



Pathological Studies on Viral Diseases of Poultry in Mizoram, India

KEYWORDS

Prevalence, poultry, viral diseases, Mizoram.

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ABSTRACT A study was conducted during the period from March 2013 to February 2014 to survey the prevalence of viral diseases of poultry in Mizoram, to study the pathology and finally to diagnose them by using common laboratory techniques. A total of 476 dead and sick birds were collected from different organized and unorganized poultry farms of Mizoram. Of these, 208 cases (i.e. 43.69%) were diagnosed as viral diseases. The diseases were diagnosed based on clinical history, signs and symptoms prior to death, gross lesions observed on post-mortem examination, histopathology and laboratory detection of viral antigens. Among the diseases, infectious bursal disease (IBD) was found in 15.13% followed by Newcastle disease (ND) in 8.40%, Marek's disease (MD) in 7.14%, lymphoid leukosis (LL) in 5.88%, hydropericardium hepatitis syndrome (HHS) in 5.04% and infectious laryngotracheitis (ILT) in 2.10% cases, respectively. Age-wise analysis showed that the maximum number of cases were recorded in the age group of 3-6 weeks (40.38%), followed by 6-9 weeks (28.84%), above 12 weeks (17.30%), 1-3 and 9-12 weeks (6.73%) age groups of poultry. The season-wise incidence of viral diseases was found to be the highest during winter season, followed by summer and rainy seasons. The present study suggests that many viral diseases are prevalent in poultry population of Mizoram which occur mostly in winter season with higher incidence in 3-6 weeks age groups.

INTRODUCTION

Poultry industry has made remarkable progress in Mizoram during the last few decades, from a backyard venture to a considerable sophisticated commercial industry. Total poultry population of Mizoram was estimated to be 12,34,150 as per 18th Livestock Census, 2007 (Anonymous, 2010). However, a negative trend in the growth was observed in last few years. The total egg production of Mizoram in 2008-09 was 411 lakh which declined to 371 lakh during 2009-10 (Anonymous, 2010). One of the most important reasons behind this is frequent incidence of diseases in poultry farm leading to huge economic losses to the poultry farmers.

The poultry health care system in Mizoram suffers due to difficult geographical terrains which lead to reduced accessibility, weaker linkages and a poor health delivery system. In rural areas, diagnosis of poultry diseases is mostly based on clinical and post-mortem examination. Often the etiology of outbreak of diseases, which cause huge loss to the poultry farmers, remains undiagnosed. The inadequate measures adopted by the stakeholders for the control of diseases in addition to inefficient management have rendered the poultry farming a risky business. Poor reporting system and lack of mass surveillance facilities are also among the major constraints for generating the data regarding exact disease status and the economic losses. Moreover, Mizoram shares borders with Myanmar and Bangladesh and is also famous for game birds, chances of disease occurrence in this area is more. However, no detailed studies have been done on poultry diseases in this area so far. So, the epidemiology, pathogenesis and pathology of the poultry diseases in this area are not fully known.

MATERIALS AND METHODS

The epidemiological data pertaining to viral diseases in

poultry from March 2013 to February 2014 were collected from both organized and unorganized poultry farms of Mizoram. Detailed information such as total birds in a flock, number of birds affected, number of birds died, age of the affected birds, month of occurrence of the disease, history of previous outbreaks of viral diseases and vaccination status were obtained from the affected flocks.

Dead/moribund birds were collected for proper necropsy. Representative tissue samples (heart, liver, spleen, lungs, kidneys, bursa of Fabricius, trachea, proventriculus, caecal tonsil, etc.) showing typical lesions were collected for histopathological examination and laboratory analysis.

Both organized and unorganized poultry farms of Mizoram were visited regularly and the morbidity, mortality, age of affection of various diseases were recorded. To assess the age-wise variations in the incidence of the diseases, the birds were grouped as 1-3, 3-6, 6-9, 9-12 and above 12 weeks old. To study the seasonal variations, the whole year was divided into conventional three seasons, namely summer (March-June), Rainy (July-October) and winter (November-February).

In case of mortality/outbreak of diseases in the poultry population, the clinical signs exhibited by the individual bird during illness were recorded in detail in a prescribed according to the description of the respective poultry farm's owner or attendant. In addition, sometimes some sick/moribund birds were kept under careful observation with feed and water *ad libitum* till death to record the detailed clinical signs along with other abnormalities.

Detailed post-mortem examination of all the dead birds was performed. At necropsy, gross tissue changes were observed and recorded carefully. Representative tissue samples (heart, liver, spleen, lungs, kidneys, bursa of Fabricius, trachea, proventriculus, caecal tonsil, brain, feather follicles, etc.) showing lesions were carefully collected in ice and in 10% formaldehyde solution. Viable tissue samples were collected aseptically

in sterile polypropylene zipper bags and stored in -80°C for further analysis.

Formalin fixed tissues (2-3 mm thick) were taken, washed overnight in running tap water and then dehydrated in ascending grades of alcohol starting from 50%, 70%, 90% and absolute alcohol I, alcohol II, alcohol III and finally cleared in xylene. These dehydrated tissue pieces were then embedded in molten paraffin. Sections were cut at 4-5 µm thick with semi-automatic rotary microtome (MRS 3500, Histoline Laboratories) and stained with Mayer's hematoxylin and eosin (Bancroft and Stevens, 1980). The stained slides were examined under a trinocular research microscope (Olympus) and the magnified images of the tissue structures were captured for further study.

The diagnosis of the diseases was made mainly basing on the clinical history, signs, characteristic gross lesions and microscopic tissue alterations. However, common laboratory techniques such as haemagglutination (HA), haemagglutination inhibition (HI) and agar gel precipitation test (AGPT) were conducted to detect the viral antigens of diseases like infectious bursal disease (IBD), Newcastle disease (ND) and Marek's disease (MD) as per the procedure described by OIE manual (2012) with modification.

RESULTS AND DISCUSSION

A total of 476 poultry carcasses were examined during the present study period, of which 208 (43.69%) cases were diagnosed as viral diseases. This present percent proportionate incidence is found higher than that Rahman and Samad (2005) who recorded 22.73% as viral

diseases. Among the viral diseases, the incidence of infectious bursal disease (15.13%) was found to be the highest followed by Newcastle disease (8.40%), which is in accordance with the findings of Rahman and Samad (2005). Whereas, the incidences of Marek's disease (7.14%), lymphoid leukosis (5.88%), hydropericardium hepatitis syndrome (5.04%) and infectious laryngotracheitis (2.10%) respectively were comparatively low. Age-wise analysis showed that the highest occurrence of viral diseases was recorded in the age group of 3-6 weeks (40.38%), followed by 6-9 weeks (28.84%), above 12 weeks (17.30%), 1-3 and 9-12 weeks (6.73%) age groups, which supports the earlier reports of Rahman and Samad (2005). Name of the viral disease, total number of cases examined, age of birds, number of confirmed cases and proportionate incidence% recorded during the present study period are shown in (Table 1).

The season-wise incidence of viral diseases was found to be the highest during winter season, followed by summer and rainy seasons (Table 2), which is contradictory to the earlier reports of Rahman and Samad (2005) who recorded the highest incidence in summer followed by rainy and winter seasons. The highest incidence in winter season could be attributed to extreme cold weather prevailing in this geographical area which predisposes the poultry to a variety of infectious diseases. The higher viability of the viruses in cold weather could also be a factor. The geographical location of this region restricts the extreme of temperature in summer season which might be responsible for lower incidence of viral diseases in the birds during summer season.

Table 1: Overall prevalence of poultry viral diseases in Mizoram: age-wise distribution and proportionate incidence.

Name of disease	Number of carcasses examined	Number of suspected cases	Age of birds (weeks)					Number of confirmed cases	Proportionate incidence%
			1-3	3-6	6-9	9-12	>12		
IBD	476	98	10 (13.88%)	50 (69.44%)	10 (13.88%)	2 (2.77%)	-	72	15.13
ND		57	4 (10.00%)	10 (25.00%)	14 (35.00%)	4 (10.00%)	8 (20.00%)	40	8.40
MD		49	-	-	28 (82.35%)	6 (17.64%)	-	34	7.14
LL		40	-	-	-	-	28 (100.00%)	28	5.88
HHS		35	-	20 (83.33%)	4 (16.66%)	-	-	24	5.04
ILT		18	-	4 (40.00%)	4 (40.00%)	2 (20.00%)	-	10	2.10
TOTAL	476	297	14 (6.73%)	84 (40.38%)	60 (28.84%)	14 (6.73%)	36 (17.30%)	208	43.69

Table 2: Overall prevalence of poultry viral diseases in Mizoram: Season-wise distribution and proportionate incidence.

Name of disease	Summer	Rainy	Winter	Number of confirmed cases
	(March-June)	(July-October)	(November-February)	
Infectious bursal disease (IBD)	22 (30.55%)	20 (27.77%)	30 (41.66%)	72 (15.13%)
Newcastle disease (ND)	4 (10.00%)	10 (25.00%)	26 (65.00%)	40 (8.40%)
Marek's disease (MD)	8 (23.52%)	8 (23.52%)	18 (52.94%)	34 (7.14%)
Lymphoid leukosis (LL)	10 (35.71%)	6 (21.42%)	12 (42.85%)	28 (5.88%)
Hydropericardium hepatitis syndrome (HHS)	12 (50.00%)	7 (29.16%)	5 (20.83%)	24 (5.04%)
Infectious laryngotracheitis (ILT)	-	-	10 (100.00%)	10 (2.10%)
TOTAL	56	51	101	208

Infectious bursal disease (IBD)

The highest incidence of IBD was recorded in 3-6 weeks old birds, which is in support of Lukert and Saif (1997) as well as the report of Mor *et al.* (2010) who found maximum cases (52.80%) in 21-30 days old birds followed by (33.13%) in 31-40 days old birds in Haryana. The younger chicks of 1-3 weeks as well as 6-9 weeks old were also found affected during the investigation, which is in conformity with the earlier reports of Fadley & Nazerian (1983) and Okoye & Uzoukwu (1981) respectively. The disease was found to occur all around the year as similarly reported by Babiker *et al.* (2008). The highest incidence was observed during the winter season followed by summer and rainy seasons. Similar observations were also reported by Jindal *et al.* (2004) and Sultana *et al.* (2008).

Post-mortem lesions of IBD affected birds included darkened discoloration of thigh and breast muscles with frequent hemorrhages, enlarged and swollen bursa with accumulation of thick creamy exudates, while in some cases; there were presence of gelatinous exudates around bursa and bursal hemorrhages with blood clots. Similar observations have been recorded by several workers (Zelege *et al.*, 2005; Dutta *et al.*, 2007; Singh, 2008; Sultana *et al.*, 2008).

Newcastle disease (ND)

During the present investigation the disease was found to affect all age groups of birds as similarly described by Shankar (2008). The highest incidence was observed during the winter season followed by rainy and summer seasons, which directly supports Nwanta *et al.* (2008) and Olabode *et al.* (2012) who also reported the highest occurrence of ND in dry season i.e. winter season. The dampness and wet environment in rainy season might cause higher susceptibility, while the pleasant climatic condition in summer season might be responsible for lower incidence of the disease in the birds of this region.

Necropsy examination of birds died of ND showed pin point hemorrhages at the tips of the proventriculus glands, hemorrhagic ulcers in intestinal wall and caecal tonsils, hemorrhagic tracheitis with congestion and catarrhal exudates, which are in conformity with earlier reports of several workers (Singh, 2008; Hadipour *et al.*, 2011; Khan *et al.*, 2011).

Marek's disease (MD)

The highest incidence of MD was found to occur in birds of 6-9 weeks followed by 9-12 weeks of age and no cases were recorded in other age groups. This observation is nearly similar to that of Rajkhowa (2005) who recorded acute MD outbreaks in commercial chickens of above 8-9 weeks of age in Aizawl, Mizoram.

The MD affected birds revealed white or grey tumors of varying sizes on liver or marked enlargement of liver with granular appearances during post-mortem examination, which supports the findings of Hablolvarid (2011) and Gong *et al.* (2013) who observed formation of tumors resulting into diffuse enlargement and white or greyish discoloration of the organs. The lesions of the sciatic nerves are in accordance with Murphy *et al.* (1999) and Duguma *et al.* (2005) who observed that the affected nerves (vagus, brachial, sciatic) were pale or greyish discoloration, enlarged two to three times than normal size and lost the cross-striations.

Lymphoid leukosis (LL)

The cases of lymphoid leukosis were only found to occur

in adult birds above 12 weeks of age, mostly at sexual maturity, which is in accordance with Rubin *et al.* (1962), Purchase and Burmester (1972) who describe that in field outbreaks, lymphoid leukosis case can occur at any time after 14 weeks but the incidence is highest at sexual maturity. The lower incidence of the disease recorded in the present study (i.e. 5.88%) might be due to the widespread occurrence of infectious bursal disease in this region, as the bursa is required as a target organ for the initial transformation to lymphoid cell (Saif, 2008).

Most of the carcasses showed severe emaciation and dehydration, which might be due anorexia and diarrhea during the disease course. Necropsy of the birds showed visible tumors on the liver surface which was soft, smooth and glistening. There was marked enlargement of liver and spleen which were almost twice the size of normal organs. The kidneys were congested, enlarged and nodular in appearance. These findings are in conformity with the earlier reports of Mathew *et al.* (2010) and Saif (2008). The bursa of few birds had congestion, enlargement and nodular in appearance, which supports the report of Cooper *et al.* (1968).

Hydropericardium hepatitis syndrome (HHS)

The highest incidence of the disease was found to occur in birds of 3-6 weeks of age, while a few cases seen in 6-9 weeks of age and no birds of the other age groups were found affected. These results are in agreement with the earlier reports (Rajkhowa, 2002; Balamurugan and Kataria, 2004; Memon *et al.*, 2006). The highest incidence of the disease was found to be more in summer followed by rainy and winter seasons, which supports the description of Asthana *et al.* (2013).

The most striking post-mortem findings were accumulation of clear or straw-coloured, watery or jelly-like fluid in the pericardial sac, a misshapen and flabby heart, which have been similarly described by many workers (Sonawane, 2000; Biswas *et al.*, 2002; Rajkhowa, 2002; Memon *et al.*, 2006). The swollen, congested and friable livers with focal hepatic necrosis, congested and oedematous lungs and pale kidneys with prominent tubules, recorded during the present study, support the observations of earlier reports (Sawale *et al.*, 2012; Thakor *et al.*, 2012; Kataria *et al.*, 2013).

Infectious laryngotracheitis (ILT)

In the present study period, higher incidences of ILT were seen in 3-6 and 6-9 weeks old birds followed by a lower incidence in 9-12 weeks old birds, while birds in the age groups of 1-3 and above 12 weeks were not affected. This result is in agreement with Fahey *et al.* (1983) and Bagust *et al.* (1986) who describes that all ages of chickens are affected but chickens older than 3 weeks are most susceptible to ILTV. All the incidences of the disease were found to occur only in winter season, which might be due to the strong dry wind and extreme cold weather prevailing in this region which predisposes the birds to get this kind of air-borne infections.

Post-mortem lesions included mucoid tracheitis, laryngitis and severe hemorrhages in the trachea which was filled with blood-mixed mucus leading to obstruction. Similar changes have been described by several workers (Aziz, 2010; Barhoom and Dalab, 2012). Tracheal sections revealed severe congestion and hemorrhagic mucosa which showed the presence of syncytial cells with intranuclear inclusion bodies, while some sections had complete desqua-

mation of the mucosa. These microscopic changes of the present study are in accordance with the earlier reports of many researchers (Aziz, 2010; Srinivasan et al., 2012; Preis et al., 2013).

The present study reveals that some viral diseases namely; IBD, ND, MD, LL, HHS and ILT are prevalent in poultry population of Mizoram and the birds aged between 3-6 weeks and winter season are most vulnerable to these diseases which needs immediate attention for prevention and control.

ACKNOWLEDGEMENTS

The authors are thankful to the Dean, College of Veterinary Sciences & A.H., CAU, Selesih, Aizawl, Mizoram for providing funds and facilities for carrying out the present study.

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