Cryptosporidium sp. infection in the broiler chickens and turkeys on farms in north central Algeria

S. Guechtouli, N. Mimoune, C.-R. Messai, O. Salhi, R. Kaidi and D. Khelef*

Abstract

This study aimed to determine the prevalence of cryptosporidiosis in broiler chickens and turkeys and the factors that can influence its development, the precision of the most frequent sites of *Cryptosporidium,* and the severity of lesions associated with the disease. From October 2019 to September 2020, the prevalence of Cryptosporidia infection was determined on 22 farms located in north central Algeria. For each farm, weekly visits were conducted during the study period and information on the type of farming and health status was collected. At these farms, 98 chickens and 22 turkeys were examined and clinical lesions recorded. Analyses of faeces, tissue samples and scrapings made from the intestine, bursa of Fabricius, trachea, cloaca and proventriculus revealed a prevalence of 55% at chicken farms. The age of chickens was found to be a significant factor, with a high frequency of the disease observed in chickens aged between 10 to 50 days with a maximum between 31 and 40 days (84.62%). The parasite was not detected in chickens younger than 7 days. The most common site of *Cryptosporidium* sp. was the bursa (24.2%), followed by the trachea (21.5%), cloaca (18.9%), intestine (16.3%), and proventriculus (6.6%). In turkeys, the overall prevalence was 41%. The bursa was the most infected organ (23.81%), followed by the cloaca (15.79%) and intestine (9%). For both avian species, data confirmed the impact of season, strain (genetic potential), and rearing conditions on the prevalence of *Cryptosporidium* sp. To conclude, our results showed that *Cryptosporidium* was very common on these farms, and was related mainly to climatic conditions and poor management, particularly hygienic measures.

Key words: *Cryptosporidiosis; broiler chicken; turkey; prevalence*

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Introduction

Cryptosporidiosis is a disease caused by small protozoa belonging to the group of Coccidia (Cavalier-Smith, 2014; Starič et al., 2020). Cryptosporidia mainly colonize the digestive epithelium, but also respiratory, biliary and urinary tracts (Ryan and Hijjawi, 2015). This is a cosmopolitan parasite, infecting a large number of mammal species (including humans) and birds, and more rarely, reptiles and fish (Ibrahim et al., 2007; Mallinath et al., 2009; Baroudi et al., 2013) causing clinical and subclinical infections (Gomes et al., 2012; Santín, 2013). Discovered in the peptic glands of the mice stomach (Naciri, 1992), they have long been considered commensal parasites, possibly responsible for opportunistic infections (Current and Garcia, 1991).

Panciera et al. (1971) described an episode of diarrhoea in an 8-month-old heifer, presumably attributable to Cryptosporidia. Since then, the pathogenic role of this parasite has been widely demonstrated in humans and animals. The development of the AIDS epidemic since the early 1980s has made cryptosporidiosis a very topical disease. Indeed, the lack of aetiological treatment for this infection makes it one of the leading causes of death in immunocompromised animals (Naciri et al., 1989).

In birds, *Cryptosporidium* is one of the most important parasites in poultry and can infect more than 30 avian species (Wang et al., 2014). The first description of *Cryptosporidium* infection among birds was reported by Tyzzer (1929) and involved the caecal epithelium of chicken (Nakamura and Meireles, 2015). Three species of *cryptosporidium* have been described: *C. baileyi*, *C. galli* and *C. meleagridis* (Nguyen et al., 2013; Ryan et al., 2014; Máca and Pavlásek, 2015). The importance of cryptosporidiosis is now better understood, and it is now accepted that it causes economic losses in the poultry industry. Thus, episodes of morbidity, even mortality, in ornamental birds are regularly described (O'Donoghue, 1995). In fact, Kichou et al. (1996) reported infection with *Cryptosporidium* spp. in 14 of 38 broiler farms, with a prevalence of 37%.

In Algeria, the sanitary and economic impact of *Cryptosporidium* infection on the poultry sector is poorly understood and deserves to be studied in depth. Therefore, the objective of this study was to assess the real prevalence of cryptosporidiosis in broiler chickens and turkeys and the factors determining its appearance, and the precision of the most frequent localisations of *Cryptosporidium* and the severity of lesions associated with the disease.

Material and methods

Study area

A total of 22 farms, 17 broiler and 5 turkey farms, located in different municipalities of Boumerdes region, were the subject of the study. This zone is located in north central Algeria, covering an area of 1,456.68 km², with a coastal strip exceeding 80 km, and a population of 831,000.

The climate in Boumerdes is Mediterranean, cold and humid in winter, hot and dry in summer. Rainfall varies between 500 and 1300 mm per year, from October to March. The most influential climatic parameters on the farms selected in this study are temperature and relative humidity, since 91% (20/22) of these farms are located in the coastal regions (Zemmouri, Corso, Cap Djinet).

The annual thermal amplitudes are generally low due to the proximity to the sea. Coastal regions are known for their mild and temperate climate. The average annual temperature is 18°C near the coast and 25°C inland, with relative humidity ranging from 60 to 80%. This rate is important because the hydraulic network of this zone is made up of a large set of wadis and also due to the proximity to the sea.

Farms

Species studied and strains

The poultry species selected for this study are listed in Table 1.

Table 1. Species studied

Species	Latin name	Strain
Chicken	*Gallus gallus*	ISA 15, Arbor acres
Turkey	*Meleagridis gallopavo*	Big 6, Nicolas 700

Farm size

The capacity of the buildings monitored varied from 2500 to 14,000 individuals.

Type of buildings

In total, 59% (13/22) of the buildings are agricultural greenhouses where farming conditions are difficult to manage, and 40.91% (9/22) are solid buildings where the minimum conditions are met. This choice was made in order to determine the influence of the type of building and farming conditions on the prevalence of Cryptosporidia.

Period of study

This study took place between October 2019 and September 2020 and included all four seasons to determine the influence of climatic parameters, humidity and temperature amplitudes (seasonal variation), on cryptosporidiosis in chickens and turkeys.

Experimental design

Sampling protocol

A total of 22 farms, 17 broiler chicken and 5 turkey farms, were monitored regularly with weekly visits.

Faeces

Faecal samples were taken immediately after emission, from the litter, in clean plastic bottles, labelled and stored at +4°C, in potassium dichromate ($K_2Cr_2O_7$), until examination in the Parasitology-Mycology Laboratory of the National High School of Veterinary Medicine (NHSVM, Algiers-Algeria). A total of 101 samples were taken from these farms. Each sample was accompanied by an information sheet.

Scraping of mucous membranes

120 subjects (98 chickens and 22 turkeys), showing signs of respiratory, digestive or depression, were autopsied. In some cases, fresh corpses were used. The lesions found were recorded and scrapings were performed on the mucous membranes of different organs: trachea, proventriculus, intestine, bursa of Fabricius, and cloaca. These were spread out, air dried and then fixed in methanol for 5 min. After scraping, the same organs were removed and fixed in 10% buffered formalin for histological examination in the Pathological Anatomy Laboratory of NHSVM.

Techniques used for the detection of *Cryptosporidium*

Examination of faeces

The oocysts present in the faeces were detected after concentration by the Ritchie technique simplified by Allan and Ridley and by the Ziehl-Neelsen stain modified by Henriksen and Pohlenz (1981). This method gives the best results regarding the prevalence of infection in flocks (in the case of asymptomatic carriers).

Ritchie concentration technique simplified by Allan and Ridley

In current practice, the simplified Ritchie method (ether-formalin) is more easily used (Gati, 1992), as a versatile technique performed systematically for each faecal sample. Its purpose is to concentrate a maximum of parasitic elements with a minimum of residue in a small volume of faeces. It is a two-phase (physicochemical) method that involves the hydrophilic-lipophilic balance of the parasite. It follows from that of Telemann (1908) who diluted the stool in an equal mixture of ether and hydrochloric acid (Mosele, 1998).

Ziehl Neelsen stain modified by Henriksen and Pohlenz (1981)

In this study, we opted for the Ziehl Neelsen stain modified by Henriksen and Pohlenz, where the contrast between the bright red staining of the parasite and the blue background allows for easy identification, and the obvious cytological characteristics make diagnosis more certain. This technique makes it possible to characterize the acid-alcohol resistance of germs having the capacity to retain fuchsin after treatment with an alcohol or an acid. These appear in red despite the use of methylene blue (Euzeby, 2002). These staining properties are explained by the structure of the cell wall, and in particular its richness in fatty acids and lipids, which makes it difficult for both colouring and bleaching agents to penetrate. On the other hand, the mycolic acids present on the wall of Cryptosporidia retain fuchsin (Solatges, 2008). It is a reference colour, fast, simple, inexpensive and easy to read. In addition, the slides can be stored, used for the detection of Cryptosporidia.

Examination of scrapings

After good spreading, drying and fixing in methanol for 5 minutes, the slides were stained with the Ziehl Neelsen stain modified by Henriksen and Pohlenz (1981) described above.

Histological examination

Histopathology is used to confirm positive cases by examining the scrapings to detect new individuals carrying *Cryptosporidium* sp. not detected and to study the associated lesions. The different stages of development of Cryptosporidia present as ovoid granules 2 to 5 µm in diameter, on the surface of epithelial cells where they appear to be attached to the brush border.

Statistical analysis

Descriptive statistics were performed using R software (version 3.5.2). The Chi² test of independence was used to detect links between the rate of *Cryptosporidium* infection and the tested parameters and also between the results obtained by the different methods. The confidence interval was calculated for each result. Data were considered significant when $P<0.05$.

Results and discussion

The $\chi 2$ independence test showed that there was no significant difference between the results obtained by the histological examination and the scraping method. We therefore present the means of the results obtained by these two methods. Coprology examination gave greater precision for the calculation of variations in the prevalence of *Cryptosporidium* sp. from one farm to another.

Faeces and scrapings examination

Cryptosporidium oocysts are identified as ovoid-shaped spheres, coloured bright pink on a pale green background (Figure 1). The oocyst load is average, approximately 6 oocysts per slide, because the samples were taken from

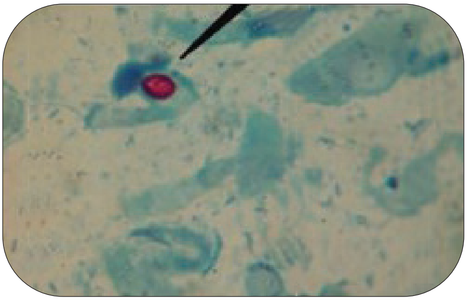

Figure 1. Cryptosporidium sp. oocyst, modified Ziehl Neelsen stain. Turkey faeces exam, x 1000.

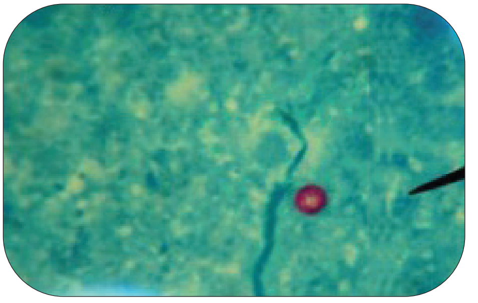

Figure 2. Cryptosporidium sp. oocyst, modified Ziehl Neelsen stain. Fabricius purse scraping examination in hens, x 100.

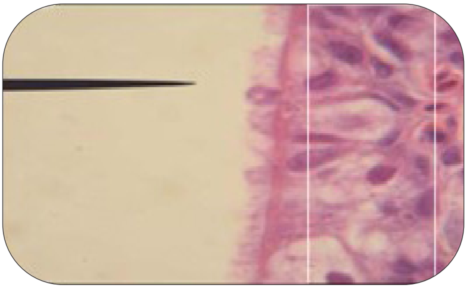

Figure 3. Attachment of Cryptosporidia to the tracheal epithelial cell, x 1000.

Figure 4. Attachment of Cryptosporidia to bursal epithelial cell, x 1000.

the litter (a mixture of droppings from healthy birds and those from sick birds).

For scraping product examinations, the degree of infestation was important for positive slides because scraping allows the parasite to detach from the mucosa and thus gives a better parasite load. Cryptosporidia appear round to ovoid, bright red on a green background. Their cytoplasm is granular, often with a lighter centre (Figure 2). All other elements are coloured green.

Histological examination

Cryptosporidia appear as ovoid elements, approximately 2 to 4 microns in diameter, abundantly covering the epithelia of the organs removed (Figures 3 and 4), especially at the level of the bursa of Fabricius which was the most affected organ.

Main histological lesions encountered

Bursa of Fabricius

The histological lesions observed in the bursa of Fabricius (Figure 5) were mainly epithelial lesions: Cryptosporidia abundantly cover the bursal epithelium, and in most cases the underlying chorion was infiltrated by inflammatory cells and epithelial hyperplasia.

These results agree well with Kichou et al. (1996) and Rhee et al. (1997), who reported that the distribution of the parasite is accompanied by infiltration of the epithelium and the underlying chorion by inflammatory lymphocyte-like cells. These epithelial changes are believed to be a tissue response to parasitic invasion by *Cryptosporidium* sp. (Goodwin et al., 1990), suggesting the immunosuppressive effect of the parasite (Rhee et al., 1997).

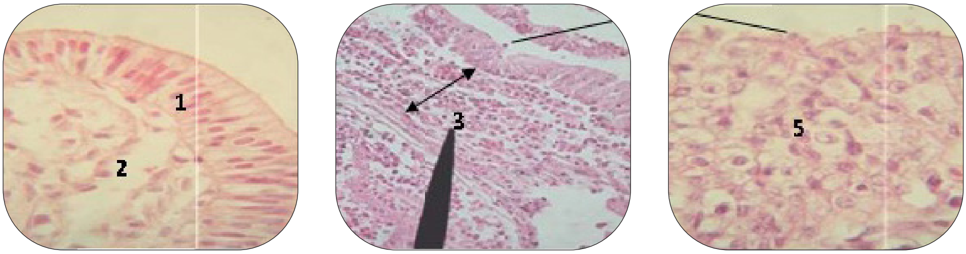

Figure 5. Comparison between healthy bursal mucosa and other infected with *Cryptosporidium* sp., Hemalun-eosin staining.
(1. Pseudo-stratified epithelium. 2. *Lamina propria*. 3. Infiltration of the *lamina propria* by inflammatory cells. 4. *Cryptosporidium* sp. attached to the surface of the bursal epithelium. 5. Epithelial hyperplasia).

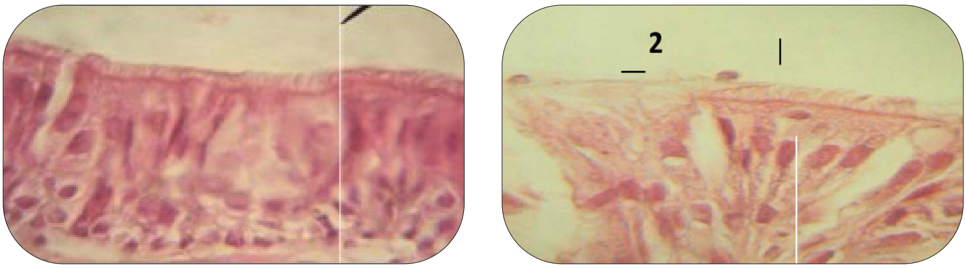

Figure 6. Comparison between healthy and infected tracheal mucosa by *Cryptosporidium* sp. H&E staining, x1000. (1. Normal pseudo-stratified epithelium of the trachea. 2. Disappearance of cilia. 3. *Cryptosporidium* sp. attached to the surface of the trachea epithelium).

Trachea

The changes observed in the trachea are hyperplasia and disappearance of the cilia from the covering epithelium. The underlying chorion is infiltrated by lymphocytes and plasma cells. These microscopic lesions are similar to those usually described (Itakura et al., 1984; Goodwin et al., 1996): epithelial hypertrophy and hyperplasia, deciliation, *lamina propria* infiltration by macrophages, heterophiles, lymphocytes, and plasma cells (Figure 6).

Intestine

The samples included all parts of the digestive tract, especially the terminal part (colon), in order to see all the lesions. The most commonly encountered abnormalities were: destruction of microvilli, villi atrophy and fusion, *lamina propria* inflammatory infiltration and hyperaemia. These lesions were the same as those described by several authors (Goodwin and Brown, 1988a; Goodwin, 1989), while Kichou et al. (1996) found no lesions in the intestine. They explained their results by the fact that their samples were taken only on the ileum, a portion which is reported to be the preferential site of *Cryptosporidium* sp. in the digestive tract (Chermette et al., 1989).

Proventriculus

The only parasitic species found in the proventriculus was *Cryptosporidium galli* (Ryan and Hijjawi, 2015). Lesions included the presence of *Cryptosporidium* spp. and infiltration of the underlying chorion by inflammatory cells.

Prevalence of infection with *Cryptosporidium* sp. in chicken

Overall prevalence

Of 98 chickens examined, 54 (55%) tested positive for *Cryptosporidium* sp. This shows a high incidence of Cryptosporidia on these farms, due to non-compliance with breeding standards and the climate of the central region of Algeria, which is favourable for the survival of the parasite (heat and relative humidity). This prevalence is higher than that reported in broilers in Korea (15%; Rhee et al., 1991), in the United States (6.4%; Goodwin and Brown, 1988a; 27.6%, Ley et al., 1988), in Morocco (24%; Kichou et al., 1996) and in Italy (26.7%; Floretti et al., 1991). In China, a low prevalence was described among broiler chickens (10%; Wang et al., 2014).

Our results are similar to those reported in broilers in the USA by Goodwin et al. (1996), where the parasite was found in 41% of lots examined, with a parasitism rate of 10 to 60%, and in Russia by Pavlasek et al. (1989), with an infection rate between 38 and 100%.

In this study, 100% of the farms surveyed were infected with *Cryptosporidium* sp. The prevalence of infection in flocks varied between 50% [40–60%] and 80% [72–88%], with 10 farms showing a frequency of 80%. This high prevalence testifies to a strong contamination between individuals of the same farm and can be explained by the hygienic conditions of the farms. This susceptibility to infection could also be due to a decrease in the immune status of animals, as cases of cryptosporidiosis have been observed in immunosuppressed chickens (Naciri et al., 1989).

Absolute prevalence

Several parameters (age, strain, breeding conditions) were examined to determine the factors that influence the Cryptosporidia infection.

Prevalence according to age

The results of *Cryptosporidium* infestation according to the age of the chickens are shown in Figure 7.

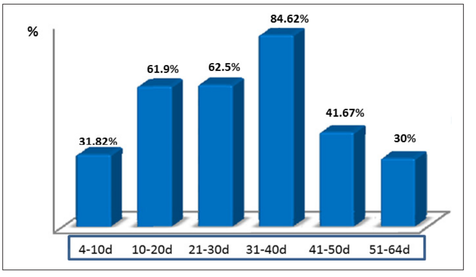

Figure 7. Prevalence of cryptosporidiosis according to the age (d: days)

The results showed a strong increase in the prevalence until the age of 40 days, then its decrease until the age of 64 days, with significant rates between 10 and 50 days of age. These results are similar to those reported by Goodwin and Brown (1988a), who found the disease in animals aged 17 to 52 days. Indeed, infection with *Cryptosporidium* sp. occurs in young chickens less than 11 weeks old (De Graaf et al., 1999) and the disease has never been reported in adult animals (Itakura et al., 1984; Goodwin and Brown, 1986), though 6 month old chickens can be experimentally infected (Goodwin, 1989).

Although the disease in chickens is not neonatal and the parasite is not found in animals less than three weeks old (Kichou et al., 1996), the rates found in our study were 31.82% [13.86– 54.87%]. This relationship can be explained by poor hygienic conditions at the hatchery as a large percentage of chicks were affected by omphalitis. These may have favoured the infestation through their debilitating action on the immune system. This rate remains significantly lower than those recorded between 10 and 50 days of age due to the resistance of animals, which likely have antibody levels against

Cryptosporidium sufficient to counteract the infection at this age.

The lowest rate, 30% [6.67–65.25%], was reported between 51 and 64 days of age and may be due to the acquisition of immunity to *Cryptosporidium* sp. Some authors confirm that a first infection allows the acquisition of subsequent resistance to the disease (Current et al., 1986; Sreter et al., 1995). The "age" and the "immunity" factors are linked and intervene concomitantly.

Prevalence according to season

Our samples were taken over a period of a year and included all seasons to distinguish the influence of climatic variations on infection with *Cryptosporidium* sp. The prevalence rates found are presented in Figure 8.

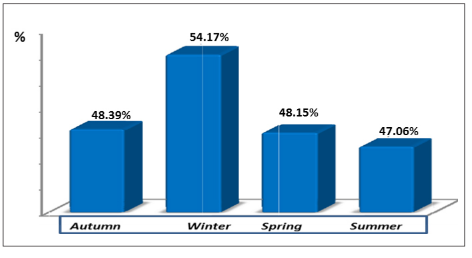

Figure 8. Prevalence of cryptosporidiosis according to season

The results show a positivity rate of 54.17% [32.82–74.45%] for the samples taken in winter. Similar rates were noticed in autumn, spring and summer (47 to 48%). Statistical analysis using the $\chi 2$ test confirmed the absence of a significant difference between the rates. Therefore, there is no seasonal variation of cryptosporidiosis and this is explained by the climate of the coastal regions, which is mitigated by the maritime climate (adequate temperature and relative humidity), therefore allowing greater resistance of the parasite in the external environment.

Our results differed from those reported by Goodwin and Brown (1988a) who noted a decrease during winter compared to other seasons, and a significant difference in the prevalence of cryptosporidiosis between northern and southern Georgia, USA (Goodwin and Brown, 1989). However, these authors did not explain the reason for these differences, and the lack of data prevents conclusions on the influence of climate and geographic location on infection.

Prevalence according to farming conditions

The next figure shows the influence of farming conditions on Cryptosporidia infection.

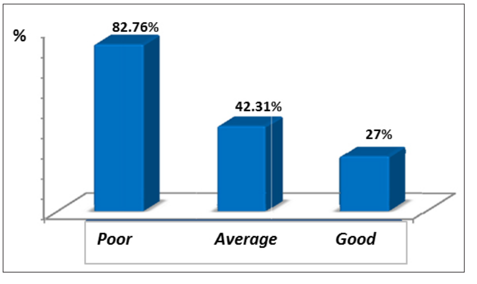

Figure 9. Frequency of *Cryptosporidium* infection according to farming conditions

Breeding conditions have a clear influence on the state of resistance and the onset of most diseases. However, it is impossible to have all the conditions, namely a suitable environment, a good general atmosphere and a good diet. Compliance with certain major conditions can, however, reduce or limit the spread of the disease and the durability of the parasite in a farm.

During this study, very few farms met the standards and it is difficult to classify them according to this parameter.

An arbitrary classification allowed for the clustering of the 17 farms into three groups:

- Those where a maximum of conditions were met (six farms): good breeding conditions.
- Those where a few conditions were met (seven farms): average breeding conditions.
- Those where the conditions were not met (four farms): poor breeding conditions.
-

Figure 9 shows a significantly high rate (82.76%) for farms where conditions are poor, compared to the rates obtained for farms where conditions are average (42.31%) or good (27.27%).

The farms classified under "poor breeding conditions" showed a lack of hygiene, suggesting that this parameter alone may be the dominant factor for the onset of infection by *Cryptosporidium* sp. substandard hygiene, in addition to promoting contamination, weakens animals through the installation of bacterial or viral diseases and compromises their immune status. The role of litter should not be overlooked. Euzeby (2002) found that Cryptosporidia retain its infectious power for 4 to 12 months, or even 18 months (Chermette and Boufassa-Ouzrout, 1988) on wet soils.

Litter is made of straw, which creates a moist and protective environment for oocysts. Thus, Moroccan farms infected with Cryptosporidia all show poor hygiene (Kichou et al., 1996). Similarly, the increase in the number of infected farms in Georgia, from 1% between 1974 and 1984 to 6% between 1984 and 1988, was due to the extension of the practice of permanent litter in poultry farming (Goodwin et al., 1990).

Apart from this factor, other parameters intervene in the onset of the disease. During this study, poor storage of feed was observed (bags against the wall, exposed to humidity) in addition to its irregular distribution (insufficient quantities and not at specific times), which generates stress that directly influences the immune state of animals. It has been shown that prolonged deficiencies in vitamin E, selenium and essential amino acids cause severe immunosuppression (Silim and Rekik, 1992).

Most of the buildings surveyed were non-compliant (agricultural greenhouses). Temperature, humidity and light intensity were unstable and difficult to control, resulting in the development of a particularly unfavourable state of stress, as cold reduces cell-mediated immunity and the transfer of passive immunity (Silim and Rekik, 1992).

Prevalence according to strain (genetic potential)

In order to demonstrate the effect of genetic selection on the prevalence of *Cryptosporidium* sp. infection, we selected two broiler strains: a heavy strain (Arbor Acres) and a light strain (ISA 15). The results are illustrated in Figure 10.

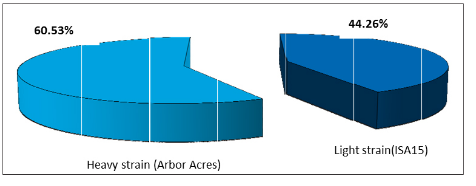

Figure 10. Frequency of *Cryptosporidium* infection according to strain (genetic potential)

Breeding programmes for farmed birds aim to maximize productivity by accelerating growth while reducing feed consumption. Although bird health is an important trait in the breeding programme, growth remains the target trait, closely followed by other economically important traits such as feed efficiency.

The rapid growth and increase in body weight lead to skeletal problems and the development of metabolic diseases (ascites). Increasingly, fast growing (heavy strain) broilers suffer from a weakened immune system, which makes them susceptible to pathogens (Qureshi et al., 1994; Rauw et al., 1998). Confirmation of these hypotheses came after comparing two strains, one heavy (Arbor Acres), and the other light (ISA 15).

During this study, 98 chickens were examined of which 65 were light strain chickens and 33 were heavy strain. Our results (Figure 10) show a non-significant difference between the rates of *Cryptosporidium* infection for the two strains, with 60.53% (23 infected chickens / 38) [44–76%] for heavy strain chickens and 44.26% (27 infected chickens / 61) [31–57%] for light strain chickens. The sex and strain effects on the onset of cryptosporidiosis have not been studied. This result is logical given the global distribution of *Cryptosporidium*. However, there are differences in the prevalence of cryptosporidiosis among countries, which may be more closely linked to epidemiological factors (age, level of hygiene, type of breeding) other than the strain itself.

Prevalence according to *Cryptosporidium* sp. localisation

Due to their low organ specificity, Cryptosporidia can grow in many of the sites where they may have reached (Figure 11).

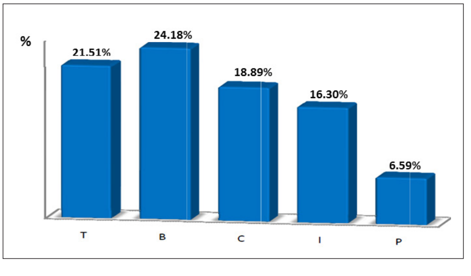

Figure 11. Frequency of ***Cryptosporidium*** localisations
(T: Trachea; B: Bursa of Fabricius; C: Cloaca; I: Intestine; P: Proventriculus)

According to Figure 11, the bursa of Fabricius was the most infected organ by *Cryptosporidium* sp., with a frequency of 24.2% [15–33%], followed by the trachea with 21.5% [13–29%], the cloaca with 18.9% [10–26%] and the intestine with 16.3% [8–24%]. The proventriculus presented only 6.6% [1–11%] of all the cases examined.

In birds, the development of *Cryptosporidium baileyi* is accompanied by colonization of the bursa of Fabricius (Mosele, 1998). Likewise, regardless of the route of inoculation, *Cryptosporidium baileyi* is found in the cloaca and bursa of Fabricius, due to ingestion of respiratory and conjunctival secretions (Lindsay and Blagburn, 1986a). This may explain the importance of the rates recorded at the level of these two organs: bursa of Fabricius (24.2%) and cloaca (18.9%). Some authors found Cryptosporidia only in the bursa (Kichou et al., 1990).

Itakura et al. (1984) reported that the tissues most often affected by *Cryptosporidium* sp. were the bursa of Fabricius (85%), the respiratory tract (41%) and the intestine (11%). Papadopoulou et al. (1988) found 52.9% of animals carrying Cryptosporidia exhibiting respiratory symptoms, 29.4% diarrhoea and 17.6% a combination of the two. Our results agree well with those reported by these authors who considered the bursa of Fabricius and the respiratory tract as the organs of choice for the development of this parasite. In birds, the respiratory tract is of great importance, with chicken cryptosporidiosis most often manifesting in respiratory form (Current et al., 1986; Goodwin et al., 1996).

Association of different localisations

The study of the relationship between infection with *Cryptosporidium* sp. from the bursa of Fabricius, trachea, intestine, cloaca and proventriculus showed that, among the 59 individuals carrying *Cryptosporidium*:

- 6 chickens (10.17%) [2–18%] presented both an infection of the bursa of Fabricius and the trachea, the highest frequency, and 8.47% both an infection of the cloaca and the trachea. In these two cases, the organs are anatomically distant. Hematogenous passage is impossible and intravenous injection of the parasite did not permit its development in any organ (Lindsay et al., 1987d). During experimental infections, oral inoculation is accompanied by colonization of the bursa of Fabricius, and the cloaca (Blagburn et al., 1987), and later of the respiratory system (Lindsay et al., 1986a, 1987a). Some authors explain this late development by aerial contamination from faeces (Lindsay and Blagburn, 1986a). When inoculated via the trachea, the parasite colonizes the respiratory system (Lindsay et al., 1986b, 1987b), the cloaca and the bursa of Fabricius (Blagburn et al., 1987). This is presumably due to ingestion of respiratory or conjunctival secretions (Lindsay and Blagburn, 1986a).
- 5 chickens (8.47%) [1–15%] present both an infection of the bursa of Fabricius and the cloaca. The anatomical location of bursa, near the cloaca, may predispose it to infection. This is because the bursa is located in the last part of the bird's cloaca, the proctodeum. It is lined by the intestinal mucosa which forms numerous folds.
- a significant rate was found for the association of the infection of the bursa of Fabricius and the intestine (6.78%) [0–12%]. This could be related to the close anatomical relationship between the intestine and the bursa of Fabricius. Once the cloaca is infected, the parasite can spread by contiguity (passage from one cell to another) to other parts of the intestine.

There is a close relationship between colonization of the bursa of Fabricius and that of the respiratory and digestive tracts. These results are similar to those of Kichou et al. (1996) but contrary to those reported by Goodwin et al. (1990), who confirmed the absence of a relationship between infection of the bursa and that of the respiratory and digestive tracts.

The relatively low rate of trachea / intestine infection (3%) [0–7%] can be explained by the fact that contamination of the digestive tract occurs by ingestion of respiratory or conjunctival secretions (Lindsay and Blagburn, 1986a), and then the parasite takes a long time to infect the intestine. We took an early sample, before the progression of Cryptosporidia and colonization of the intestine.

For the association of the infection of the proventriculus with the other organs, the recorded rates were relatively low (2%), which is due to the life cycle of *Cryptosporidium galli* which only develops at the level of the proventriculus.

Cryptosporida infection can spread to several organs, though rates remain low due to the organ specificity and the route of inoculation of avian *Cryptosporidium* species. However, experimental infections, carried out with oral isolates of *Cryptosporidium baileyi*, lead to development of the parasite in the terminal intestine and the bursa of Fabricius (Current et al., 1986; Blagburn et al., 1987).

Prevalence of cryptosporidiosis in turkeys

Overall prevalence

Of the 22 subjects examined, 9 turkeys (41%) [20.71–63.65%] were positive for *Cryptosporidium* sp. This high prevalence could be explained by the climatic conditions (heat and humidity) in addition to poor breeding conditions (non-compliant facilities, wet litter, overdensity, poor disinfection, and non-compliance of the sanitary vacuum delay).

This prevalence was similar to that reported (33.8%) in the USA (Woodmansee et al., 1988), and higher than that reported (14.8% in Romania). (Radu and Dan, 1985). These rates show the wide distribution of Cryptosporidia.

Absolute prevalence

Prevalence according to breeding conditions

As with all species, husbandry conditions are a contributing factor in the development of most diseases. For the six turkey farms studied, all facilities were non-compliant, and therefore it was difficult to classify them according to this parameter.

The six farms, as with chickens, were classified into two groups:

- Those where the standards were more or less respected (four farms): average breeding conditions.
- Those where the standards were not respected (one farm): poor breeding conditions.

The chi-square test revealed that the distribution of samples for the two types of breeding conditions showed a significant fluctuation ($P<0.05$). The rate of *Cryptosporidium* infection in farms with poor breeding conditions was significantly high (54%) [25.13–80.78%] compared to those with average conditions (33%) [7.49–70.7%].

In addition to hygiene condition, facilities were overpopulated, which promotes contact between animals and enables better propagation of the parasite. Storing feed in poor conditions and high temperatures were recorded at these farms, which promotes the occurrence of stress and directly influences the resistance and immune status of the animals.

Prevalence according to *Cryptosporidium* sp. localisation

The prevalence of the parasite differed from organ to organ, depending on the parasite species and the route of inoculation.

The bursa of Fabricius represents the most infected organ by *Cryptosporidium* sp., 23.81% [8.22–47.17%], followed by the cloaca and the intestine with respective frequencies of 15.79% [3.38–39.58%] and 9% [1.12–29.16%]. The least affected organs were the trachea and proventriculus with 4.55% [0.12–22.84%].

These results were contrary to reports that the respiratory form is the most frequent site of infection in turkeys (Hoerr et al., 1978; Tarwid et al., 1985). In contrast, other authors reported episodes of diarrhoea associated with Cryptosporidia (Goodwin et al., 1988b; Wages and Ficken, 1989). Although the results of experimental infections showed that the respiratory form was the most frequent in turkeys, they remain the reference host of *Cryptosporidium meleagridis* suspected of causing episodes of enteritis in birds (Mosele, 1998).

Prevalence of *Cryptosporidium* sp. depending on strain

Economic demands have encouraged a selection of strains with broad breasts, rapid growth and high weight. During this study, 22 turkeys were examined, of which 9 were semi-heavy strain turkeys (Nicolas 700) and 13 heavy strain (Fig 6). The data showed a significant difference between the rates of infection with *Cryptosporidium* sp. between the two strains; 46% [19.22–74.87%] for the heavy strain and 11% [0.28–48.25%] for the semi-heavy strain.

Contrary to what was observed in chickens, the "strain" trait (genetic potential) influenced the prevalence of Cryptosporidia infection in turkeys. This may be the result of selection as weight gain in chickens is uniform, while in turkeys, breast width is the trait targeted by breeders. The increase in chest size shifts the centre of gravity of the trunk, increasing the effort needed to maintain balance while standing. This increases the tension required to stay upright and for movement (Hafez, 2000). This extra effort is the cause of stress and consequently of immunosuppression, resulting in aggression by various germs including Cryptosporidia.

The genetic selection of heavy strains with rapid growth represents a major cause at the origin of an increase in locomotor pathologies (Hafez, 2000), hence difficulty in accessing feeders, resulting in a weakened organism predisposed to infection. Thus, it has been shown that prolonged deficiencies in vitamin E, selenium and essential amino acids cause severe immunosuppression (Silim and Rekik, 1992). Genetic factors therefore appear to be involved in the appearance of several pathologies. However, cryptosporidiosis can be attributed to varying causes, most often difficult to identify.

Clinical signs and lesions

In chickens from flocks infected with *Cryptosporidium*, the clinical signs observed were general non-specific signs, namely rales (28%), cachexia (22%) and diarrhoea (20%). The macroscopic lesions observed in the bursa of Fabricius consist of atrophy of the bursa or the presence of caseous exudate in its lumen. In the respiratory system, the presence of an abundant mucoid exudate on the surface of the tracheal mucosa and an opalescence of the air sacs were noted (Figure 12). In the intestine, lesions were limited to simple congestion of the mucous membrane. Our results differ from those reported by Kichou et al. (1996) who found a predominance of digestive symptoms, with diarrhoea (30%), and respiratory symptoms, with rales (18%). These symptoms are general and do not reflect the clinical picture of the disease. The same lesions were found in turkeys.

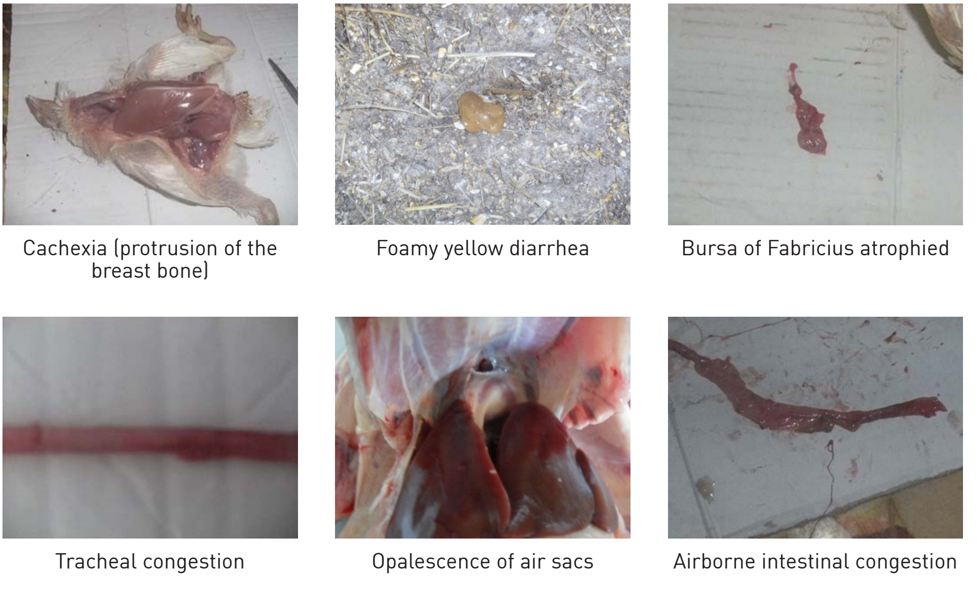

Figure 12. Clinical signs and lesions encountered during infection with *Cryptosporidium* sp.

Conclusions

This study highlights the importance of cryptosporidiosis on farms in north central Algeria. During the study, a few risk factors were identified, making it possible to establish a close link between the appearance of cryptosporidiosis and other parameters, namely age, season, strain, target organ and breeding conditions. Finally, the authors recommend the need for systematic monitoring for these parasites in the poultry industry, facilitated by simple diagnostic, both during pathological episodes and during routine checks. This would allow for more precise identification of their distribution in the poultry sector, and in particular in breeding farms for which few data are currently available. Because of the economic losses (weight loss and mortality), cryptosporidiosis must be taken seriously by all staff in the poultry sector: breeders, technicians and veterinarians.

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Infekcija s *Cryptosporidium* sp. u tovnih pilića i purana na nekim farmama na središnjem sjeveru Alžira

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Cilj ove studije bio je ustvrditi prevalenciju kriptosporidioze u tovnih pilića i purana te čimbenike koji mogu utjecati na njezin razvoj, ali i preciznost najčešćih lokacija *Cryptosporidium* i ozbiljnost lezija povezanih s bolešću. Stoga je u razdoblju od listopada 2019. do rujna 2020. ustvrđena prevalencija infekcije s kriptosporidijima na 22 farme smještene na središnjem sjeveru Alžira. Za svaku su farmu obavljeni tjedni izvidi tijekom razdoblja studije i prikupljene su informacije o vrsti uzgoja i zdravstvenom statusu peradi. Na tim je farmama istraženo 98 pilića i 22 purana u kojih su zamijećene kliničke lezije. Analiza fekalija, uzorci tkiva i strugotine crijeva, Fabricijeve burze, dušnika, kloake i proventrikulusa otkrile su prevalenciju od 55 % na farmama pilića. Dob pilića ima važnu ulogu. Stoga je velika učestalost bolesti zamijećena u pilića u dobi od 10 do 50 dana, s maksimalnom vrijednošću između 31 i 40 dana (84,62 %). Parazit nije otkriven u pilića mlađih od 7 dana. Najčešća lokacija *Cryptosporidium* sp. bila je burza (24,2 %), zatim dušnik (21,5 %), kloaka (18,9 %), crijeva (16,3 %) te proventrikulus (6,6 %). U purana je sveukupna prevalencija bila 41 %. Burza je bila najinficiraniji organ (23,81 %), zatim kloaka i crijeva, s učestalošću od 15,79, odnosno 9 %. S druge strane, za obje vrste peradi podatci su potvrdili utjecaj sezone, soja (genetski potencijal) i uvjeta uzgoja na prevalenciju *Cryptosporidium* sp. Zaključno, naši rezultati su pokazali da je *Cryptosporidium* vrlo čest na našim farmama, a to je uglavnom povezano s klimatskim uvjetima, lošim upravljanjem i posebice higijenskim mjerama.

Ključne riječi: *kriptosporidioza, tovni pilići, purani, prevalencija*