Middle East Research Journal of Agriculture and Food Science
ISSN: 2789-7729 (Print) & ISSN: 2958-2105 (Online)
Frequency: Bi-Monthly
DOI: 10.36348/merjafs.2023.v03i02.003

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Seroprevalence and Associated Risk Factors of Infectious Laryngotrachitis in Small Scale Chicken Production in Nekemte Town and Selectted Districts of East Wollega Zone, Oromia, Ethiopia

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Abstract: Infectious laryngotracheitis (ILT) is a disease of high economic consequence to the poultry sector. Infectious laryngotracheitis virus (ILTV), under the genus Iltovirus, and the family Herpesviridae, is the agent responsible for the disease. The aims of current study were, therefore, to estimate the seroprevalence of ILTV in chickens and identifying associated risk factors. A cross-sectional study was conducted from April 2022 to October 2022 in East Wollega zone specifically in Nekemte town and three surrounding districts (Diga, Guto Gida and Leka Dulecha). The districts were selected by purposive sampling, however, cluster-sampling technique was used to select the smallest administrative units (villages) and farms/households, and the study element (chickens) was selected by simple random sampling method. Logistic regression analysis was utilized in order to analyse the collated data. A total of 384 serum samples were collected from Nekemte town and the three selected districts of east Wollega zone. Indirect ELISA was used to assay for the existence of anti-ILTV antibodies in the serum. A questionnaire survey was used to identify the potential risk factors. Out of the 384 samples, 15.36% (95% CI: 12.1–19.3) tested positive for anti-ILTV antibodies, with 21.88%, 14.44%, 11.11% and 10.53% prevalence obtained from Nekemte town, Guto Gida, Diga and Leka Dulecha respectively. Univariable logistic regression analysis showed that the chicken source and Sasso breed were found to be significantly associated with ILT seropositivity. Chickens obtained from day old chicks distributers was 2.22 (COR: 2.22, 95% CI: 1.23-4.00) times more likely seropositive than those bought from market. Multivariable logistic regression analysis of potential risk factors revealed that exotic Sasso breeds of chicken were found 9.68 (AOR: 9.68, 95% CI: 1.96-47.78) more likely to be seropositive than local breed. It was concluded that the seroprevalence obtained in this study indicated the circulation of this economically important poultry disease in the study sites and, hence warrants prevention and control.

Keywords: Chickens, East Wollega zone, Seroprevalence, Infectious laryngotracheitis, risk factors.

Research Paper

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How to cite this paper:
Teshome Kidane Balcha, Begna Bulcha, Monenus Etefa (2023). Seroprevalence and Associated Risk Factors of Infectious Laryngotrachitis in Small Scale Chicken Production in Nekemte Town and Selectted Districts of East Wollega Zone, Oromia, Ethiopia. *Middle East Res J. Agri Food Sci., 3*(2): 27-35.

Article History:
| Submit: 20.07.2023 |
| Accepted: 25.08.2023 |
| Published: 26.08.2023 |

INTRODUCTION

Poultry farming is one of the rapidly developing sectors; playing an important role in the global food security (Gowthaman *et al*., 2020). In Ethiopia, chicken production is widely spread with almost every rural family rearing chickens as a valuable source of family protein and income (Tadelle *et al.,* 2003). As of the Ethiopian Central statistical agency (CSA, 2021) report, the country's chicken population was estimated to be 57 million. Nevertheless, this huge potential is unable to satisfy the growing domestic demand for chicken products, and the economic contribution of the sector remained marginal for various reasons (Bekele *et al.*, 2015). Owing to the rapid population growth and change in living standards, the demand for chicken meat and eggs in Ethiopia is expected to be rise (FAO, 2019). As a coping strategy, the Ethiopian government has outlined policies for intensifying the poultry production system by introducing exotic breeds and advanced technologies (Shapiro *et al.,* 2015). As a result, many government-owned poultry multiplication and distribution centers along with nongovernmental organizations have been

importing and distributing exotic breeds to augment the intensification process (Pagani and Wossene, 2008). However, there was a growing concern of introduction of diseases of various etiologies into the poultry sector concurrently with the importation of exotic breeds (Dawit *et al.,* 2009). Among the infectious diseases of potential damage to the poultry sector is infectious laryngotracheitis (ILT).

Infectious laryngotracheitis is a highly contagious respiratory disease of chickens caused by infectious laryngotracheitis virus (ILTV), *Gallid alphaherpesvirus* 1 (GaHV-1), which belongs to the genus *Iltovirus* of subfamily *Alphaherpesvirinae*, and the family *Herpesviridae* (Davison *et al.,* 2009). The natural portal of entry of ILTV is respiratory and ocular routes. The initial replication takes place in the epithelium of the conjunctiva, respiratory sinuses, larynx and upper respiratory tract largely (Guy and Bagust, 2003). Clinically it is characterized by dyspnea and gasping with an extension of the head and neck. Coughing, rattling, and gurgling also noticed when the birds try to expel the clotted blood and debris from the obstructed trachea (Blakey *et al*., 2019). Severe form of the disease results in respiratory depression, gasping, and expectoration of bloody exudates with high rates of morbidity and mortality up to 70% in an acute form of infection (Parra *et al.,* 2016). The morbidity and mortality vary depending on the virulence of circulating field strains of ILTV (Devlin *et al*., 2006; Oldoni *et al*., 2009), viral load and concurrent infections with other respiratory pathogens (Guy and Garcia, 2008). Although birds over three weeks of age are reported to be highly susceptible to ILTV infection (Dufour-Zavala,2008), almost all ages starting from eight days to four years of age (Linares *et al*.,1992) are prone to the infection. High intense poultry rearing, mixing of the different type of birds in the same geographical area and a breach in biosecurity often lead to outbreaks of ILT in many parts of the world (Bagust *et al*., 2000).

In spite of the economic implications of the disease and its high contagiousness, there are limited scientific reports on the status of ILTV in Ethiopia. However, the evident clinical signs on the field suggestive of the disease and the increased demand of the government to commercialize the poultry production have invoked a national need to identify the disease urgently and plan an appropriate intervention. Only few reports are existing in the country. For instance, in recent years, Tesfaye *et al*., (2019) and Roba *et al*., (2020) reported serological evidence of ILTV infection in Central and South Ethiopia (19.4%) and Ada'a district in Oromia region (54.7%), respectively and additionally (Birhan *et al.,* 2022) reported prevalence of ILTV infection (59.1%) in central Gondar, West Gojjam, and South Gondar of Amhara regional state, northwestern Ethiopia. This disease causes production losses due to increased morbidity, moderate mortality, decreased weight gain, reduced egg production and expenses spent on vaccination, biosecurity measures and therapy to counteract secondary infection by other avian pathogens (Guy and Bagust 2003; Guy and Garcia 2008; Jones 2010; Garcia *et al*., 2014). Although the disease has paramount economic significance and highly contagious, there are limited scientific reports on the status of ILTV in Ethiopia, and more specifically no evidence of ILT report in East Wollega zone. Thus, the current study designed with the objectives of estimation of the sero-epidemiological status and associated risk factors for the occurrence of Infectious Laryngotracheitis in chickens at Nekemte town and three surrounding districts of East Wollega Zone.

MATRIALS AND METHODS

Description of the study Areas and study period

The study was conducted in at Nekemte town and three surrounding districts namely Guto Gida, Diga, and Leka Dulecha of Eastern Wollega zone of Western Oromia, Ethiopia (**Figure 1**). Diga district is located at a distance of 343 km from Addis Ababa and 12km from Nekemte, administrative town of East Wollega zone. There are about population of 69,965 cattle, 16,424 ovine, 11,857 caprine, 39,730 poultry, and 80,099 people. Its potential land area coverage is 59,545.43 hectares (Diga District Agricultural office, 2022). A Guto Gida district is located at a distance of 320 km southwest of Addis Ababa, the capital city of Ethiopia. The district surrounds Nekemte town having livestock population of 13, 6005 cattle, 22,004 ovine, 23,349 caprine, 837,095 poultry, and 101,189 human populations (Guto Gida District Agriculture office 2022). Leka Dulecha-district is found in south direction from Nekemte town, the capital of East Wollega zone, having distance of 27 km from the town and 355km from Addis Ababa. The climate alternates with long summer rain fall (June-September) and winter dry season (October-May) with mean annual rain fall 1950-1100mm, with daily temperature of 18.5-27.5^{o}C. The district consists of 19 villages and total human population of 73,970 (CSA, 2020). The total livestock population of the area is 603,110 in which 323954 are cattle, 45723 sheep, 32528 goats, 14690 equines and 186215 are poultry (CSA, 2020). The study was conducted between the periods of April 2022 to October 2022.

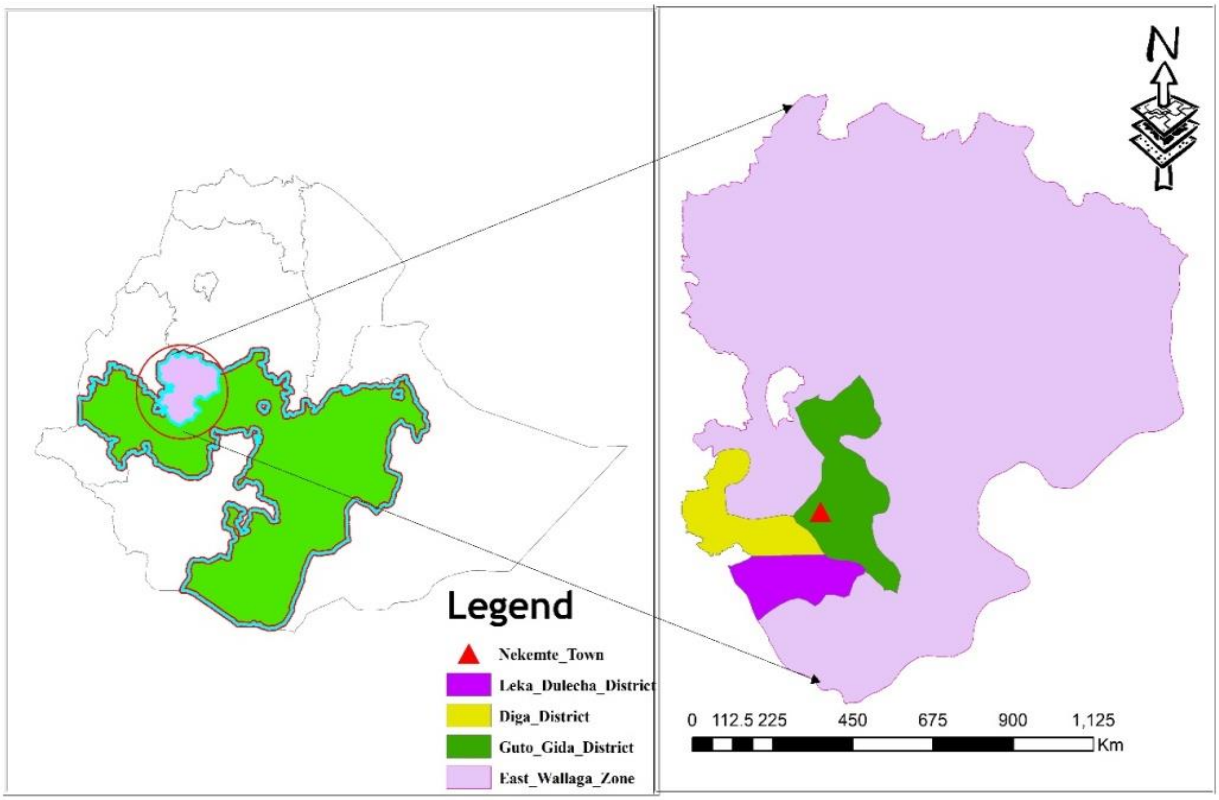

Figure 1: Map of the study Areas (Created by ArcMap 10.7, WGS 1984)

Study Population

The study populations involved in the present study were both existing exotic and local chicken breeds with age ranges from chicks to adult birds. Apparently healthy and non-vaccinated chickens against ILT were the study elements considered. The production purpose of the Chickens was range widely from breeders, egg production, and meat production. The sources of the study chickens were either from market or from Ethio-chicken production centers.

Study Design, sampling techniques and Sample Size determination

A cross-sectional study design was applied from April 2022 to October 2022, with the aims to determine the sero-prevalence of ILTV in chicken and identification of the associated risk factors. Purposive sampling method was executed to select the study districts however; cluster-sampling technique was used to select the smallest administrative units (villages) and farms/households. Villages were used as primary sampling units. Farms/households/flocks were taken as secondary sampling units. Livestock experts and extension staff of the Agricultural office of the districts facilitated the selection. Then, lastly, simple random sampling technique was used to select individual chickens. The sample size was determined by the formula described in Thrusfield, (2018) at 95% confidence interval and 5% of precision and considering that there was no such previous study in the study area (50%) in case of ILT. Thus, the minimum overall sample size required for the study was 384 by using the formula is:

$$n = \frac{Z^2 - \exp P(1-\exp P)}{d^2};$$

$$n = \frac{1.96^2 - 0.5(1-0.5)}{0.05^2} = 384$$

Where, Z= standard Z-value; expP= expected prevalence, d= required precision and n= total sample size.

Data collection

Blood Sample Collection

Blood samples were collected aseptically from the wing vein of each chicken. About 2-3 ml of blood samples were collected using a sterile syringe with a 22-gauge needle. The blood samples in the syringe were allowed to clot in a slant position overnight to separate the sera. Subsequently, the sera was transferred into 1.5 ml Eppendorf cryovial tubes and kept at -20°C during transport by ice box and until the serological analysis for the presence of infectious Laryngotracheitis virus antibodies done. The laboratory procedures were performed at Animal Health institute (AHI), previously known as National Animal Health Diagnostic and Investigation Center (NAHDIC), serology laboratory.

Questionnaire Survey

Sem-structured questionnaire was used to assess all necessary risk factors and information related

to each chicken including age, breed, sex, management system, production type, nutrition status, litter management, carcass management, batch management and ventilation status was recorded on the data-recording sheet by interviewing the owner and by observation during the visit in the farm. Each farmer and local leaders stay 20 minutes to respond to the questionnaire survey.

Laboratory Analysis

The presence of anti-ILTV antibodies in Chicken serum was detected by using commercial indirect ELISA kit (ID Screen® ILT Indirect, 310 rue Louis Pasteur, 34790 Grabels, France) following the procedure provided by manufacturer. In brevity, each serum was tested at a final dilution of 1:500 in Dilution Buffer 14, such that 1:50 pre-dilution followed by 1:10 dilution in the micro plate. About 245µl Dilution Buffer 14 was added to all wells except to the control well (A1, B1, C1, and D1), following addition of 5µl of serum to be tested, in the predilution plate. About 100µl of negative control were added to wells A1 and B1 and 100 µl of positive control were added to wells C1 and D1. Apart from the control wells, 90µl of dilution buffer 14 and 10µl pre-diluted sample were added into the micro-plates, and then the plates were incubated at 21°C for 60 minutes after covering the plate. Then, approximately 300µl of wash solution 1X was used for washing the wells 3 times after the content of the wells is drained, while washing, the wells plate drying was avoided between plate washings and prior to the addition of conjugate. After washing and drying of the wells, 100 µl of conjugate 1X, which has been prepared by diluting the concentrated conjugate 10X in to 1:10 of Dilution Buffer solution, was added into each wells and incubated at room temperature of 21°C for 60 minutes after covering the plate. Following draining and washing of the wells for 3 times with wash solution 1X, 100µl of substrate solution was added into appropriate each wells and incubated at room temperature of 21°C for 15 min by placing the plane in a dark place. Finally, 100µl of stop solution were added to the each well to stop the reaction. Then, the micro-titer ELISA plate was placed in the ELISA reader and the optical densities (ODs) were measured photometrically at a wavelength of 450nm. Sample positivity or negativity was determined by calculating the sample (diluted sera) to positive (S/P) ratio according to the methods provided by the manufacturer as follows:

$$\frac{S}{P} = \frac{ODS - ODNC}{ODPC - ODNC}$$

where "S/P": sample to positive ratio, "ODS": optical density of a given sample, "ODNC": optical density of the negative controls, "ODPC": optical density of the positive controls. Accordingly, sample to positive (S/P) ratios of ≤0.3 and > 0.3 were read as negative and positive, respectively.

Data Management and Analysis

The data collected from field level and laboratory investigation was collected and entered into a Microsoft Office Excel 2019 spreadsheet. The data were checked for errors of entry, coded, and then imported to STATA for descriptive and further analyses. All statistical analyses were done by using Statistical tool that is STATA version 15 software.

Descriptive statistics involving frequency and percentage was used to determine the sero-prevalence of the disease. Logistic regression analysis was used to identify potential risk factors associated with ILT. First, univariable logistic regression analysis with the flock as a random effect was performed and potential risk factors (explanatory variables) with P-values <0.25 were screened for the multivariable logistic regression. Variables with a P value > 0.05were removed from the model, backward elimination procedure, until variables remaining in the final model all reached a P value ≤ 0.05 and these variables was considered as potential risk factors. The strength of the association between outcome and explanatory variables was determined using the crude and adjusted odds ratios (OR).

RESULTS

In the current study, a total of 384 samples were tested for anti-ILTV antibodies using an indirect ELISA. The overall sero-prevalence of ILTV was 15.36% with the highest prevalence observed in Nekemte town commercial poultry farm (21.88%), whereas the lowest seroprevalence was detected in Leka Dulecha district (10.53%) (Table 1).

Table 1: Seroprevalence of ILTV in chickens by Study Districts

Study Districts	Numbers of Chickens:		Prevalence (95% CI)
	Examined	Tested Positive	
Guto Gida	90	13	14.44 (7.92- 23.43)
Leka Dulecha	76	8	10.53 (4.65 – 19.69)
Diga	90	10	11.11 (5.45 – 19.48)
Nekemte town	128	28	21.88 (15.05 – 30.04)
Total	**384**	**59**	**15.36 (11.90 - 19.36)**

The following (Table 2) provides summary of the potential risk factors recorded from the study sites and their respective proportions. As shown, in the table Sasso breed, Ethio-Chicken source of chickens were found significantly associated with the sero-positivity of ILT in the current study areas.

Table 2: Seroprevalence of anti-ILT antibodies analysis across different categorical variables in Chickens in and around Nekemte town

Variables	Categories	No of Chickens		Prevalence (95% CI)	Univariable analysis	
		Examined	Positive		COR (95%CI)	P-value
Age	≤ 1 year	51	7	13.72 (5.70 - 26.25)	Ref	
	1 year	333	52	15.61 (11.88 – 19.96)	1.16 (0.49-2.72)	0.728
Sex	Male	87	10	11.49 (5.65 – 20.12)	Ref	
	Female	297	49	17.56 (13.2 - 22.54)	1.52 (0.74-3.14)	0.258
Breed	Local	186	19	10.21 (6.26 – 15.49)	Ref	
	Bovans	70	11	15.71 (8.11 - 26.37)	0.38 (0.20-0.72)	0.226
	Sasso	128	29	22.65 (15.72 – 30.89)	1.66 (0.73-3.64)	0.003
Source	Market	186	19	10.22 (6.26 - 15.49)	Ref	
	Ethiochicken	198	40	20.20 (14.84 - 26.47)	2.22 (1.23-4.00)	0.008
Production Purpose	Broilers	85	10	11.76 (5.78 – 20.57)	Ref	
	Layers	253	41	16.20 (11.88 - 21.33)	-0.68 (0.32-1.44)	0.324
	Dual	46	8	17.39 (7.82 – 31.41)	1.08 (0.47 – 2.50)	0.842
Hygiene	Medium	145	15	10.34 (5.90 – 16.48)	Ref	
	Poor	101	13	12.87 (7.03 – 21.00)	0.78 (0.35-1.72)	0.540
	Good	138	31	22.44 (15.80 – 30.34)	1.96 (0.96-3.97)	0.062
Management system	Intensive	213	28	13.14 (8.91 –18.43)	Ref	
	Extensive	171	31	18.12 (12.66 – 24.73)	1.46 (0.83-2.55)	0.180

Note: CI: confidence Interval; COR: Crude Odds ratio

External (environmental) factors such as, feed source, chicken batch management method, house ventilation, litter management, chickens nutritional status and carcass management were evaluated as potential risk factors for the seroprevalence of ILTV. As illustrated in (Table 3), seroprevalence showed a statistically significant variation among study sites with Nekemte (OR: 2.38, 95CI: 1.02 – 5.53) and Diga district (OR: 1.43, 95%CI: 0.56 – 3.67) having a higher odd of seropositivity as compared to Leka Dulecha district. Apart from the study districts, none of the extrinsic risk factors evaluated exhibit no statistically significant relationship with ILTV seropositivity (Table 3).

Table 3: Relations of environmental risk factors with ILTV seroprevalence

Variable	Category	Number of chickens		Prevalence (95% CI)	Univariable analysis	
		Sampled	positive		COR (95% CI)	P-value
Feed source	Mixed	10	15	14.15 (8.14-22.26)	Ref	0
	Homemade	212	34	16.04 (11.36-21.68)	1.15(0.60- 2.23)	0.661
	Commercial	66	10	15.15 (7.51-26.10)	1.08 (0.45-2.57)	0.856
Nutritional Status	Well fed	87	10	11.49 (5.65-20.12)	-	-
	Poorly fed	181	29	16.02 (11.00-22.19)	1.46 (0.68 -3.17)	0.327
	Medium	116	20	17.24 (10.86-25.36)	1.60 (0.70 -3.62)	0.256
Batch management	All-in- all-out	97	46	13.40 (7.33-21.82)	Ref	
	Different batch in one house	287	46	16.03 (11.97-20.79)	1.23 (0.63 -2.39)	0.536
Litter management	Used as fertilizer	137	14	10.22 (5.70-16.55)	-	
	Burning	66	13	19.70 (10.92-31.32)	2.15(0 .94-4.89)	0.067
	Accumulate to the nearby free space	181	32	17.68 (12.41-24.03)	1.88 (0.96 -3.69)	0.064
Carcass management	Burying	106	13	12.26 (6.69-20.05)	-	
	Feed to dogs	30	4	13.33 (3.75-30.72)	0.23(0.07-0.72)	0.111
	Throw away	248	42	16.94 (12.48-22.19)	0.31 (0.18 -0.51)	0.8811
District	L/Dulecha	76	8	10.53(4.65 – 19.69)	-	
	Diga	90	10	11.11 (5.45 –19.48)	1.06(0.39– 2.84)	-0.451
	G/Gida	90	13	14.14 (7.92-23.43)	1.43(0.56 –3.67)	0.044
	Nekemte	128	28	21.88 (15.05-30.04)	2.38(1.02 –5.53)	
Ventilation	Partially	50	7	14.00 (5.81-26.73)	-	-
	Well ventilated	334	52	15.57 (11.85-19.91)	1.13(0.48– 2.65)	0.774

Note: COR=Crude odds ratio

Seroprevalence of ILTV in relation to host-specific risk factors (breed, sex, and age) was analyzed as the proportion of affected chickens out of the total examined. The multivariable analysis showed that exotic Sasso breed chickens (AOR: 9.68; 95%CI: 1.96 - 47.78) had a higher odds of seropositivity to ILTV as compared to both Bovans and local breeds (Table 4).

Table 4: Multivariable logistic regression analysis result

Variables	Categories	No of Chickens		% Prevalence (95% CI)	Multivariable analysis	
		examined	Positive		AOR* (95%CI)	P-value
Breed	Local	186	19	10.21 (6.26 – 15.49)	-	
	Bovans	70	11	15.71 (8.11 - 26.37)	2.11 (0.92- 4.84)	0.226
	Sasso	128	29	22.65 (15.72 – 30.89)	9.68 (1.96 - 47.78)	0.005

*AOR: Adjusted Odds Ratio

DISCUSSION

Infectious laryngotracheitis (ILT) is a contagious viral disease with high economic consequence to the poultry industries in developing countries like Ethiopia. To estimate anti- ILT antibody, this cross- sectional study was performed in East Wollega zone where the disease was yet not reported and no history of vaccination against the disease. The present study revealed an overall seroprevalence of 15.36% (95% CI= 11.90 - 19.36). Chickens may seroconvert in response to infection (Hidalgo, 2003) or vaccination with ILT vaccines (Izuchi *et al*., 1984). Infectious laryngotracheitis vaccination in Ethiopia is yet not approved and hence, the current finding can be considered as an indication of possible circulation of the virus in the study areas and warrants the need to isolate and confirm the virus from clinical cases or outbreaks indicative of ILT. The present finding agrees with the finding of Mohamad Inkyas *et al*., (2014) who reported 17.33% from Chitta gong districts of Bangladish and Tesfaye *et al*., (2019) with finding of 19.4% from central and southern parts of Ethiopia. On the other hand, the current finding was lower than the reports of Owoade *et al*., (2006), Roba *et al*., (2020); Salhi *et al*., (2021); Johnson *et al*., (2004); Madsen *et al*., (2013); Derksen *et al*., (2018), Birhan *et al*., (2022); who reported a seroprevalence of 20%; 59.1%; 54.7%, 56.25%, 57.1%, 49%; 45% in Nigeria, Ada'a district of Oromia region in Ethiopia, in Algeria; in Delmarva, Maryland, and Northwestern part of Ethiopia respectively. This variation might be due to vaccination in those developed countries where ILT vaccination is utilized. Higher finding mentioned above were reported from studies conducted in commercial production systems where contagiousness nature of ILT gets opportunity to raise the prevalence reported.

In contrary, the current report (15.36%) is higher than the of findings Uddin *et al.*, (2014) who reported 13% in selected areas of Bangladesh; Pohjola *et al*., (2016) with report of 4% in Finland; Shittu *et al.*, (2016) who reported 13% in broiler flocks of 1.2% in north central Nigeria; Ghalyanchi *et al.*, (2020) who reported 13% in broiler flocks of Iran and Tadiose *et al*., (2022) who reported 11% prevalence of ILTV from clinical cases in Addis Ababa respectively. In this study, seroprevalence varied significantly ($p < 0.05$) among the different study sites, with the highest seroprevalence recorded in Nekemte town followed by Guto Gida, Diga and the least in Leka Dulecha. Similarly, Bhuiyan *et al*., (2019), Roba *et al.*, (2020) and Birhan *et al*., (2022) reported a significant variation in prevalence of ILTV between the different study areas they considered. Differences in management practices including biosecurity and vaccination could explain the differences in different study areas. In contrary to the present finding, Tesfaye *et al*., (2019) and Salhi *et al.*, (2021) did not observe a statistically significant difference among their study sites.

Among the host-related risk factors, only breed showed a significant association with seroprevalence ($p<0.05$). Sasso breed chickens had 9.68 times more likely in the odds of seropositivity as compared to local breed and Bovans exotic breeds (AOR: 9.68, 95%CI: 1.96 -47.78) This study was in line with finding of Birhan *et al*., (2022) who reported that local chickens had a 62% reduction in the odds of seropositivity as compared to exotic breed. Studies have shown that local breeds of chickens have better environmental adaptability and disease resistance traits than exotic breeds (Wong *et al*., 2017). Other intrinsic factors such as age, sex, and production purpose of chickens did not show significant association.

Chickens included in the study were either from market or from Ethio-chicken. It was found that the source of chickens was statistically associated with seroposivity of ILTV at univariable analysis. Accordingly, the likelihood of seropositivity to ILTV was 2.22 times higher in chickens obtained from Ethio-chicken compared to those from market source. In Ethiopia, vaccination against ILT is still under controversy as there is some rumor circulating that some commercial poultry farms in the country were vaccinating their flock against the disease though the vaccine is not officially allowed to be given in Ethiopia. Thus, the high seroprevalence in chickens obtained from Ethio-chicken might due to this confidentially and concealed vaccination by the company.

CONCLUSIONSAND RECOMMENDATIONS

This study revealed a seroprevalence of 15.36% in backyard and commercial chickens in Nekemte town and three surrounding districts of East Wollega zones of Oromia region, Ethiopia. As to the knowledge of the authors, this is the first report about ILTV in the East Wollega zone. Hence, the present result can be considered as an important signal that prompts further investigations about ILT near the study zone and the

country as well. In this study, source of chickens and breed were found to be statistically associated with occurrence of ILT. Therefore, all stakeholders and concerned bodies should play their roles in the investigation and planning of an appropriate intervention mechanisms as well as application of stringent biosecurity procedures that would have an utmost impact on the control of the disease. Furthermore, further epidemiological investigation and Molecular confirmation and characterization of the virus from ILT suggestive cases should be considered to justify the use of ILT vaccines.

Acknowledgements

Firstly, all glory should be to God for reaching this milestone in my life. I am grateful to express my thanks and appreciation to my advisors Dr. Begna Bulcha for his encouragement, continuous guidance and overall assistance.

We would like to appreciate and thank Animal Health Institute (AHI) staffs, especially Dr. Demeke Zewde and Mr. Chala Dima who provided us invaluable assistance during serological test and, the owners of poultry farmers who allow to collect the data.

Ethical Approval

Before starting data collection, ethical approval was obtained from the Research Ethics Committee of the School of Veterinary Medicine, Wallaga University, and dated 05/04/2022 with a reference no. VMERC 18/14/04/2022. Data was collected after getting the permission from animal owners to collect sample from animal. During the period of data collection, good veterinary practice was applied, the information was anonymous, and confidentiality of data was assured. The purpose of the study was explained to the animal owners, and oral informed consent was received.

Consent

The purpose of the study was clearly explained to the poultry owners and veterinary officers, and informed verbal consents were obtained.

Funding

The research did not receive any specific funding from this study.

Conflicts of Interest

The authors declare that they have no conflict of interest.

Authors' Contributions

TKB and BBG are involved in idea generation proposal development, data processing, and editing, and MEA is involved in proposal development and data collection.

List of abbreviations

AOR: Adjusted Odds Ratio; COR: Crude Odds Ratio; CSA: Central Statistics Agency; ELISA: Enzyme linked immune sorbent assay; FAO: World Food Organization ILT: Infectious Laryngotrachitis

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