process is still ongoing on most farms at day 35, as well as a regeneration process on some of them. On the microscopic side, the intestinal lesions observed coincide with the macroscopic ones, and the predominant lesions in the gut portion were mainly exhibiting notable inflammation within the intestinal epithelium and the lamina propria and high levels of enterocyte proliferation.

The bacterial translocation method used on the livers of 11 and 35 days old chicks shows that all the tested broilers carried bacterial entities on their livers. These results are logical and comply with the type of microscopic lesions observed. Indeed, the bacteria found in the liver allude to a breach in the intestinal barrier which allowed the passage of pathogens. The breach in question is actually the loosening of the tight junctions that keeps the enterocytes coapt together, which prevents the crossing of the bacteria to the bloodstream and adjacent organs (Camilleri, 2019), and in this case, to the liver. This phenomenon is identified as the "Leaky gut" (Kinashi & Hase, 2021).

The bacteria found on the livers of the broilers from all of the farms were pathogens such *as Escherichia coli (E. coli), Salmonella* spp., *Staphylococcus aureus, Staphylococcus* spp., *and Yersinia enterocolitica*. These bacteria are pathogens that can be harmful to human consumption and health in general.

Bacteria such as *E. coli* and *Staphylococcus* spp. are commensal entities widely encountered in the gastro-intestinal microbiota, but their presence in the liver is synonymous in most cases with a systemic infection. It is not only harmful to the birds (by inducing colibacillosis) but also to consumers, as some strains of *E. coli* that produce the Shiga toxin can be highly deleterious (Kim et al., 2020). In the same manner *Staphylococcus aureus* is a pathogen that is known to induce Staphylococcal Food Poisoning through its production of the Staphylococcal enterotoxin B (Abolghait et al., 2020). *Salmonella* is also a well-known pathogen that is the causable agent of Salmonellosis and is one of the major agents of food-borne infections (Ehuwa et al., 2021). This bacterium has been found in nearly 39% of the farms studied. Although *Yersinia enterocolitica* was only detected on one farm, it isn't a pathogen that should be glossed over, seeing that it causes Yersiniosis which is a highly detrimental infection for the animal as well as for the consumer (Galindo et al., 2011; Shoaib et al., 2019).

These findings support the general idea that animal health and welfare are closely related to humans' health. Poultry products are considered to be one of the major food poisoning sources (Centers for Disease Control and Prevention, 2021). It goes without saying that securing livestock's good health is primordial, and adheres to the One Health policies claiming that global health security must be addressed and that human health cannot be separated from animal and ecosystem health.

One of the most widespread infections in poultry is coccidiosis, and despite the implementation of strict biosecurity measures and because *Eimeria* species are ubiquitous in the environment, it is extremely hard to keep broiler flocks free of coccidiosis (Allen & Fetterer, 2002). Granted, few severe clinical coccidiosis cases are encountered nowadays due to the broad use of anticoccidials; nonetheless, mild and subclinical infections are still pretty common.

During our study, a search for coccidial oocysts was conducted primarily through the analysis of mucosal scrapings from different gut portions using a microscope, and using this approach it has been found that 11 days old chicks did not show any sign of coccidiosis infection; nevertheless, at day 35 all the farms presented different levels of detection of oocysts. After the analysis of the histology slides containing the duodenal, ileal, and caecal portions, coccidiosis infection was indeed detected in 28% of the farms on day 11, as gametocytes were observed at a cellular level, embedded into enterocytes.

The absence of detection of a coccidiosis infection in most of the farms on day 11 could be explained by the following parameters: the first one being the use of anticoccidials incorporated in the feed of young chicks; and the second one being the climate in which most of the samples were taken. Indeed the samples gathered from the farms were taken between the months of March and July. Variation in temperatures as well as in humidity levels varied during this period of time, which takes in the seasonal parameter into account. The farms in which coccidiosis infection was detected in 11 days old chicks were sampled last, during the month of June when the temperatures in the Doukkala area were high as well as the humidity levels, both being imperative parameters needed by the Eimeria species in order to thrive. These findings are supported by the study done by (Awais et al., 2012), in which the impact of the seasonal parameters on coccidiosis infection prevalence was demonstrated. Indeed it was observed that the prevalence of this infection soared during the season of autumn and summer.

Although the coccidial infections that we encountered during our study were mostly sub-clinical, they aren't without consequences. In fact, during the schizogonic and gametogonic phases (Taylor et al., 2016), the implementation of the gametes into the mucosa and the releasing of the oocysts causes lesions to the mucosal barrier that predispose the broiler to bacterial infections such as those caused by clostridium perfringens.

Clostridia are anaerobic germs that colonize the caeca of healthy birds and adopt a commensal interaction with the host. As stated before, a clostridial population at the caecal level is normal, contrarily in the ileum, in which the proliferation of clostridia bacteria shouldn't happen in healthy birds. In this study, all the farms tested presented different levels of clostridial presence in the caeca, and 22% of the farms displayed the presence of *Clostridium* spp. in the ileum. The presence of *Clostridium* spp. in the ileum could indicate the occurrence of a shift in the microbial communities of the ileum, indeed shifts in the gut's microbiota could happen following multiple causes, and stress is considered one of the major ones. It can be induced by heat, feed composition/processing/additives, litter management, or use of antibiotics, and although the mechanisms are yet to be elucidated, increasing evidence incriminates it when it comes to altering host-pathogens interactions and thus causing shifts into the gut's microbiota (Weiss & Hennet, 2017; Oviedo-Rondón, 2019). These shifts observed within the farms exhibiting clostridial presence at the ileum level could be considered as early red flags, signaling the start of a certain imbalance and weakening of the immune system, which in turn facilitates the colonization of the gastrointestinal tract (GIT) by a variety of not only pathogenic but also zoonotic bacteria. During this study, parameters such as antibiotic use, feed programs, additive use, and disease history were difficult to inquire about and thus weren't taken into account, making it somewhat difficult to properly justify some elements and results.

Although lesions such as thinning of the intestinal walls and the presence of pseudo membranes coating the intestinal mucosa were observed, along with inflammation of the liver and occasional kidney injury, which are all direct damages characterizing necrotic enteritis (Abd El-Hack et al., 2022). *Clostridium perfringens* was only detected on 22% of the farms using the bacterial isolation method and identification.

These results do not eliminate the possibility of infection by *Clostridium perfringens* in the other farms, as bacterial isolation is not an infallible method for its detection. Molecular tools such as the Polymerase Chain Reaction are more efficient when it comes to *Clostridium perfringens* detection, seeing that they rely on the detection of toxins produced by the pathogen and therefore are more reliable for necrotic enteritis diagnosis.

On one hand, when it comes to macroscopic and microscopic lesions, an augmentation was observed on day 35 compared to day 11 in the D group and the G group, where 85% and 83.3% of farms observed an increase in terms of macroscopic lesions. 71.4% and 83% of farms saw a rise in microscopic lesions in the intestinal portions as well as the liver, in the D group and G group respectively. The results found in these groups are approximately similar. The majority of the farms sustained more lesions in the GIT on day 35 compared to day 11. The rise in microscopic lesions indicates the ongoing of an inflammatory process in the guts, which takes a toll on the production of the farms as a considerable amount of the broilers' energy is spent in order for the birds' immune system to counter the challenges that they faces, whether they be of infectious or non-infectious causes.

On the other hand, the P group seems to stand out in the percentage of farms that exhibit a decrease in microscopic lesions. Indeed, while a uniform augmentation of macroscopic lesions was observed in all the farms of the P group, 60% of the farms showcased a fall in microscopic lesions. It can be speculated based on the microscopic lesions found that the farms belonging to this group have already entered the regenerative phase that follows an inflammatory process.

Microscopic lesions found on the liver at day 35 such as hepatocytes vacuolization are characteristic of inflammation within the liver caused by toxins produced by *C. perfringens*. It has been shown that vacuolization of hepatocytes is a phenomenon occurring after toxin exposition of the cell, as a mechanism of protection, the cells are then resistant to degradation but cannot regenerate. The presence of immune cells within the liver tissue disseminated but also in clusters around bile ducts and portal spaces are also strong indicators of ongoing inflammation within the organ (Nayak et al., 1996; Germolec et al., 2018). The increase of gut lesions on a microscopic scale on day 35 compared with day 11, like inflammatory cell infiltration within the epithelium and the lamina propria, as well as the thickening of the epithelium and proliferation of goblet cells that lead to a boost of mucus production are all markers of the inflammation phase (Kellermann & Riis, 2021).

No trend in coccidiosis infections was observed in both the D and the P groups; both showcased different levels of infection in the different intestinal portions.

On the other hand, the G group stood out when it comes to coccidiosis oocyst detection. Indeed, 66% of the farms in that group exhibited infection in the caeca only, and 33% of the farms showed infection in the caeca and ileum. These results could be related to better litter management in the farms of this group. As a matter of fact, wet litter is known to be a predisposing factor that enhances the chances of infection by *Eimeria* species. Ventilation and temperature modulating tools are also of higher quality and tend to be more efficient in this category, which helps to reduce the impact of coccidiosis infection in broilers.

Four different foodborne pathogens were detected in both the D and G groups, versus (five) 5 species in the P group farms, indicating that no important difference in the diversity of pathogens is exhibited between the three groups. The presence of a clostridial population in the ileum being highly unusual, and all the farms of the P group exhibiting it leads us to believe that the level of biosecurity might be of importance when it comes to limiting the colonization of the ileum with these bacteria. Higher levels of biosecurity encountered in the D and G groups are likely to have led to higher chances of keeping the ileum of broilers intact from clostridial species.

Depending on the level of biosecurity of the farms, the performances of broilers can be impacted on different levels and to different degrees. Higher biosecurity levels might be helpful in avoiding early onset of gut inflammation and clostridial colonization of upper gut portions. Lower biosecurity levels might have the opposite effect with early onset of inflammation, which leads to an earlier regeneration process in the birds' lifespans, which consumes a lot of energy and thus lowers production performances. Data gathered in this study might not be sufficient in order to set writing on stone when it comes to the impact of biosecurity levels on broilers, but what can be stated is that high biosecurity and management alone are not the only factors affecting the health of the animals and thus farm production. In truth quite a number of parameters must be accounted for, such as the level of technical efficiency of the breeder, the experience degree of the farm workers, as well as the quality of 1-day old chicks and feed.

CONCLUSION

Biosecurity measures play a critical role in maintaining gut health and ensuring better animal performance. The study found that broilers aged 11 and 35 days old exhibited intestinal discomfort and gut lesions, which could be linked to the rapid growth of the broiler industry. Bacterial translocation tests revealed that all tested broilers carried harmful bacterial entities, such as *Escherichia coli, Salmonella spp., Staphylococcus aureus, Staphylococcus spp., and Yersinia enterocolitica*, on their livers, indicating potential risks to human health. This underscores the importance of implementing effective biosecurity measures to ensure livestock's good health and, consequently, human health. Furthermore, the study also detected coccidiosis infection in broilers aged 35 days old, demonstrating that maintaining flocks free of coccidiosis can be challenging, even with the use of anticoccidials.

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CONFLICT OF INTEREST

The authors have no conflicts of interest to declare. All co-authors have seen and agree with the contents of the manuscript and there is no financial interest to report.

STATEMENT OF COMPLIANCE WITH STANDARDS OF RESEARCH INVOLVING ANIMALS

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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NOVELTY STATEMENT

Integrating an advanced biosecurity management approach, leveraging comprehensive analysis tools, and conducting rigorous risk evaluations empower professionals in the broiler industry to establish and adjust parameters based on their available means. This study reveals the significant role of biosecurity measures in maintaining gut health and enhancing animal performance. The findings in this study underscore the importance of implementing effective biosecurity measures to ensure the well-being of livestock and, consequently, human health. This research emphasizes the need for proactive management strategies, comprehensive risk evaluation tools, and continuous monitoring. By employing biosecurity evaluations, professionals can establish and adapt parameters according to their available resources, facilitating targeted interventions and promoting sustainable practices in the broiler industry.

AUTHORS CONTRIBUTION

The authors of this study, Dr. Skenndri Safae and Dr. Charrat Nadia, made equal contributions to both the research and the development of this manuscript. Their collaborative efforts and shared expertise enhanced the outcomes of the study. Dr. Jmiai Mehdi played an active role as a participant, providing valuable insights and contributing to the experimental design. Additionally, Professor Nassik Saadia provided invaluable guidance and supervision throughout the project. The collective efforts of all authors have led to the completion of this study and the preparation of this manuscript.

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