ISSN: 1220-3173; E-ISSN: 2457-7618

RETROSPECTIVE EPIDEMIOLOGICAL STUDY OF INFECTIOUS LARYNGOTRACHEITIS IN BROILERS FROM 2011 TO 2019

STUDIU EPIDEMIOLOGIC RETROSPECTIV AL LARINGOTRAHEITEI INFECȚIOASE LA BROILERI, DIN 2011 PÂNĂ ÎN 2019

Houria RAMDANI[1)], M. ZEGHDOUDI[1),2),*)], Latifa MERDACI[1),2)], MardjaTAHRI[3)], Seloua MADI[1),2)], Leila AOUN[1),2)]

ABSTRACT | REZUMAT

Infectious laryngotracheitis (ILT) is responsible for significant productivity and costs losses for diagnostic tests, vaccines, and treatments. The protective immune system against ILT and the virulence infection mechanisms used by the disease was usually poorly established. Our objective was to analyse the key epidemiologic features of ILT linked to its cyclic appearance and spontaneous disappearance to better fluency this pathology. To achieve this investigation, a total of 784500 chicks were set up between 2011-2013 and 2017-2019 in eastern Algeria where 540 blood serum samples from 54 flocks between 2011 and 2013, and 600 samples from 59 flocks between 2017 and 2020 were used for the ELISA test. The ILT appeared between the 35th and 43rd days old with morbidity and mortality rates of 80% and 17.1%, respectively. The symptoms and the lesions were characteristics of the disease's acute form. The spatio-temporal emergence and re-emergence of the ILT with regular latency periods confer a specific epidemiological character. Studies on the viral pressure during the latency period until the emergence of the disease could help to better understand the infectious nature of this disease. These results will contribute to the ILT monitoring and control for the prevention success of this disease. Until now, biosecurity measures remain the only way to fluency this disease.

Keywords: biosecurity, control, ELISA, emergence, ILT

Laringotraheita infecțioasă (LTI) produce pierderi semnificative prin scăderea productivității și creșterea costurilor asociate testelor de diagnostic, vaccinurilor și tratamentelor. Mecanismul imunitar de protecție dezvoltat de organism împotriva LTI și mecanismele de virulență prin care acționează patogenul nu au fost pe deplin stabilite. Obiectivul nostru a fost să analizăm cele mai importante aspecte epidemiologice ale ILT referitoare la ciclicitatea apariției și dispariției spontane în vederea elaborării unor programe de combatere ale acestei patologii. Acest studiu s-a derulat în perioadele 2011-2013 și 2017-2019, pe un efectiv de 784500 pui din estul Algeriei. Pentru testarea ELISA, între 2011 și 2013 au fost utilizate 540 probe de ser de sânge de la 54 efective și între 2017 și 2020 au fost utilizate 600 probe de la 59 efective. LTI a apărut la puii cu vârsta de 35 - 43 de zile, cu o rată a morbidității de 80% și a mortalității de 17,1%. Simptomele și leziunile au fost caracteristice formei acute de evoluție a bolii. Emergența și reemergența spațio-temporală a LTI, cu perioade de latență regulate, conferă acestei boli un caracter epidemiologic specific. Studiile asupra presiunii virale în perioada de latență până la apariția bolii ar putea ajuta la o mai bună înțelegere a naturii infecțioase a acestei boli. Aceste rezultate vor contribui la controlul LTI pentru succesul prevenirii și combaterea acestei boli. Până în prezent, biosecuritatea rămâne singura modalitate de luptă împotriva acestei boli.

Cuvinte cheie: biosecuritate, control, ELISA, emergența, LTI

Avian respiratory diseases are the major problem of poultry industries and the most important such as infectious bronchitis, Newcastle disease, mycoplasmosis, infectious Coryza and aspergillosis are controlled through rigorous vaccination plans or effective antibiotic treatments (1). Infectious laryngotracheitis due to a herpesvirus is liable for significant losses in productivity, diagnostic tests, vaccines, antibiotic treatments and understanding of protective immune systems against ILT is lacking. The virulence infection mechanisms used by the disease were usually poorly established or disregarded (20).Thus, currently available ILT vaccines manufactured with live modified viruses generate a latent infection of the disease (5). Most ILT field isolates studied in western European countries were closely linked to vaccines administered against this pathology (25). The disease was first described in 1925 by May and Tittsler

1) Department of Veterinary Sciences, ChadliBendjedid University, El Tarf-36000, Algeria
2) Laboratory of ESPRETCADE ChadliBendjedid University, El Tarf-36000, Algeria.
3) Department of Biology, ChadliBendjedid University, El Tarf-36000, Algeria
*) *Corresponding author: mouradzeg@yahoo.fr*

and then reported in many countries where poultry production is intensive with a high concentration of poultry farms, particularly in North and South America, China, Europe, Australia and Southeast Asia(16). Since the 1980s, over 200 ILT reports have been issued on the recurrence and sporadic appearances of ILT in the United States and Canada (1). In the Maghreb (Northern Africa), isolation and characterization of ILT virus in Tunisia were achieved in 2013 (26) and in 2016 (21) in broilers. In Morocco, the first cases of ILT were noted in laying hens in 2003 (14).Despite the improvement in biosafety and vaccination programs in recent years, the ILT keeps appearing regularly (13). Currently, most researches are concentrating on virus testing strategies or trials for the development of new ILT vaccines. The present study is focused on the epidemiological character analysis of this disease including its spatio-temporal dynamics linked to its endemic aspect and its spontaneous disappearance.

MATERIALS AND METHODS

History, Site and Animals

In the years 2003, respiratory disorders refractory to several antibiotics administered and vaccination plans applied against infectious bronchitis (IB) and Newcastle disease (ND) were reported in broilers in eastern Algeria. The disease occurred between the 40th and 60thday old and the average mortality rate was 15%. Clinical signs were respiratory distress, grumbles, purulent discharge and primary lesion of haemorrhagic laryngotracheitis (3). During this period, this pathology was not sufficiently studied due to a lack of diagnostic materials and the short disease evolution that has spontaneously disappeared the following year.Between 2011 and 2013, the appearance of these same respiratory disorders enabled us to realize an epidemiological study of the disease in Annaba locality-eastern Algeria. In this area, there is a huge concentration of chicken rearing where broiler represents 90% of the total. Over that time, the sampling consisted of 10 broiler farms with a capacity of 3000 to 5500 chickens each for a total of 390500 chicks. The breeding farms received 2, 3 or 4 times per year chicks from different hatcheries located in several regions of centre and eastern Algeria. These farms applied a strict immunization protocol against ND, IB, and Gumboro disease.

In 2013, when the disease disappeared again, we investigated throughout local veterinarians who have constituted an epidemiological surveillance network in order to detect the timeframe of re-occurring disease. Thus, in February 2017 new outbreaks of ITL were declared in broiler farms and the same diagnostic and epidemiological protocol was carried out on 10 other farms until January 2020 when the ILT again disappeared. A total of 540 blood serum samples were collected from 54 flocks between 2011 to 2013 and 600 samples from 59 flocks between 2017 to 2020 (ELISA test was realized from 2011-2013 and 2017-2020). In Algeria, vaccination against ILT was not instituted in broiler or in any other type of farming. In suspected farms ILT, we identified clinical signs and changes in morphopathologic in morbid subjects and necropsied carcasses respectively as well as the main epidemiological characteristics. At the slaughter age (between the 50th and the 60th day), we calculated the mean live weight in the livestock building.

ELISA test

Blood samples from morbid animals were immediately transported under aseptic conditions to the laboratory ESPRETCADE of Chadli Bendjedid University-El Tarf. A total of 10 serum samples were taken from diseased chickens in each flock to detectthe antibodies against infectious laryngotracheitis virus(ILTV). The blood serum samples were studied using a commercial ELISA kit (HIPRA-CIVTEST AVI-ILT, Spain) including microtiter plates 96-well U bottom. The test procedure was performed according to the manufacturer's recommendations. The absorbance at 450 nm was read by an ELISA reader and the relative amounts of antibodies in chicken samples was calculated by reference to the positive control. This relationship wasexpressed as S/P ratio (Sample to Positive ratio). Samples with an S/P of 0.5 or greater contain anti-ILT antibodies and then were considered positive; the opposite case, they were considered negative.

RESULTS AND DISCUSSION

Clinical signs and lesion

The first observed symptoms were sneezing without deterioration of the stock general condition. Gradually, these disorders become increasingly accented with prostration, respiratory distress with beak open, intense groans, nasal and conjunctival purulent mucus, sometimes the presence of blood in the oral cavity. Contamination between congeners was very rapid and death occurs after 2 to 3 days.

The necropsy was performed on fresh carcasses of less than 12 hours throughout disease evolution. Initially, the primary lesion was located at the laryngo-tracheal mucosa which appears bright red colour, haemorrhagic along its length, sometimes containing an abundant purulent exudate or whitish casein at the entrance to the trachea. In some individuals, we found blood clots in the trachea overflowing into the oral and nasal cavity. All the serums collected from sick animals with clinical signs and lesions of ILT were tested positive to the ELISA test. A total of eight farms in ten during the period 2011-2013 and nine farms in ten in the period 2017-2019 were positive to the ELISA test (Table 1 and 2).

Table 1

Livestock and main epidemiological data of ILT (2011-2013)

Farms	1	2	3	4	5	6	7	8	9	10
Capacities	3500	4500	3500	5000	4000	4500	5000	4000	35000	4500
Number of flocks	12	9	9	9	11	8	8	9	11	9
Effective set up	39500	40500	31500	45000	43000	36000	40000	36000	38500	40500
Total effective / total number of flocks	390500/95									
Number of infected flocks / infected effective	8/28000	5/22500	6/27500	7/35000	9/34000	7/31500	-	5/20000	-	7/31500
Total number of infected flocks / total infected effective	54/ 230000									
Epizootiological characters of ILT										
Age occurrence interval of ILT (days)	38-43	38-40	36-41	35-41	38-42	37-43	-	40-36		37-42
Mean mortality rate during the disease (%)	15.6	20.1	17.8	15.5	14.9	19.1	**2.7**	16.3	**2.2**	14.7
Age interval of ILT evolution in days	15-21	16-20	18-21	15-22	16-21	17-20	-	16-21	-	17-22
Mean Weight at slaughter (kg)	2.3	2.2	2.0	2.1	2.2	2.3	**2.8**	2.3	**2.7**	2.2

Farms 7 and 9: unaffected

Table 2

Livestock and main epidemiological data of ILT (2011-2013)

Farms	1	2	3	4	5	6	7	8	9	10
Capacities	4000	3500	4500	5500	5000	5500	3500	5500	5000	4000
Number of flocks	11	12	10	9	8	9	12	9	8	10
Effective set up	43000	42000	43500	48500	40000	48000	41500	49000	40000	40000
Total effective / total number of flocks	435500/98									
Number of infected flocks / infected effective	6/23000	8/28000	7/30500	6/31000	-	6/33000	6/21000	8/43500	6/30000	7/28000
Total number of infected flocks / total infected effective	59/268000									
Epizootiological characters of ILT										
Age occurrence interval of ILT (days)	35-40	36-41	35-43	38-42	-	36-40	35-42	16-40	38-43	37-42
Mean mortality rate during the disease (%)	15.1	20.0	17.2	12.8	**1.2**	19.1	20.2	16.9	15.7	11.8
Age interval of ILT evolution (days)	16-20	17-19	16-18	15-19	-	15-20	16-19	18-20	16-20	15-19
Mean Weight at slaughter (kg)	2.3	2.2	2.0	2.1	**3.2**	2.3	2.1	2.3	2.2	2.2

Farm 5: unaffected

Epidemiological characters

Between 2011 and 2013, diseased animals showed clinical signs and lesions of ILT from 35 to 43 days old. The disease lasted at least 2 weeks and 3 weeks maximum with mortality rates have increased steadily for two weeks to slowly regress during the third week. The morbidity rate was about 80% and the mortality rate has varied between 14.7 % and 20.1 % (Table 1). Aggravating factors that we have observed are represented mainly by the microclimate parameters that are not sufficiently mastered and a non-respected health barrier in contrast to the two other healthy farms (7 and 9) where the infrastructure quality, management and biosecurity were strictly observed.

The mean slaughter weight was well below the standards except for healthy livestock (Table 1).

During this period, the ILT has spread throughout the country exclusively affecting broiler flocks.

At the end of 2013, the cases of ILT became extremely rare to disappear suddenly after and we didn't observe any case of disease until the beginning of 2017 when cases of ILT reappeared. The obtained results based on the same diagnostic and the same epidemiological protocol previously performed showed similar values to those obtained between 2011 and 2013 with the difference that on the ten farms only one that hashigh-performance equipment was not infected by the ILT (Table 2).

Discussion

In the present study, the history showed that the ILT in Algeria would initially appear in 2003 to disappear spontaneously a few months later. After a latency period -nearly 6 years-, the ILT reappeared in 2011 with similar epidemiological and pathological characters to disappear two years later without any specific prophylactic use. In 2017 and after five years of absence, this disease is manifested again still in its acute form what confirms its emergence and re-emergence character with regular latency periods. Simultaneously, cases of ILT appeared in Morocco in 2004 (14), in Tunisia in 2012 (26) and in 2016 (21), which shows that ILT has cross-border transmission. Other epidemics of ILT occurred in several continents during this period. In 2001, an infection by the ILT virus is declared in herds of broiler chickens in the southeast of the United States, in the Mississippi state in 2002-2003 (31). In 2007 and 2015, epidemics of ILT occurred in Australia, United States and India (27). Between May 2007 and October 2008, 34 ILT outbreaks are reported in chicken flocks of flesh in Italy (24). In Turkey, the first case of ILT in a group of laying hens has been reported in 2003 then the epidemics have been announced in 2014 and have affected the herds for 2 years (32). In November 2010, an ILT epidemic has been described in an installation of laying hens muILT-age in Brazil; the previous ILT epidemic has been reported only in 2002 (28). At the same time, cases of ILT are found in several countries of Central and South America (4), in Peru between 2008 and 2014 (23) and in Argentina between 2009 and 2011(10). Well before 1981, (6) reports the occurrence of sporadic outbreaks of ILT that laid a persistent problem in the Australian poultry industry. The recurrence of a benign form of ILT in England has also been announced in 1983 (11). The ILT identified in many countries remains a serious pathology likely to achieve important avian populations with a virus geographic spread pattern (7). The ILT is speedy evolving with very high transmissibility but also a great capacity to spread in free areas(2). Those epidemiologic data are in accordance with our spatio-temporal analysis on the ILT emergence that appears simultaneously in many countries and at cyclical periods. Given the absence of vaccination in Algeria of avian flocks, the herpesvirus responsible cannot be from vaccine strain although several evidence than most of the epidemics are caused by viruses indistinguishable from the vaccine strains of embryonic origin. The start of the disease is age 35 and 43 days, the evolution is between 2 and 3 weeks, high morbidity and mortality rates are the main characteristics noted in our investigation. As a general rule, most of the chickens are recovering in 10-14 days, with extremes of 4 weeks (12).The morbidity and mortality mean rates recorded were 50% and 17% respectively. The serious epizootic forms can cause morbidity between 90 and 100%; and mortality from 5 to 70% but in general it varies between 10 and 20% (18).

Brandlyin 1936(8) found that 36 series of transfers of the ILT virus in the hen's embryos have not reduced its virulence or its ability to immunize. In broilers, this mortality varies from 0.7% to 50%; the total mortality of herds may be underestimated because they are often marketed during an epidemic (17). In this study, we have recorded poor growth and insufficient slaughter weight. The economic importance of the ILT isn't determined with precision; however, the United States poultry industry has suffered losses of several million dollars each year from the induced mortalities and losses in production (29). We observed that the microclimate parameters like the ambient temperature, the moisture level, the noxious gases and the density have played a role in encouraging the virus aggressiveness and the spread of this pathology. Clinical signs and lesions of the severe form are readily distinguished for the ILT diagnostic (30). The clinical signs may vary depending on the virus strain, other environmental factors and the microclimate (9). Experiences have been set up to assess the effects of natural and artificial stress on the re-excretion of the ILT virus in chickens infected. Thus, the start laying had a significant effect on the total loss rate of birds carrying. Nine birds on ten have eliminated the virus after the start laying, against only two in the three and a half previous weeks (19). We noted an epidemiological analogy between the human coronavirus (SARS-CoV, MERS-CoV, COVID-19) and the ILT concerning the emergence and re-emergence at regular periods, the speedy cross-border spread in countries with large polluting economies where the circulation of people and traffic transport are intense. The spontaneous disappearance of these diseases is explained by a gradual weakening of the aggressiveness of the virus and viral pressure. To this, it can be added that it is very unlikely to find a vaccine given the spatio-temporal mutation of the virus and the risk that in this type of pathology, vaccines can on the contrary cause the appearance of the disease.

CONCLUSIONS

This study showed that the ILT is an emergent and re-emergent typical disease with spontaneous occurring and disappearing, regular and cyclic latency periods besides its regional and cross-border nature that confer specific characteristics in the field of epidemiology. The occurring and reoccurring of the ILT linked with the latency period remain the main factor to explain. Studies on the viral pressure during the latency period until the emergence of the disease could help to better understand the infectious nature of this disease. Research should focus on prophylactic biotechnological mechanisms to mitigate the aggressiveness of the virus and viral pressure in the external environment.

Finally, these results will contribute to the ILT monitoring for the prevention success and the control of this disease where the biosecurity measures remain the only way to fluency this disease. ILT can represent a study model for emerging human diseases such as the coronavirus.

Acknowledgements

This study was fully supported by the Laboratory of Epidemiology Research (ESPRETCADE) of Chadli Bendjedid University, El Tarf, Algeria. We thank the general direction of scientific researchand technological deve-lopment (DGRSTD).

REFERENCES

1. *Adams W.W., Hargreaves R.C., Hughes E., Murphy, C.D., Newman J.A., Stein W.T.,* (1986), Summary of disease reports. American Association of Avian Pathologists. Avian Dis,30:856-892
2. *Agnew-Crumpton R., Vaz P.K., Devlin J.M., O'rourke D., Blacker-Smith H.P., Konzak-Ilievski B., Hartley C.A., Noor mohamedi A.H.,* (2016), Spread of the newly emerging infectious laryngotracheitis viruses in Australia. Infect Genet Evol, 43:67-73
3. *Annual health report*, (2003), Regional Office of Avian diseases in the eastern Algeria. Bulletin no12
4. Ayala *S.*M., Revelov A., Barragang A., (2014), Detection and characterization of infectious laryngotracheitis virus from an outbreak of respiratory disease in Ecuador. J Appli Res Vet Med, 12(3):245-U1
5. *Bagust T.J., Jones R.C., Guy J.S.,* (2000), Avian infectious laryngotracheitis. Rev sci tech Off int Epiz*,* 19(2):483-492
6. *Beveridje W.I.B.,* (1981), Viral Diseases of Farm Livestock. Animal Health in Australia, Vol.1, (Ed.) Australian Government Publishing Service, Canberra, Australia, 158-161
7. *Biggs P.M.,* (1982), The world of poultry disease. Avian Pathol, 11:281-300
8. *Brandly C.A.,* (1936), Studies on the egg-propagated virus of infectious laryngotracheitis and fowl pox. J Am Vet Med Assoc, 88:587-599
9. *Cover M.S., Benton W.J.,* (1958), The biological variation of infectious laryngotracheitis virus. Avian Dis*,* 2:375-383
10. *Craig M.I., Rojas M.F., Van der Ploeg C.A., Olivera V., Vagnozzi A.E., Perez A.M., Konig G.A.,* (2017), Molecular characterization and cluster analysis of field isolates of avian infectious laryngotracheitis virus from Argentina.Frontiers in Veterinary Science, 4:212
11. *Curtis P.E., Wallis A.S*., (1983), Infectious laryngotracheitis. Vet Rec, 112-486
12. *Davison S., Eckroader R., Miller K*., (1988), Laryngotracheitis. The Pennsylvania Experience, Proceedings of the 23rd National Meeting on Poultry Health and Condemnations. Ocean City, Maryland, USA, 14-19
13. *Dufour-Zavala L.,* (2008), Epizootiology of infectious laryngotracheitis and presentation of an industry control programme. Avian Dis, 52:1-7
14. *El Houadfi M., Kichou F., Tazi R.M., Mouahid M., Kissi B.,* (2005), First report of avian infectious laryngotracheitis in Morocco. Vet Rec,156:520-521
15. *Guerin J.L.,* (2008), La laryngotrachéiteinfectieuse. Avicampus. Available from: www.avicampus.fr/bacterio.html
16. *Hidalgo H.*, (2003), Infectious Laryngotracheitis: a review. Brazilian J Poult Sci, 5:157-168
17. *Hillbink F.W., Oei H.L., Roozelaar D.J.,* (1987), Virulence of five live virus vaccines against infectious laryngotracheitis and their immunogenicity and spread after eye drop or spray vaccination. Vet Q*,* 9:215-225
18. *Hinshau W.R.,* (1931), A survey of infectious laryngotracheitis of fowls. Calif Agric Exp Stn Bull, 520:1-36
19. *Hughes C.S., Gaskell R.M., Jones R.C., Bradbury J.M., Jordan F.T.W.,* (1989), Effects of certain stress factors on the re-excretion of infectious laryngotracheitis virus from latently infected carrier birds. Res Vet Sci, 46: 247-276
20. *Jordan F.T.W.,* (1980), Immunity to infectious laryngotracheitis. In: Avian Immunology. Proceedings of the 16th Poultry Science Symposium. Edinburgh, UK, 245-254
21. *Kaboudi K., Nciri J., Amara A., Laarabi I., Moalla N., Bouzouaia M.,Ghram A.,* (2016), Histopathological and molecular diagnosis of infectious laryngotracheitis in Tunisia - First Report. Int J Livest Res, 6(10):34-45
22. *Linares J.A., Bickford A.A., Cooper G.L., Charlton B.R., Woolkock P.R.,* (1994), An outbreak of infectious laryngotracheitisin California broilers. Avian Dis*,* 38:188-192
23. *Morales R.S., Bendezu E.J., Montesinos R, Tataje-Lavanda L., Fernandez D.M.,* (2018), Full-genome sequence of infectious laryngotracheitis virus (gallidalphaherpesvirus 1) strain VFAR-043 isolated in Peru. American Society for Microbiology, 6-10
24. *Moreno A., Picciorillo A., Mondin A., Morandini E., Gavaazzi L.,Cordioli P.,* (2010), Epidemic of infectious laryngotracheitis in Italy: Characterization of virus isolates by PCR–restriction fragment length polymorphism and sequence analysis. Avian Dis, 54:1172-1177
25. *Neff C., Sudler C., HoopR.K.,* (2008), Characterization of western European field isolates and vaccine strains of avian infectious laryngotracheitis virus by restriction fragment length polymorphism. Avian Dis, 52:278-283
26. *Nsiri J., Farhat M., Khchini R., Ammouna F., Ettriki S., Elbehi I., Larbi I., Lachheb J. and Ghram A.,*(2014), Isolation and characterization of avian infectious laryngotracheitis virus in Tunisa. Institut Pasteur Report. Tunis, 170-173
27. *Oldoni I., Garcia M.,* (2007), Characterization of infectious laryngotracheitis virus isolates from the USA by polymerase chain reaction and restriction fragment length polymorphism of muILTple genome regions. Avian Pathol, 36:167-176
28. *Preis I.S., Braga J.F.V, Couto R.M., Brasil B.S.A.F., Martins N.R.S., Ecco R.,(*2013), Outbreak of infectious laryngotracheitis in large muILT-age egg layer chicken flocks in Minas Gerais Brazil,Pesq Vet Bras, 33:591-596
29. *Saif Y.M.,* (2003), Diseases of poultry American Association of Avian Pathologists. (11th edn.), sect I chap 15, (Ed.) Iowa state press, USA, 421-469
30. *Sillim A.,* (1992), Laringotracheitis Infectious. In: J. Brugère -Picoux. Manuel, A. Silim (ed.), Manuel de pathologie aviaire. (Ed.) ENV d'Alfort, France, 129-132
31. *Volkova V., Thornton D., Hubbard S.A., Magee D., Cummings T., Luna L., Watson J., Wills R.,*(2012), Factors associated with introduction of infectious laryngotracheitis virus on broiler farms during a localized outbreak. Avian Dis, 56(3):521-528
32. *Wells B.,* (2004), New South Wales outbreaks of ILTV. In: Proceedings of the Australian Veterinary Poultry Association Scientific Meeting, Melbourne, Australia, 52-54
33. *Zeki A., Yavuz O.,Sanioglu G.,*(2018), Occurrence of infectious laryngotracheitis outbreaks in commercial layer hens detected by ELISA. J Immunoass Immunoch, 2:190-195.