



Concomitant Vitamin A Deficiency Following Suspected Fowl pox virus Infection Leading to Esophageal Gland Metaplasia in a Layer Flock

Rajanbir Singh Randhawa¹, Sidhartha Deshmukh^{1*}, Harmanjit Singh Banga¹ and Navdeep Singh²

¹Department of Veterinary Pathology, College of Veterinary Science, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, INDIA

²Veterinary Officer, Department of Animal Husbandry, Punjab, INDIA

*Corresponding author: S Deshmukh; Email: sid0159@yahoo.com

Received: 01 Sept, 2018

Revised: 25 Nov., 2018

Accepted: 28 Nov., 2018

ABSTRACT

The present communication pertains to an incidental occurrence of concomitant vitamin A deficiency and fowl pox infection in a layer flock. The lesions were characterized by typical presence of pustule like eruptions in cranial part of esophageal mucosa and histologically with metaplastic and keratotic glands with distinctly evident intracytoplasmic inclusion bodies within few intact epithelial cells. Specialized staining techniques i.e. Triple shorr's confirmed its presence at palatine mucosal gland, esophageal gland and tracheal epithelium. To author's knowledge, the evidence of eosinophilic intracytoplasmic inclusion bodies deeply within tracheal epithelial surface is rare and one of the few histologically documented case where unusual location of inclusion bodies at splanchnic surface was described, in addition to the description of vitamin A deficiency with accelerated, pre-altered tissue morphology preceded by fowl pox virus infection.

Keywords: Vitamin A deficiency, Fowl Pox infection, esophageal gland, Inclusion bodies

Incidences of vitamin A deficiency in poultry operations in recent days have become almost negligible with few isolated occurrence. The reason being awareness among farmers with practical sense of scientific farming. Despite of it, some complexities still exists in the management and operational aspects, which insidiously, results in obvious clinical symptoms of either infectious or non infectious disease. At several instances in past, there had some uncertainty exist over with regards to identification of primary cause of affection due to their identical pattern and disturbances, which warranted thorough investigation and such delay unknowingly had resulted serious fallout in the form of severe disease outbreaks (Wahab *et al.*, 2017). Here in through this communication, we intend to report a typical case where overlapping of clinical appearance of Vitamin A deficiency and Fowl pox infection, confounded diagnostic ability, thus inadvertently missed crucial etiology that resulted contigal problem of inanition, sluggish mortality and marked production drop in some layer flocks. The affected chicken flocks, were previously

affected by fowl pox virus, probably have ensued post pox virus complications that putatively lend favorable contributions to this additional vitamin A affiliated anomaly in the given case. To our knowledge, this is a rare or incidental report where post fowl pox virus infection in chicken has potentiated the problem of vitamin A deficiency due to its inadvertent missing in the ration. Further, our attempts to characterize the histological alterations owing to concomitant non-infectious etiology and infectious anomaly had certainly added supplementary information, which is unavailable. Incidentally, we found a rare evidence of intracytoplasmic eosinophilic inclusion bodies in tracheal epithelial lining and esophageal mucosal surface, which probably has never been discussed before in the annals of veterinary science.

MATERIALS AND METHODS

The clinical sign of swollen eye with cheesy materials along with ruffled feathers initially appeared among 80

weeks aged layer birds with eventual spread to younger lots i.e. 56 weeks aged chicken. The disease further developed with blackening and drying of combs with marked dehydration possibly owing to decreased feed and poor watering intake. Approximately a total of 5% of birds in flock exhibited above cited eye complications along with 60% drop in egg production. An average of 2-3 % mortality was noticed over a period of fortnightly long illness. Affected birds had poorly recovered from the illness with a record of 70% return in the total egg production. Thereafter, few affected and moribund birds were sent to laboratory, at Department of Veterinary Pathology, GADVASU, Ludhiana for necropsy. On post mortem examination, the gross lesions were mainly noted confining to cranial part of esophagus characterized as white to yellowish tiny discrete nodules of nearly 1 mm in size over the mucosa (Fig. 1).



Fig. 1: Esophagus, showing small whitish pustule like eruptions in mucosal surface

There were no additional gross pathological lesion noted in major organs like eye, liver, kidney and spleen. The lungs exhibited moderate degree of hyperemia and consolidations. Requisite tissue samples like esophagus, trachea and lungs were collected in 10% Formalin for histopathology. Following fixation, the tissues were processed through graded changes of alcohol followed by xylene treatment and paraffin embedding. Tissue sections were cut at 3-4 μ thickness and were finally subjected to hematoxylin and eosin staining (Luna, 1965). The

stained slides were mounted with cover slip using DPX mountant and examined under bright field microscope for histopathological alterations.

RESULTS AND DISCUSSION

The rare occurrence of fowl pox virus infection proceeding with accentuated vitamin A deficiency in a layer operation is reported. On opening, esophagus from some birds, showed typical presence of tiny nodular eruptions resemblances to pustules in the mucosal surfaces. The frequency of distribution of such pustule(s) on the mucosal lining were mainly confined to the cranial part of esophagus, pharyngeal mucosa and were comparatively lesser in numbers as reported by Prabhu *et al.* (2015) in a non descript duck. On cut, such pustule like eruptions revealed, almost inspissated exudative content(s). Accidentally, we noted very few small sized, indistinct presence of mucosal bulging over the trachea in some birds, which were later confirmed histologically. The lungs on either side exhibited reddening and consolidation which were characterized by marked infiltration of mononuclear cells, hemorrhages as well as proliferation of regional alveolar epithelial cells that resulted in near constriction of available air spaces. The histological section of esophagus mainly revealed cystically dilated metaplastic and hyperkeratotic glands containing degenerated, necrotic debris with several numbers of intact and dying heterophils (Fig. 2A). Such metaplastic and keratotic gland looks characteristics to lesion of vitamin A deficiency, mainly affecting the epithelia of digestive, respiratory and urinary tracts (Howell, 1970; Aye *et al.*, 2000; Cortes *et al.*, 2006). On close examination of certain metaplastic and exfoliated cells within the esophageal glands, presence of indistinct eosinophilic inclusion bodies were noted (Fig. 2B). Similar outstanding bodies were also seen scattered in tracheal epithelial cells and esophageal gland, which was confirmed through triple shorr's staining. (Fig. 2C). This indicates the remnant of fowl pox virus intracytoplasmic inclusions within epithelial cells that might have facilitated the progression of lesions with more accentuation to vitamin A deficiency. Presumably, the damage taken place with fowl pox virus infections like excessive proliferations of epithelial cells of esophageal gland as well as excoriation of inclusion inflicted cells in conjunction with missed vitamin A supplementation had further worsen tissue architecture to an extent that has

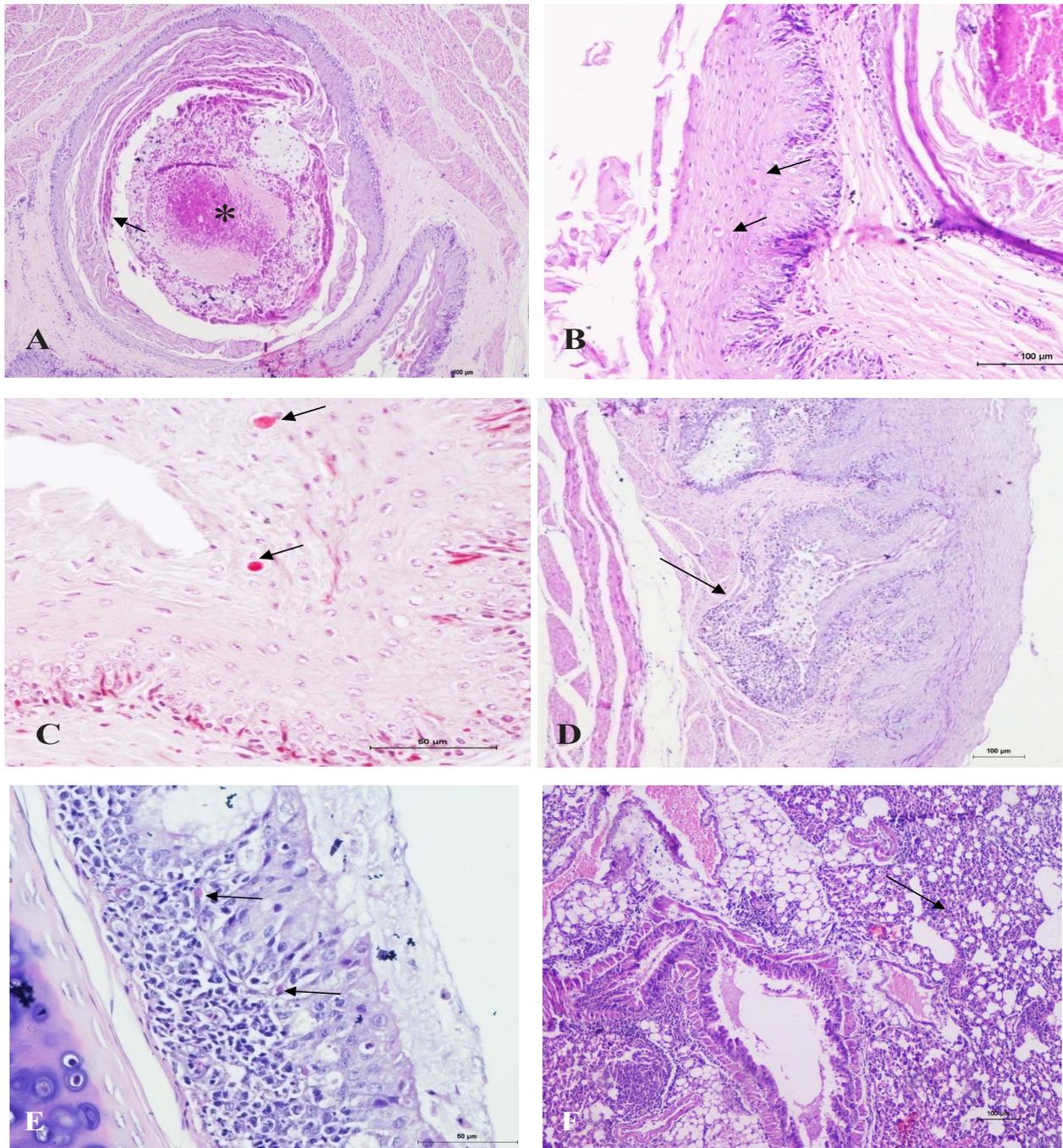


Fig. 2. (A): Esophageal gland with metaplastic and keratotic glandular lining containing inspissated exudates (H&E $\times 10$), (B): Highly stratified epithelial surface showing presence of intracytoplasmic inclusion bodies (H&E $\times 20$), (C): Same as 2b showing distinctly delineated reddish orange intracytoplasmic inclusion bodies within epithelial cells (Triple Shorr's $\times 40$), (D): Palatine gland with squeezed outline due to surrounding inflammatory reactions (H&E $\times 10$), (E): Trachea, mucosal zone inundated with mononuclear cells particularly with lymphoid cells. Presence of few interspersed eosinophilic intra-cytoplasmic inclusion bodies is noted. (H&E $\times 20$), (F): Lungs, showing massive inflammatory cell infiltrations causing obliterations of alveolar air spaces (H&E $\times 10$).

led to absolute clogging of esophageal gland at several places. Very recently a report had indicated co-existence of Fowl pox virus infection with *Candida albicans* infection in chickens where unusual pox lesions were described in bursal follicle epithelium (Ogaswara *et al.*, 2016). A similar attempt to ascertain and identify other possible factors responsibly augmenting the condition of hypovitaminosis A and worsening the organ's textural alterations were delved into and found no significant findings. Considering this, one can easily establish that there is a possibility of fowl pox infection progressing simultaneously and resulting in flaring up some infectious and non-infectious etiologic cause of disease.

The initial or cranial part of esophagus containing few adjacent palatine glands were also found to be squeezed with peripheral inflammation especially infiltrated with lymphoid cells (Fig. 2D). Additionally, the sub-epithelial lining of trachea showed intense lymphocytic infiltrations with encapsulated nodular like aggregations containing macrophages and some interspersed lymphocytes (Fig. 2E). Lungs showed massive cellularity with loss of air spaces following heterophilic and mononuclear cell infiltrations (Fig. 2F). The involvement of palatine mucosa in this case also supports the fact that the initiation of vitamin A deficiency pustules was probably in their early stages of formation, which was also seen in cranial part of esophagus with possible early phase (i.e. marginal degree) of avitaminosis. Normally in cases of early or marginal vitamin A deficiency, the involvement of oropharynx and cranial part of esophagus were mainly noted and reported (Aye *et al.*, 2000).

Further, following report of probable vitamin A deficiency and immediate restoration of supplemented vitamin A premix in the feed, there had been a significant alleviation of problem in the flock.

CONCLUSION

Interestingly, this observation has clearly demonstrated possible concomitant and potentiated aggravation of vitamin A deficiency associated tissue damages following fowl pox infections in layer flock. It would be further advisable to maintain the fact, that such inadvertent

missing of important fat soluble vitamins in feed/ rations of productive flocks (birds) may accentuate the birds towards an increased diseased incidence, decreased productivity or contiguous sluggish mortality and should be avoided. Hence based on gross and histopathological lesions, the case here may be considered as condition of early vitamin A deficiency following fowl pox infections.

ACKNOWLEDGEMENTS

The authors are thankful to Dean, College of Veterinary Sciences, GADVASU, Ludhiana, as well as Director of Research, GADVASU for providing necessary help to accomplish this work. The authors are also grateful to Head, Department of Veterinary Pathology, GADVASU, Ludhiana for extending help in the completion of this work.

REFERENCES

- Abd El-Wahab, A., Visscher, C., Ratert, C., Kölln, M., Diephaus, D., Beineke, A. and Kamphues, J. 2017. Outcome of an experimental study in growing Turkeys suspected of having a diet related, uncommon and uncoordinated gait. *Vet. Sci.*, **4**: 49.
- Aye Morishita, T., Saif, Y., Latshaw, J., Harr, B. and Cihla, F. 2000. Induction of Vitamin A deficiency in Turkeys. *Avian Dis.*, **44**(4): 809-817.
- Cortes, P.L., Tiwary, A.K., Puschner, B., Crespo, R.M., Chin, R.P., Bland, M. and Shivaprasad, H.L. 2006. Vitamin A deficiency in Turkey Poults. *J. Vet. Diagn. Invest.*, **18**(5): 489-494.
- Howell, J.M. 1970. Lesions in nervous tissue and bone in ataxic vitamin A deficient quail. *Acta Neuropathol.*, **16**: 285-292.
- Luna, L.G. 1968. Manual of histologic staining methods of the Armed Forces Institutes of Pathology, *American Registry of Pathology*, McGraw-Hill Book Company, New York, USA, Third edition, pp. 258.
- Ogasawara, F., Yamamoto, Y., Sato, Y., Fukunari, K., Murata, K., Yaegashi, G., Goto, M. and Murakami, R. 2016. Concurrent fowl pox and Candidiasis diseases in Backyard chickens with unusual pox lesions in the Bursa of Fabricius. *Avian Dis.*, **60**(3): 705-708.
- Prabhu, S.N., Gangwar, N.K., Kumar, R. and Giri, D.K. 2015. Squamous metaplasia of esophageal mucous glands in a non-descript duck. *Indian J Poult Sci.*, **50**(2): 229-230.