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Serological Survey of Avian Metapneumovirus Infection in Broiler Breeder Chicken Farms in Tamil Nadu

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Abstract Avian metapneumovirus (aMPV) is an important poultry pathogen causing an acute highly contagious upper respiratory tract infection in chickens leading to swollen head syndrome. The disease can cause significant economic losses in turkey and chicken flocks, particularly when exacerbated by secondary pathogens. The purpose of this study was to determine the prevalence of avian metapneumovirus antibodies in broiler breeder flocks in Tamil Nadu, India. Twenty numbers of broiler breeder farms located in Tirupur district of Tamil Nadu were selected randomly and blood samples were collected. A total of 485 blood samples were collected from 20 broiler breeder chicken flocks (aged between 4 and 72 weeks). The serum samples were tested for the presence of antibodies against avian metapneumovirus by using a commercial enzyme-linked immunosorbent assay kit (IDEXX APV Ab test, Liebefeld-Bern, Switzerland) which was able to determine antibodies against A, B and C subtypes of avian metapneumovirus. Out of 485 serum samples, 165 (34.02%) were positive to avian metapneumovirus antibodies, which represented 14 of 20 (70%) examined broiler breeder flocks. All the chickens had not been vaccinated against avian metapneumovirus in India and these results indicate that commercial poultry birds are exposed to this important poultry pathogen. This is the first report of serologic evidence of AMPV in India. Its prevalence has to be investigated in other parts of India. Future work may and should include the use of molecular methods and isolation of the virus. Isolation of avian metapneumovirus will allow the possibility of controlling the disease.
Keywords *ELISA; Seroprevalence; Swollen Head Syndrome*

1. Introduction

Avian metapneumovirus (aMPV) is recognized as an important pathogen in many commercially poultry producing countries. It belongs to the family *Paramyxoviridae*, subfamily *Pneumovirinae*, genera *Metapneumovirus*, and has an enveloped unsegmented single-stranded negative sense RNA

[1]. aMPV causes Turkey Rhinotracheitis (TRT) in turkeys and Swollen Head Syndrome (SHS) in chickens. aMPV can cause damage to the upper respiratory tract (trachea), such as, lack of cilia movement and/or cilia loss—damage that may lead to respiratory clinical signs such as nasal discharge, coughing, sneezing and more complicated respiratory problems. This stress on the cilia and upper respiratory tract can facilitate the multiplication of *E. coli* and other bacterial infections such as *Mycoplasmas, Pasteurella, Bordetella sp* and *Ornithobacterium rhinotracheale*, etc. that lead to a respiratory syndrome called swollen head syndrome [2]. aMPV plays a role in the multiplication of infectious bronchitis virus (IBV) in the upper respiratory tract. aMPV can also affect the reproductive tract, impacting egg formation in turkey breeders, broiler breeders and egg-type chickens, resulting in an increase in the percentage of egg abnormalities and a drop in egg production [3, 4]. aMPV was first detected in turkeys in South Africa in the late 1970s [5]. Later it was reported in chickens in South Africa [6]. By 1993, Alexander described TRT in Israel, France and Great Britain. Serological evidence of aMPV is now available from many countries of the world [7, 3]. To our knowledge, there is no published information on aMPV in India. ELISA is the most common serological way of diagnosing aMPV infection in chicken and turkeys [3]. The present study was conducted to find out the status of aMPV infection in broiler breeder chickens in India by ELISA test.

2. Materials and Methods

2.1. Seroprevalence Survey

This serological survey was conducted as part of post graduate research by the department of Veterinary Microbiology, Veterinary College and Research Institute, Namakkal, Tamil Nadu Veterinary Animal Sciences University, India, to elucidate the incidence of aMPV in chickens.

2.2. Chicken Flocks and Serum Samples

A total of 485 blood samples were collected randomly from 20 broiler breeder chicken flocks aged between 4 and 72 weeks. Chicken serum was extracted by centrifugation at 1,500 × g for 10 min at 4°C and kept at −20°C before use.

2.3. ELISA

Chicken serum samples were tested individually using a commercial Enzyme-Linked Immunosorbent Assay (ELISA) for the detection of antibody against avian metapneumovirus (Avian Pneumovirus Antibody Test Kit, IDEXX Laboratories, Liebefeld-Bern, Switzerland). Positive and negative control sera were included for each test. Absorbance (ABS) was measured and recorded using an ELISA reader (Bio Rad, Japan) at 650 nm. Based on the instruction manual of the ELISA kits, serum samples with Sample to Positive (S/P) ratios of less than or equal to 0.20 should be considered negative. S/P ratios greater than 0.20 (titres larger than 396) should be considered positive and indicate exposure to aMPV.

$$S/P = \frac{\text{Sample ABS} - \text{Negative Control ABS}}{\text{Positive Control ABS} - \text{Negative Control ABS}}$$

3. Results

The results of present study showed 14 flocks (70%) were infected by aMPV infection. Totally 165 blood sera samples (34.02%) out of 485 blood sera samples collected from 20 flocks, have different

level of specific antibody titer against aMPV infection. Mean titer of positive samples were 2553±150.62 (Mean±SE) and negative samples mean were 99.40±7.75 (Mean±SE).

4. Discussion

The present study was an initiative to record the serological presence of aMPV in India. This was conducted by using an ELISA kit, which is commercially available. The results revealed that 34.02% of the tested birds were serologically positive for SHS. A higher rate of seropositivity for aMPV in chickens observed in India is in agreement with previous reports from other part of the world [8, 9]. The aMPV started to infect chicken in Japan before 1988 and was widespread thereafter [10].

In Poland, Minta *et al.* [11] also used ELISA to detect seroprevalence to avian pneumovirus in sera collected from 39 broiler breeder flocks aged 12-96 weeks, 56.4% of broiler breeder flocks were positive. In this study, birds showed no clinical signs of SHS at time of blood collection, but titre of antibodies against SHS according to ELISA results were clearly positive in 34.02% samples. This is in agreement with previous reports from other parts of the world. aMPV has been isolated from chicken flocks without clinical signs [12] and chicken flocks free of clinical signs may have antibodies for aMPV [13, 14, 15]. In broilers, aMPV has been associated with clinical signs of swollen head syndrome. Layers infected with aMPV in the early phase of lay do not reach peak production, whereas layers in their late phase of lay suffer a drop in egg production [16, 3]. A positive detection of antibodies confirmed that the birds were exposure to the aMPV, but a negative result does not rule out the exposure. The aMPV infected chickens may not necessarily produce humoral antibodies, or antibodies may be at very low levels at the time of the sampling [17].

Chickens are not vaccinated for aMPV in India and the seropositivity for aMPV indicates that commercial chickens in India are exposed to this important poultry pathogen. aMPV may cause serious economic losses in turkeys and chickens especially in the presence of concurrent bacterial and viral infections.

On the basis of these results it is concluded that avian metapneumovirus is present in India. It is also concluded that more work is required to isolate and characterize AMP virus. Results of such a study would be strategic for institution of preventive measures against aMPV infection.

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Effect of Supplementation of Licorice Root (*Glycyrrhiza Glabra L.*) Extracts on Immune Status in Commercial Broilers

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Abstract An experimental study was designed with the objective of exploring the effect of supplementation of *Glycyrrhiza glabra* extracts on immune status in commercial broilers. Fifty four, day old, straight run broiler chicks were randomly allotted to 3 treatment groups (T_1 - unsupplemented; T_2 - supplemented with 1% *G. glabra* crude extract powder and T_3 - supplemented with 0.1% *G. glabra* standardized extract powder) consisting of three replicates of six chicks each. The birds were reared under standard and uniform management conditions throughout the experimental period. Ranikhet-LaSota strain was used to produce antibodies against Ranikhet disease virus. All the birds were also immunized against Sheep Red Blood Cells (SRBC). HI titre values against Ranikhet (LaSota) and HA titre values against SRBC antigens were estimated to assess the humoral immunity. The Serum Biochemical Parameters viz., serum total protein and albumin were estimated and serum globulin and albumin:globulin ratio were calculated. To assess the cell mediated immunity, total and differential white blood cell counts were performed. The group supplemented with 0.1% licorice standardized extract has shown significantly ($p<0.01$) higher HI titre value against Ranikhet disease virus, than the other groups, on day 42, whereas, there was no significant differences among the groups on days 7, 14, 21, 28 and 35 in HI titre values. Licorice root standardized extract supplemented group performed better than other groups in increasing HI titre values throughout the study period. Against SRBC antigen, significant differences ($p<0.01$) were observed among the groups on days 21, 28, 35 and 42 in HA titre values ($p<0.01$). Licorice root standardized extract supplemented group performed better than other groups in increasing HA titre values throughout the study period. No significant differences were noticed among groups in 2 weeks and 6 weeks except on 4 weeks in which significant ($p<0.01$) differences were noticed in serum albumin, serum globulin and albumin:globulin ratio. No significant differences were observed in the total WBC and differential cell counts throughout the study period among different treatment groups. This shows that licorice root extracts do not have any direct effect on cell mediated immunity.

Keywords *Broilers; Glycyrrhiza glabra; Immune Status*

1. Introduction

Licorice root (*Glycyrrhiza glabra L.)* is reported to be having anti-inflammatory, immunostimulant, antiviral, demulcent, expectorant, anti-catarrhal, antiulcer (PUD), hepatoprotective, spasmolytic and laxative properties (CSIR, 1985 and Nadkarni, 1996). The aim of the present study was to explore the effect of supplementation of licorice root extracts on immune status in commercial broiler chicken.

2. Materials and Methods

The study was conducted in fifty four, straight run, commercial broiler chicks of "cobb" strain, belonging to a single hatch obtained from local hatchery. Day old chicks were weighed, wing banded and randomly allotted to 3 treatment groups. Each treatment group was consisting of three replicates of six chicks each. One group served as negative control, fed with basal diet only (**T_1**) and other two groups served as treated groups, fed basal diet supplemented with **1% licorice root crude extract powder** (**T_2**) and the other group, fed basal diet supplemented with **0.1% licorice root standardized extract powder** (Standardized to contain up to 25% *Glycyrrhizin*), (**T_3**). In a gable roofed, open sided house the birds were reared in broiler cages in standard and uniform management conditions throughout the experimental period of six weeks. The broiler starter and finisher mashes were fed *ad libitum*. All the birds were immunized against Ranikhet-LaSota strain and also against Sheep Red Blood Cells (SRBC). Primary immunization was done on 7^{th} day and secondary immunization was done on 28^{th} day. The HI titre values against Ranikhet-LaSota antigen by microdilution technique (Giamborne, 1982) on 7^{th}, 14^{th}, 21^{st}, 28^{th}, 35^{th} and 42^{nd} days and HA titre values against SRBC (Van der Zijpp, 1983) on 14^{th}, 21^{st}, 28^{th}, 35^{th} and 42^{nd} days, were estimated to assess the humoral immunity. The biochemical parameters viz., serum total protein and albumin were estimated as per modified Biuret and Dumas method (Varley, 1980) and serum globulin and albumin:globulin ratio were calculated. To assess the cell mediated immunity, total and differential white blood cell counts were performed (Campbell, 1995). The results were statistically analyzed (Snedecor and Cochran, 1994).

3. Results and Discussion

Table 1 shows the **HI titre values *(log 2)* against Ranikhet-LaSota antigen** of days 7, 14, 21, 28, 35 and 42. No significant differences were observed among HI titre values of days 7, 14, 21, 28 and 35. On day 42, the group supplemented with 0.1% licorice standardized extract has shown significantly ($p<0.01$) higher HI titre value than the other groups. Throughout the study period, licorice root standardized extract supplemented group performed better than other groups in increasing HI titre values.

Table 1: *Effect of Supplementation of Licorice on HI titre Values (log 2) against Ranikhet-LaSota Antigen*

	T_1	T_2	T_3
7 days	4.222 ± 0.129	4.000 ± 0.140	4.278 ± 0.135
14 days	6.111± 0.137	6.056 ± 0.151	6.222 ± 0.129
21 days	4.611 ± 0.183	4.944 ± 0.151	5.056 ± 0.127
28 days	3.667 ± 0.181	3.833 ± 0.167	4.111 ± 0.137
35 days	5.944 ± 0.151	5.667 ± 0.162	6.000 ± 0.140
42 days	3.389 ± 0.118^{B}	3.611 ± 0.118^{B}	4.278 ± 0.109^{A}

Means with different alphabets as superscripts differ significantly ($p<0.01$).

In Table 2, the **HA titre values *(Log 2)* against SRBC antigen** of days 14, 21, 28, 35 and 42 are given. Except on day 14, significant differences ($p<0.01$) in HA titre values against SRBC antigen were observed among the groups on days 21, 28, 35 and 42. In increasing HA titre values, licorice

root standardized extract supplemented group performed better than other groups, throughout the study period.

The result of the present study is in agreement with the (Zhang QuiJun *et al*., 2002) who noticed a positive effect on immunity in chicks fed with *Zengmiansan* (consisting of 10 herbal Chinese medicines including *Glycyrrhiza) and* conversely, not in agreement with (Fulzele *et al*., 2003) who found that *Haridradi ghrita*, HG (*Panchagavya* based polyherbal formulation containing *G. glabra* as a constituent) preferentially stimulated the components of cell mediated immunity and did not show any effect on humoral immunity in rats.

Table 2: *Effect of Supplementation of Licorice on HA titre Values (Log 2) against SRBC Antigen*

	T_1	T_2	T_3
14 days	2.333 ± 0.114	2.167 ± 0.090	2.444 ± 0.121
21 days	1.056 ± 0.056^{B}	1.111 ± 0.076^{B}	1.444 ± 0.121^{A}
28 days	0.167 ± 0.090^{A}	0.056 ± 0.056^{B}	0.500 ± 0.121^{A}
35 days	1.944 ± 0.056^{B}	2.167 ± 0.090^{AB}	2.500 ± 0.167^{A}
42 days	0.944 ± 0.056^{B}	1.000 ± 0.081^{B}	1.389 ± 0.118^{A}

Means with different alphabets as superscripts differ significantly (p<0.01)

In Table 3, the estimated mean **serum total protein, albumin, globulin and albumin: globulin ratio of 2nd, 4th and 6th week** are given. Except on 4th week (p<0.01), no significant differences were noticed among groups, in serum albumin, serum globulin and albumin:globulin ratio. No significant difference was noticed in serum total protein among the groups throughout the study period.

Table 3: *Effect of Supplementation of Licorice Root Extracts on Serum Biochemical Parameters*

	Serum Total Protein (g/dL)			Serum Albumin (g/dL)			Serum Globulin (g/dL)			Serum Albumin: Globulin		
	T1	**T2**	**T3**	**T1**	**T2**	**T3**	**T1**	**T2**	**T3**	**T1**	**T2**	**T3**
2 weeks	3.037 ± 0.029	3.037 ± 0.037	3.019 ± 0.027	0.988 ± 0.019	0.988 ± 0.016	0.988 ± 0.012	2.049 ± 0.035	2.050 ± 0.043	2.031 ± 0.033	0.486 ± 0.016	0.487 ± 0.016	0.490 ± 0.013
4 weeks	3.621 ± 0.026	3.574 ± 0.024	3.574 ± 0.028	1.256^{B} ± 0.016	1.293^{AB} ± 0.013	1.336^{A} ± 0.012	2.364^{A} ± 0.035	2.281^{AB} ± 0.024	2.238^{B} ± 0.025	0.534^{B} ± 0.013	0.568^{AB} ± 0.009	0.599^{A} ± 0.009
6 weeks	4.162 ± 0.021	4.111 ± 0.024	4.153 ± 0.031	1.370 ± 0.013	1.352 ± 0.011	1.352 ± 0.013	2.792 ± 0.026	2.759 ± 0.026	2.801 ± 0.032	0.492 ± 0.008	0.491 ± 0.007	0.484 ± 0.008

Means with different alphabets as superscripts differ significantly (p<0.01)

The mean total and differential white blood cell count on 7, 14, 21, 28, 35 and 42 days are given in Table 4. No significant differences were observed in the total WBC and differential cell counts throughout the study period among different treatment groups. This shows that licorice root extracts do not have any direct effect on cell mediated immunity.

Table 4: *Effect of Supplementation of Licorice Root Extracts on Leucogram (Per µl) of Commercial Chicken*

	Total WBC Count			Heterophil			Lymphocyte		
	T1	T2	T3	T1	T2	T3	T1	T2	T3
7 days	20299.67 ± 7.97	20283.17 ± 10.13	20283.67 ± 9.56	9864.33 ± 4.13	9855.00 ± 2.46	9849.83 ± 3.46	7167.00 ± 2.88	7164.50 ± 3.37	7170.17 ± 3.34
14 days	21070.67 ± 9.49	21071.67 ± 11.54	21100.00 ± 8.56	8664.33 ± 6.01	8653.33 ± 7.15	8661.50 ± 2.05	8873.33 ± 2.79	8876.00 ± 3.48	8875.50 ± 4.54
21 days	21682.00 ± 8.55	21691.33 ± 9.98	21689.67 ± 5.17	8452.00 ± 2.22	8454.17 ± 7.16	8454.00 ± 3.31	9405.00 ± 4.08	9405.83 ± 3.52	9403.17 ± 4.81
28 days	21461.50 ± 12.66	21458.33 ± 17.83	21467.33 ± 3.50	7448.67 ± 6.08	7444.83 ± 6.07	7448.50 ± 3.37	9956.00 ± 5.04	9957.17 ± 5.57	9954.00 ± 5.54
35 days	26346.00 ± 13.33	26195.00 ± 149.62	26335.83 ± 6.61	6438.83 ± 4.71	6441.00 ± 4.75	6441.00 ± 4.37	15002.83 ± 5.68	14856.00 ± 152.39	15000.50 ± 3.65
42 days	28180.00 ± 107.98	28286.33 ± 5.16	28305.50 ± 9.10	6499.33 ± 3.47	6493.67 ± 5.60	6507.17 ± 3.41	16505.00 ± 104.06	16608.67 ± 5.12	16618.00 ± 5.42
	Monocyte			Eosinophil			Basophil		
	T1	T2	T3	T1	T2	T3	T1	T2	T3
7 days	638.67 ± 1.02	636.17 ± 3.94	640.00± 2.58	730.33 ± 1.65	731.33 ± 1.82	728.17 ± 0.95	1899.33 ± 3.61	1896.17 ± 4.06	1895.50 ± 6.49
14 days	651.33 ± 1.84	650.50 ± 3.70	650.00 ± 4.99	740.17 ± 3.75	745.50 ± 2.51	748.00 ± 3.04	2141.50 ± 2.93	2140.50 ± 3.78	2149.83 ± 4.20
21 days	797.00 ± 2.90	799.00 ± 2.62	798.17 ± 2.26	769.33 ± 1.15	770.83 ± 1.89	772.67 ± 0.56	2258.67 ± 2.54	2261.50 ± 1.26	2261.67 ± 0.76
28 days	901.33 ± 4.88	903.00 ± 4.02	902.00 ± 4.40	884.33 ± 6.68	885.33 ± 5.92	890.17 ± 3.63	2271.17 ± 3.78	2268.00 ± 4.23	2272.67 ± 4.12
35 days	1261.83 ± 4.42	1259.33 ± 4.90	1258.33 ± 4.53	1339.33 ± 3.77	1345.33 ± 3.62	1345.33 ± 2.60	2303.17 ± 4.38	2293.33 ± 2.26	2290.67 ± 2.70
42 days	1359.00 ± 4.16	1364.67 ± 2.72	1362.33 ± 2.53	1406.00 ± 4.76	1403.33 ± 5.75	1401.50 ± 1.59	2410.67 ± 3.30	2416.00 ± 1.51	2416.50 ± 2.26

4. Conclusion

The present work was carried out to study the influence of supplementation of licorice in crude and standardized extract forms on the immune potential in commercial broiler chicken. The results revealed that the supplementation of licorice standardized extract at 0.1% level improved the humoral immunity by increasing antibody titres against specific and non-specific antigens in broilers. Further research is recommended to confirm the positive influence of licorice root on immune potential and to optimize inclusion levels of licorice in broiler diets and to determine their possible physiological interfering effects and cost effectiveness.

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Growth Promoting Potentials of Indigenous Drugs in Broiler Chicken

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Abstract This study was conducted to explore the growth promoting potentials of indigenous drugs *viz*., *Glycyrrhiza glabra*, *Phyllanthus niruri* and *Aloe vera*. A total of seventy two straight run 1-day old broiler chicks (Cobb strain) were randomly allotted to 4 treatments with 3 replications of set 6 chicks in each. Treatments were included of control (T_1) and the inclusion of 1% of *Glycyrrhiza glabra* (T_2), *Phyllanthus niruri* (T_3) and *Aloe vera* (T_4) with basal diet. The results indicated that T_3 had a significantly higher body weight followed by T_4 and T_2. *A. vera* supplementation increased the feed intake significantly than other groups. Feed conversion efficiency was significantly higher with T_3 followed by T_4 and T_2. It is concluded that *P. niruri* and *A. vera* can be used as growth promoters at 1% level in feed.

Keywords *Broiler; Phyllanthus Niruri; Glycyrrhiza Glabra; Aloe Vera; Growth Performance*

1. Introduction

Intensive and scientific poultry rearing introduced the use of various growth promoters in feed to achieve the higher body weights at an early age. In previous years antibacterial drugs were tried to promote the growth. But facing the potential threats of drug residues in poultry meat and the resultant drug resistance in bacterial population causing diseases in both human and animals, the usage of these drugs in various countries have been banned. At this point of time the scientific and medical world has changed their notion on various herbal drugs and keeps trying these drugs for various ailments in humans as well as animals. Further public, academic and government interest in traditional medicines are growing exponentially due to the increased incidence of adverse drug reactions and economic burden of the modern system of medicine (Dubey, *et al*., 2004). WHO estimated that approximately 80% of the earth's inhabitants rely on traditional medicine for their primary health care needs, and most of this therapy involved the use of plant extracts or their active components. These plants and their components are perceived as natural and safe by consumers (Mehmet Ciftci, *et al*., 2005).

Glycyrrhiza glabra (Yasti-madu, Licorice) is a hardy plant growing well in deep, rich sandy soil and full sun. Dried liquorice roots are found in all the bazaars of India. *Glycyrrhiza* is documented for its anti-inflammatory, immune-stimulant, antiviral, demulcent, expectorant, anti-catarrhal, antiulcer (PUD), hepatoprotective, spasmolytic and laxative medicinal use (CSIR, 1985 and Nadkarni, 1996). Zhang QuiJun, *et al*., 2002 concluded that *Zengmiansan*, a traditional Chinese medicine consisting of 10 herbs including *Glycyrrhiza glabra* produced a positive effect on immunity and increased live weight gain.

Phyllanthus niruri (Bhoomyamalakee) prefers rocky, calcareous salts in humid tropical regions and usually found in central and southern India (CSIR, 1985 and Nadkarni, 1996). Haribabu and Panda (1993); Prajapati (1997); Gopinath, *et al*. (2001); Mihir Sarma, *et al*. (2001) and Dolly Bhaskar, *et al*. (2003) studied the effects of poly herbal formulations (containing *Phyllanthus* as one of the components) on growth performance of broilers and concluded that they improved weight gain.

Aloe vera (Ghrita-kumari) is cultivated throughout India in many varieties some of which run wild as on the coasts of Bombay, Gujarat and South India (CSIR, 1985 and Nadkarni, 1996). Many therapeutic properties of *A. vera* have been described (Grindlay and Reynolds, 1986) as anti-hyperglycaemic (Boudreau, 2006), anti-cancer (Steenkamp and Stewart, 2007), angiogenic and immune system stimulator (Sa, *et al*., 2005). Salary, *et al*., (2014) concluded that inclusion of 0.4 per cent *Aloe vera* extract in drinking water to broiler chicken had positive effects on performance.

In this juncture the present study is aimed at exploring the growth promoting potentials of crude powder of indigenous plant drugs *viz*. *Glycyrrhiza glabra, Phyllanthus niruri and Aloe vera* in commercial broiler chicken.

2. Materials and Methods

One day old broiler chicks of straight run, cob strain, purchased from a local hatchery were used for this experiment. On arrival, seventy two chicks were weighed, wing banded and randomly distributed to four groups (three replicates) of eighteen each. One group was assigned to basal diet only [Negative control (T_1)]. The other three groups were fed with basal diet supplemented (at the inclusion level of 1%) with *Glycyrrhiza glabra* crude powder (T_2), *Phyllanthus niruri* crude powder (T_3) and *Aloe vera* crude powder (T_4), obtained *as gratis* from M/s Dabur Ayurvet Ltd., New Delhi, India. All birds were fed with broiler starter (up to three weeks of age) followed by finisher mash (three to six weeks). Feed and water were offered *ad libitum* throughout the experiment. The birds were reared in table top broiler cages maintained in a gable roofed and open sided house. Uniform management conditions were maintained throughout the study period. The birds were immunized against Newcastle viral disease on day seven and twenty eight. The experimental design was approved by Institutional Animal Ethical Committee (IAEC).

Body weight and feed intake were recorded every week to measure production performance of birds. From the recorded data, weekly body weight gain and feed conversion efficiency of the birds were calculated.

Mortality was recorded daily throughout the study period.

All data were analyzed as per the methods of (Snedecor and Cochran, 1994).

3. Results and Discussion

The effects of supplementation of indigenous drugs on weekly body weight of birds and their weekly body weight gain are presented in Table 1 and 2 respectively.

Table 1: *Body Weight (In Gms)*

	T_1	T_2	T_3	T_4
1 day	48.8 ± 0.167	49.1 ± 0.179	48.8 ± 0.191	48.7 ± 0.135
7 days	162.8 ± 1.368^{A}	164.3 ± 0.709^{AB}	169.2 ± 0.550^{C}	167.2 ± 0.720^{BC}
14 days	388.7 ± 0.605^{A}	390.0 ± 2.254^{A}	396.7 ± 1.377^{B}	392.3 ± 1.355^{AB}
21 days	750.0 ± 6.458	747.2 ± 2.407	755.8 ± 0.809	752.7 ± 1.655
28 days	1107.5 ± 4.041^{A}	1129.3 ± 2.204^{B}	1158.0 ± 2.232^{C}	1137.2 ± 2.451^{B}
35 days	1545.7 ± 3.594^{A}	1554.8 ± 4.287^{A}	1622.5 ± 0.638^{C}	1604.7 ± 2.813^{B}
42 days	1966.3 ± 6.630^{A}	2005.5 ± 11.760^{B}	2059.2 ± 6.744^{C}	2044.5 ± 3.421^{C}

Values are Mean ± SE (n=18)
Means with different alphabets as superscripts between columns differ significantly (p<0.01)

Throughout the study period it is observed that the body weight of control birds (T_1) fed only with basal diet was lower compared to that of other groups supplemented with *Glycyrrhiza glabra* crude powder (T_2), *Phyllanthus niruri* crude powder (T_3) and *Aloe vera* crude powder (T_4) except on 21 days, in which it was able to match with other groups. Among the treated groups, *Phyllanthus niruri* supplemented group (T_3) topped the total body weight at 4, 5 and 6 weeks followed by *Aloe vera* and *Glycyrrhiza glabra* crude powder supplemented groups (T_4 and T_2). The final body weight of T_3 and T_4 were similar and significantly differed from T_2 and T_1.

Table 2: *Weekly Body Weight Gain (In Gms)*

	T_1	T_2	T_3	T_4
1st week	114.0 ± 1.455^{A}	115.2 ± 0.761^{AB}	120.4 ± 0.525^{C}	118.4 ± 0.805^{BC}
2nd week	225.8 ± 1.014	225.7 ± 1.898	227.5 ± 1.661	225.2 ± 0.954
3rd week	361.3 ± 6.315	357.2 ± 1.581	359.2 ± 1.396	360.3 ± 2.117
4th week	357.5 ± 9.150^{A}	382.2 ± 3.698^{B}	402.2 ± 2.447^{C}	384.5 ± 1.655^{BC}
5th week	438.2 ± 3.976^{A}	425.5 ± 5.374^{A}	464.5 ± 1.872^{B}	467.5 ± 2.622^{B}
6th week	420.7 ± 5.158	450.7 ± 12.504	436.7 ± 7.040	439.8 ± 1.133

Values are Mean ± SE (n=18)
Means with different alphabets as superscripts between columns differ significantly (p<0.01)

While analyzing the weekly body weight gain achieved by birds, it further supported the incremental body weight gain achieved by T_3 and T_4 at 1, 4 and 5 weeks, which was significantly higher. *Glycyrrhiza glabra* treated group (T_2) also could catch up weight gain in the last week, though it was not significant.

Table 3: *Cumulative Feed Intake (In Gms)*

	T_1	T_2	T_3	T_4
1st week	112.0 ± 0.577	111.7 ± 0.882	113.0 ± 1.000	113.0 ± 0.577
2nd week	409.0 ± 1.528^{A}	408.3 ± 1.667^{A}	416.7 ± 1.667^{B}	419.3 ± 0.882^{B}
3rd week	910.0 ± 2.887^{A}	910.0 ± 2.887^{A}	920.0 ± 2.887^{AB}	926.3 ± 1.202^{B}
4th week	1571.7 ± 4.410^{A}	1576.7 ± 1.667^{AB}	1586.3 ± 1.856^{B}	1590.0 ± 2.887^{B}
5th week	2486.7 ± 4.410^{A}	2490.0 ± 2.887^{A}	2494.7 ± 1.856^{A}	2516.3 ± 4.096^{B}
6th week	3563.3 ± 6.009^{A}	3558.3 ± 4.410^{A}	3578.7 ± 1.856^{A}	3601.7 ± 4.410^{B}

Values are Mean ± SE (n=18)
Means with different alphabets as superscripts between columns differ significantly (p<0.01)

Throughout the experiment cumulative feed intake was significantly high in group supplemented with *Aloe vera* (T_4) when compared to control. There was no significant difference in feed intake between control (T_1) and *glycyrrhiza* supplemented group (T_2). *Phyllanthus* supplementation (T_3) has shown significant increase in cumulative feed intake only at 2nd and 4th weeks when compared to control, similar to T_4 (Table 3).

This reflected as better feed conversion efficiency in T_3 followed by T_4 and T_2, and finally T_1 (Table 4).

Table 4: *Feed Conversion Efficiency*

	T_1	T_2	T_3	T_4
1st week	0.982 ± 0.005^{B}	0.969 ± 0.008^{AB}	0.939 ± 0.007^{A}	0.954 ± 0.005^{AB}
2nd week	1.204 ± 0.005^{a}	1.198 ± 0.004^{a}	1.198 ± 0.005^{a}	1.220 ± 0.003^{b}
3rd week	1.298 ± 0.004^{a}	1.304 ± 0.004^{a}	1.301 ± 0.004^{a}	1.316 ± 0.002^{b}
4th week	1.484 ± 0.004^{C}	1.460 ± 0.002^{B}	1.430 ± 0.002^{A}	1.461 ± 0.003^{B}
5th week	1.661 ± 0.003^{C}	1.654 ± 0.002^{C}	1.585 ± 0.001^{A}	1.617 ± 0.003^{B}
6th week	1.858 ± 0.003^{D}	1.819 ± 0.002^{C}	1.780 ± 0.001^{A}	1.805 ± 0.002^{B}

Values are Mean ± SE (n=18)

Means with different alphabets (capitals) as superscripts between columns differ significantly ($p<0.01$)

Means with different small alphabets as superscripts between columns differ significantly ($p<0.05$)

Ultimately the results of the present study show that *Phyllanthus niruri* has improved the broiler performance by increasing the body weight without affecting feed intake, which was followed by *Aloe vera* which simultaneously increased body weight along with feed intake. *Glycyrrhiza glabra* improved performance of broilers as seen with increase in body weight and FCE with no alteration in feed intake when compared to control. Contrary to the findings of the present study, Nguyen Hieu Phuong and Nguyen Quang Thieu reported that using different levels (0.25 to 1.5%) of *Phyllanthus amarus* powder in the diets had no effects on the growth performance of chicken. But, Natsir, *et al*. (2013) suggested that 0.8 per cent encapsulated combination of garlic and *Phyllanthus niruri* in broiler diet improved performance. Sedghi, *et al*. (2010) recorded that dietary licorice extract supplementation did not have any negative effects on body weight or FCR of broiler chicken. The result of other study using licorice extract through drinking water indicated no considerable effect on broilers' growth performance (Naser Moradi, *et al*., 2014). Mimereole (2011) in the evaluation of the dietary inclusion of *Aloe vera* as an alternative to antibiotic growth promoter in broiler production concluded that as a growth promoter, *A. vera* was comparable to antibiotic growth promoters. Salary, *et al*. (2014) found that the inclusion of licorice and *A. vera* extracts at the levels of 0.4 per cent in drinking water have positive effects on the performance of broiler chicken.

4. Conclusion

It can be concluded that use of *Phyllanthus niruri* and *Aloe vera* at 1% inclusion level as crude powder had positive effects on growth performance of broilers. Based on the observations with *G. glabra*, it is suggested to conduct further studies to identify the inclusion level.

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Research Article **Open Access**

Pharmacokinetics of Enrofloxacin after Single Intravenous and Oral Bolus Administration in Broiler Chicken

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Abstract The pharmacokinetics and bioavailability of enrofloxacin was compared in Cobb strain broiler chicken after intravenous and oral administration of enrofloxacin at the rate of 10mg.kg^{-1}. The concentration of enrofloxacin at various time intervals in plasma was determined by HPLC and the pharmacokinetic parameters were calculated by non compartmental approach. AUC was significantly high in i.v. route (32.72±1.15 *vs* 25.35±1.92μg.h.mL^{-1}) whereas highly significant increase in MRT (15.81±0.54 *vs* 8.86±0.23h), $V_{d\ area}$ (4.69±0.16 *vs* 3.04±0.09 L.kg^{-1}) and $t_{1/2\beta}$ (10.57±0.35 *vs* 6.84±0.15h) were noticed in oral route when compared to i.v. route. The C_{max} of 1.63±0.12μg.mL^{-1} was attained at t_{max} of 3.58±0.61h and absolute bioavailability was 77.47±5.86% after oral administration. PK/PD integration revealed that the dose (10mg.kg^{-1}) was capable of treating only moderately sensitive organisms with MIC ≤0.125μg.mL^{-1} and increase in dosage is needed for less sensitive organisms.

Keywords *Bioavailability; Broiler Chicken; Enrofloxacin; Pharmacokinetics; PK/PD Integration*

1. Introduction

Enrofloxacin, a fluoroquinolone antibacterial developed exclusively for veterinary use is still one of the highly used antibacterial in poultry to treat outbreaks of various bacterial diseases especially chronic respiratory disease, mycoplasmosis *etc.* Overuse of enrofloxacin has resulted in development of resistant bacterial populations and may also reduce the clinical efficacy (Sumano and Gutierrez, 2001). Though many pharmacokinetic studies were reported for enrofloxacin, the present study was

conducted in order to assess whether the dosage regimen followed is sufficient to obtain clinical cure in Cobb strain of broiler chicken reared in and around Namakkal region of Tamil Nadu, India, since wide variation in pharmacokinetic parameters were noticed between various studies.

2. Materials and Methods

2.1. Experimental Birds

The study was conducted in 24 commercial six weeks old apparently healthy Cobb broiler chicken of either sex weighing 2 to 2.5kg. The birds were purchased from commercial poultry farm at the age of four weeks and acclimatized for two weeks period. The birds were reared in individual cages under standard and uniform conditions with natural day-night cycle and fed *ad libitum* feed and water free of antibacterial. The experimental trial on birds was approved by Institutional Animal Ethics Committee, Veterinary College and Research Institute, Namakkal-2.

2.2. Drug Administration

The birds were divided into two groups of 12 each and enrofloxacin (M/s. Himedia, India) was administered at the rate of 10mg.kg^{-1} body weight through i.v. and oral route. The drug was dissolved in 0.1N NaOH to prepare 1.5 per cent solution and further diluted in normal saline and drinking water for i.v. and oral administration, respectively. The method of drug administration and collection of blood samples are mentioned in Table 1.

Table 1: *Experimental Design*

Group	Route	Method	Timing of Blood Collection
I	IV	Medial Metatarsal vein	0, 0.08, 0.167, 0.25, 0.5, 0.75, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0, 8.0, 10.0, 12.0, 24.0, 36.0 and 48.0h
II	Oral	Intra crop with semi rigid plastic tube	0, 0.25, 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0, 8.0, 10.0, 12.0, 24.0, 36.0 and 48.0h

Blood sample was collected in heparinised vials and plasma was separated by centrifuging at 950g for 15 min and stored at -80°C until analysis.

2.3. HPLC Analysis

Plasma samples were assayed for enrofloxacin concentration by reverse phase high performance liquid chromatography. The HPLC system (Schimadzu, Japan) consisted of an isocratic pump (LC-10AT double pump), a rheodyne manual injector with 20µL loop, C_{18} column (5µ particle size, 4.6 x 250mm length, Lichrosphere, Merck), Column oven (CTO- 10 AS vp) maintained at 40°C, Photodiode array UV-Vis detector and LC solution chromatopak software.

The plasma samples were extracted as per the method of (Nielsen and Hansen, 1997) and quantified as per (Kung *et al.*, 1993). The isocratic mobile phase consisted of acetonitrile: methanol: water (17:3:80, v/v/v) with 0.4% triethylamine, 0.4% orthophosphoric acid (85%, v/v) and pH adjusted to 3.0 with triethylamine. The scan range was 220 to 400nm and the detection wavelength was 278nm. The flow rate of mobile phase was 1.0mL.min^{-1} and run time was 10min.

The concentration of enrofloxacin in chicken plasma was obtained from the calibration curve. Standard curve was prepared for concentrations ranging from 0.01 to 10µg.mL^{-1} by spiking enrofloxacin in drug free chicken plasma and the linearity of the method was examined by linear

regression analysis of the standard curve. The recovery of enrofloxacin was 97.11 per cent and coefficient of variation (CV) was 4.22 per cent. Limit of detection and quantification were 0.01 and 0.025μg.mL^{-1}, respectively. The method was found to be linear and reproducible in the concentration range of 0.025 to 10μg.mL^{-1} for enrofloxacin. The intra- and inter-day assay CV was 3.59 and 4.08 per cent, respectively.

2.4. Pharmacokinetic Analysis

Pharmacokinetic parameters were calculated from the concentration of enrofloxacin detected in plasma by non compartmental analysis based on statistical moments theory (Singh, 1999) by using PK functions software.

2.5. Statistical Analysis

The plasma concentration and pharmacokinetic parameters are expressed as mean±SE and between groups comparison was made by students paired t test (Snedecor and Cochran, 1994).

3. Results and Discussion

The mean (±SE) plasma concentration *vs* time data of enrofloxacin after single i.v. and oral bolus dose is presented in Table 2. The mean plasma concentration was 6.01 ±0.26μg.mL^{-1} at 0.08h after i.v. administration and the concentration decreased gradually to 1.74±0.15μg.mL^{-1} at 6h until which it differed significantly from the concentration obtained from oral route. There after no significant difference in plasma concentration was noticed between i.v. and oral route and concentration was detected up to 36 and 48h in i.v. and oral route, respectively. In oral route plasma concentration was detected from 0.25h (0.36±0.04μg.mL^{-1}) which increased gradually and reached maximum plasma concentration of 1.48±0.10μg.mL^{-1} at 4h after which it declined to 0.09±0.01μg.mL^{-1} at 48h. Further, mean concentration exceeding 0.5μg.mL^{-1} was present up to 12h in both the routes.

Table 2: *Comparison of Mean Plasma Concentrations (μG.Ml^{-1}) after Single IV and Oral Bolus Dose of Enrofloxacin in Broiler Chicken*

Time (h)	Mean±SE	
	IV	Oral
0.08	6.01 ±0.26	-
0.167	5.64±0.28	-
0.25	5.16**±0.26	0.36±0.04
0.5	4.74**±0.24	0.56±0.02
1	3.96**±0.20	0.87± 0.04
1.5	3.63**±0.20	1.19±0.07
2	3.17**±0.14	1.46±0.11
4	2.54**±0.14	1.48±0.10
6	1.74*±0.15	1.33±0.09
8	1.32^{NS}±0.12	1.11^{NS}±0.07
10	0.84^{NS}±0.06	0.90^{NS}±0.07
12	0.54^{NS}±0.04	0.66^{NS}±0.06
24	0.29^{NS}±0.02	0.34^{NS}±0.04
36	0.10^{NS}±0.01	0.16^{NS}±0.03
48	ND	0.09±0.01

– Sample not collected
ND- Not detected
* Significant (p<0.05)
** Significant (p<0.01)

(Anadon *et al.*, 1995) reported higher concentration of 24.06±0.43μg.mL^{-1} at 10min whereas (Jakubowski *et al.*, 2010) reported slightly lower concentration of 4.17μg.mL^{-1} at 5min after i.v administration. Concentration exceeding 0.5μg.mL^{-1} persisted for about 12h in both the studies which was similar to the present study whereas it was up to 24h in the study conducted by (Silva *et al.,* 2006). These differences might be due to the difference in the strain and age of bird which emphasize the need to conduct pharmacokinetic studies in specific species in their own environment rather than mere extrapolation.

The pharmacokinetic parameters of enrofloxacin are presented in Table 3 for single i.v. and oral bolus dose. The elimination rate constant was significantly higher ($p<0.01$) in i.v. when compared to oral bolus dose which was reflected by significantly lower ($p<0.01$) half life in i.v. (6.84±0.15h) than oral dose (10.57±0.35h). The half life in the present study is lower than reported values of 10.57±0.35h (i.v.) and 14.23±0.46h (oral) by (Anadon *et al.*, 1995). A comparable $t_{1/2\beta}$ of 5.56h (Knoll *et al.,* 1999) in chicken and 6.64h (Dimitrova *et al.,* 2007) in turkey after i.v. administration had been reported. (Silva *et al.*, 2006) recorded $t_{1/2\beta}$ of 14h after oral dose in chicken which was higher than the present study. In all the studies half life after oral dose was comparatively longer than i.v. route suggesting that the drug is eliminated faster after i.v. route. The half life in both the routes suggests that chicken eliminate enrofloxacin slowly and hence dosing interval may be prolonged.

Table 3: *Comparison of Mean Pharmacokinetic Parameters after Single IV and Oral Bolus Dose of Enrofloxacin in Broiler Chicken*

Parameters	Units	Mean±SE	
		Group I	Group II
β	h^{-1}	0.101**±0.002	0.065±0.002
$AUC_{0-\infty}$	μg.h.mL^{-1}	32.72*±1.15	25.35±1.92
$AUMC_{0-\infty}$	μg.h^2.mL^{-1}	282.83±15.45	405.06*±43.29
MRT	h	8.86±0.23	15.81**±0.54
MAT	h	-	6.95±0.54
$V_{d\ area}$/F	L.kg^{-1}	-	6.17±0.38
$V_{d\ area}$	L.kg^{-1}	3.04±0.09	4.69**±0.16
Cl_B/F	L.h^{-1}.kg^{-1}		0.41±0.03
Cl_B	L.h^{-1}.kg^{-1}	0.31±0.01	-
$t_{1/2\beta}$	h	6.84±0.15	10.57**±0.35
C_{max}	μg.mL^{-1}	-	1.63±0.12
t_{max}	h	-	3.58±0.61
F	%	-	77.47±5.86

*Significant (P<0.05)
**Significant (P<0.01)

The C_{max} obtained in the study was 1.63±0.12μg.mL^{-1} at 3.58±0.61h (t_{max}) and was concurrent with the findings of (El-Aziz *et al.,* 1997 (1.69μg.mL^{-1} at 2.52h); Knoll *et al.,* 1999 (1.9μg.mL^{-1} at 1.5h) and Silva *et al.,* 2006 (1.5 μg.mL^{-1} at 9h)) in chicken.

The mean AUC value was significantly higher ($p<0.05$) after i.v. when compared to oral administration (32.72±1.15 *vs* 25.35±1.92μg.h.mL^{-1}). (Anadon *et al.,* 1995) reported almost similar AUC of 34.51±1.30 and 22.26±0.69μg.h.mL^{-1} for i.v and oral administration. (Jakubowski *et al.,* 2010) reported lower value of 25.09μg.h.mL^{-1} for i.v. and (Silva *et al.*, 2006) reported higher value of 35.00μg.h.mL^{-1} for oral route. The AUC observed in the study are quite high which could be attributed to longer stay of drug in the body in both the routes and longer absorption phase in case of oral route. The MRT value was significantly low ($p<0.01$) in i.v. (8.86±0.23h *vs* 15.81±0.54h) than oral route which indicates the absorption phase was longer as evidenced by MAT of 6.95±0.54h in oral route.

The MRT of (Anadon *et al.,* 1995) was 9.65h for i.v and 15.40h for oral route and (Silva *et al.*, 2006) reported 15.64h which is almost similar to the present study.

The $V_{d\ area}$ obtained in this study after i.v. and oral route were 3.04±0.09L.kg^{-1} and 4.69±0.16L.kg^{-1}, respectively which differed significantly ($p<0.01$). Comparatively higher $V_{d\ area}$ of 4.31L.kg^{-1} (i.v.) and 5.94L.kg^{-1} (oral) were reported by (Anadon *et al.,* 1995) and 5.0L.kg^{-1} (i.v.) by (Knoll *et al.,* 1999) in chicken. Drugs with apparent volume of distribution greater than 1L.kg^{-1} were considered to be widely distributed in the body tissue (Baggot, 1977) and in the present study also enrofloxacin was found to be widely distributed in the body fluids and tissues of chicken as reflected by higher apparent volume of distribution.

The mean Cl_B of enrofloxacin obtained in the present study after i.v. was 0.31±0.01 L.h^{-1}.kg^{-1} and was comparable to (Anadon *et al.,* 1995) (0.29±0.01L.h^{-1}.kg^{-1}) and (Jakubowski *et al.,* 2010) (0.4L.h^{-1}.kg^{-1}) whereas (Knoll *et al.*, 1999) reported higher clearance of 0.62L.h^{-1}.kg^{-1} for the same dose. After oral administration the Cl_B/F was 0.41±0.03L.h^{-1}.kg^{-1} which was higher than the clearance reported by (Anadon *et al.,* 1995) (0.288±0.001L.h^{-1}.kg^{-1}).

The absolute bioavailability was 77.47±5.86 per cent and was comparable to 80.1 per cent reported in chicken (Bugyei *et al.,* 1999) and 80.35 per cent in turkey (Tansakul *et al.,* 2005). This value was higher than the reported bioavailability of 64 per cent (Anadon *et al.,* 1995), 59.6 per cent (El- Aziz *et al.,* 1997) in chicken, and 69.2 per cent (Dimitrova *et al.,* 2007) in turkey. The results confirmed that enrofloxacin was well absorbed after oral administration in chicken. However, the bioavailability calculations are only estimates, since different group of birds were used rather than a crossover design.

3.1. PK/PD Integration

The ultimate aim of pharmacokinetics study is to suggest appropriate dosage regimen which can produce clinical cure. Integration of PK variables such as AUC and C_{max} obtained in the study with PD variables *viz.* MIC (hypothetical values of 0.05, 0.125, 0.25, 0.5μg.mL^{-1}) revealed that the AUC/MIC was 236.37±7.78 and 155.62±9.63 for i.v. and oral route, respectively and C_{max}/MIC was 13.00±0.94 for oral route for microorganisms with MIC of 0.125μg.mL^{-1} (Table 4).

Table 4: *PK/ PD Integration of Enrofloxacin Based on Hypothetical MIC Values*

Ratio	MIC (μg.ml^{-1})	IV	Oral
AUC_{0-24}/MIC	0.05	590.92± 19.43	389.05±24.08
	0.125	236.37± 7.78	155.62±9.63
	0.25	118.18±3.89	77.81±4.82
	0.5	59.09±1.94	38.91±2.41
C_{max}/MIC	0.05	-	32.50±2.34
	0.125	-	13.00±0.94
	0.25	-	6.50±0.47
	0.5	-	3.25±0.23

In order to maximize clinical efficacy and minimize the development of resistance AUC/MIC>100-125 and C_{max}/MIC>8-12 should be achieved (Andes and Craig, 2002). In this study i.v route obtained AUC/MIC of 118.18±3.89 for MIC of 0.25μg.mL^{-1} but after oral route it was low. Hence based on AUC/MIC and C_{max}/MIC, enrofloxacin @ 10mg.kg^{-1} is sufficient to treat only moderately sensitive

organisms with MIC of 0.125μg.mL^{-1} after oral bolus administration whereas i.v route can treat organisms with MIC ≤0.25μg.mL^{-1}. For less sensitive organisms the dosage of enrofloxacin need to be increased as per the clinical situation.

4. Conclusion

The present study concludes that the bioavailability of enrofloxacin after oral administration was 77.47±5.86 per cent and desirable pharmacokinetic parameters could be attained. However the dose 10mg.kg^{-1} through oral route is sufficient to treat only moderately sensitive organisms with MIC≤0.125μg.mL^{-1} and dosage need to be increased based on the sensitivity of the microorganism.

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Case Study | Open Access

A Rare Case Study on Feline Mycoplasmosis

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Abstract This paper discusses the case report on Mycoplasma infection in cat and its timely diagnosis by blood smear examination and haematology. It also discusses the treatment and response of the cat for the disease. Haemobartonellosis in cats is caused by *Mycoplasma haemofelis*, formerly known as *Haemobartonella felis*. An eight months old Persian cat was received in the Small Animal Clinics, Out Patient Ward, Medicine department, Madras Veterinary College with the history to suspect for feline Mycoplasmosis. Peripheral blood smear and whole blood sample was collected and subjected to blood smear examination and whole blood for routine haematological study. It revealed codocytosis, anisocytosis and hypochromasia. Few ghost cells also were seen. Nearly 80–85% of the RBCs revealed darkly stained small organism at the rim or periphery of the cells.

Keywords *Haemobartonella; Feline Mycoplasma; Feline Anemia; Cat Anemia*

1. Introduction

Haemobartonellosis is a disease of cats caused by *Mycoplasma haemofelis,* formerly called as *Haemobartonella felis*. *M. haemominutum*, also causes infection in cats but less likely to cause disease. These mycoplasma infections are not caused by typical bacteria, but by a group of microorganisms called mycoplasma. Since, *M. haemofelis and M. haemominutum* are blood (hemo)-associated (tropic), they termed as "hemotropic mycoplasmas" or "hemoplasmas" (Small and Ristic, 1967).

The disease transmits through infected fleas and ticks by feeding on infected animal to the non-infected animals. Thereby, mycoplasmas are passed on in the environment. Moreover, they live in the blood cells; they could be spread via a blood transfusion from an infected animal to a non-infected one. Vertical transmission from the queen (mother cat) to her kittens also the possible route of

transmission. They may also even spread through cat bites - male cats, cats that roam. Cats less than 4-6 years of age appear to be at high risk of becoming infected (Small and Ristic, 1971).

2. Materials and Methods

An eight months old Persian cat was received in the Small Animal Clinics, Out Patient Ward, Medicine department, Madras Veterinary College with the history of depression, loss of appetite, dehydration, pale conjunctival mucous membrane, weight loss, fast heart and respiratory rates and treated locally for a week. Peripheral blood smear subjected to blood smear examination as per the methodology of Houwen (2000). 2 ml of whole blood sample was collected in a vacutainer with EDTA as anticoagulant and is subjected for routine haematological study using Mindray BC Vet 2800, automated haematology analyser. Differential counts were calculated manually.

3. Results and Discussion

On blood smear examination, it revealed codocytosis, anisocytosis and hypochromasia. Few ghost cells also were seen. Nearly 80-85% of the RBCs revealed darkly stained small organism at the rim or periphery of the cells suggestive of feline mycoplasma caused by *Mycoplasma haemofelis* (Figure 1). Some of the RBCs revealed multiple organisms. On whole blood analysis haematological value of 4.2g/dL haemoglobin, 13% PCV, 2.1 m/Cmm total RBC, 7200/Cmm total WBC and 280000 total thrombocyte counts were noticed. The differential counts of 72% neutrophils, 22% lymphocytes, 3% each of monocytes and eosinophils were seen. Red blood cells are destroyed by the cat's own immune reactions to the parasites (Bobade et al., 1988).

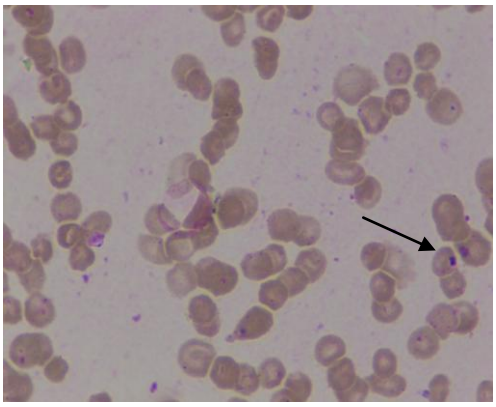

Figure 1: *Stained Blood Smear Showing Mycoplasma Organism in the Periphery of the Red Blood Cells (1000 X)*

Hence, the cat was treated for the disease with Doxycycline @ 5-10 mg/kg bwt and Vitamin B12 @ 50mg SID for a week and kept under observation (Novotny, 2012). After day 3 of the treatment the cat has improved and animal was found to be active and start taking feed. A number of diseases and toxic agents interfere with the production of red blood cells in the bone marrow. Infection with *Mycoplasma haemofelis* (formerly called *Hemobartonella felis*) is more common in cats. This blood parasite is primarily transmitted to cats through tick and flea bites, and also by vertical transmission. Hence the owner was advised to isolate the animal to prevent its mingling with other cats.

Mycoplasma haemofelis may also work in concert with feline leukemia virus to stimulate bone marrow cancers (Grindem et al., 1990). Cats with this type of infectious anemia are often weak and may have fever. Some cats practice pica by eating dirt or their litter in an attempt to add minerals to their diet. If left untreated, up to 30 percent of affected cats may die.

4. Conclusion

The present paper discussed the case study on mycoplasma infection in cat and its timely diagnosis by blood smear examination and haematology. It also discussed the treatment and response of the cat for the disease. In future molecular diagnosis using PCR with specific primers to *Mycoplasma haemofelis* can be more accurate and early for diagnosis of this disease.

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Research Article | Open Access

An Outbreak of Classical Swine Fever in Indigenous Pigs in Tamilnadu, India

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Abstract Classical swine fever is a highly contagious viral disease of swine causing economic losses due to heavy mortality and reproductive problems. The present study was conducted at Nakalapuram village, Villathikulam taluk of Thoothukudi District, Tamilnadu where there was a suspected outbreak of swine fever among the indigenous pigs reared under scavenging system. Ninety pigs out of one hundred and ten pigs of all age groups without any vaccination died within two to three days period. The ailing animals showed clinical signs such as high fever, staggering gait, frothy excessive salivation, severe respiratory distress, paddling of legs with convulsions followed by death. The post mortem examination was carried out in two pigs and the histopathological examination was carried out on representative necropsy samples like liver, spleen, kidney and mesenteric lymph node. Based on the necropsy findings such as haemorrhagic lymphadenitis, petechial haemorrhages in kidney and spleen and by NS5B gene based RT-PCR the disease was confirmed as classical swine fever.
Keywords *Indigenous Pigs; PCR; Classical Swine Fever*

1. Introduction

Classical swine fever is a highly contagious viral disease of pigs caused by the genus Pestivirus belongs to the family Flaviviridae, which also includes bovine viral diarrhoea virus (BVDV) and border disease virus (BDV) (Wengler et al., 1995). Depending upon the virulence of the virus, host and environmental factors, age and breeds of the pigs the course of the disease in affected pigs may be per-acute, acute, subacute, chronic or atypical (Moennig et al., 2003). Swine fever causes heavy losses due to reproductive problems and mortality in affected pigs (Sarma et al., 2008). In India, the disease was recorded in Punjab for the first time in 1961 and subsequently from Maharashtra and Uttar Pradesh in 1962. During the past few years the outbreaks of swine fever have been recorded from the states of Nagaland, Manipur, Tripura, West Bangal and Tamilnadu (Rahman, 2011). Swine fever causes heavy losses due to reproductive problems and mortality in affected pigs (Sarma et al., 2008). The present paper describes the outbreak of classical swine fever in non-descript indigenous pigs in Tamilnadu.

2. Materials and Methods

2.1. Disease Investigation

The present study was conducted at Nakalapuram village, Villathikulam taluk of Thoothukudi District, Tamilnadu where there was a suspected outbreak of swine fever among the indigenous pigs reared under scavenging system. The detailed history of the feed and housing, number of pigs affected, mortality pattern, vaccination status and clinical findings were recorded. The post mortem examination was carried out in two pigs.

2.2. Histopathology

Pieces of tissues from liver, spleen, kidney and mesenteric lymphnode were collected and fixed in 10 per cent formal saline. The tissues were subjected for histological processing and finally embedded in paraffin. Paraffin embedded tissues were sectioned at 5 µm thickness and stained with haematoxylin and eosin (H & E) for histological examination (Bancroft and Stevens, 1996).

2.3. Polymerase Chain Reaction

The NS5B gene based reverse transcriptase-polymerase chain reaction as described by Rathnapraba et al., (2013) was carried out at the Department of Animal Biotechnology, Madras Veterinary College, Chennai from the samples of liver, spleen, kidney and mesenteric lymphnodes for confirmatory diagnosis of CSF.

3. Results and Discussion

Classical swine fever may occur in peracute, acute, subacute, and chronic forms, with the acute form occurring most commonly. In the acute form, high fever, depression, anorexia, and conjunctivitis appear 2 to 4 days post exposure, followed by vomiting, bacterial pneumonia, paresis, paralysis, tremor, and convulsions. Nearly all pigs in a unit become affected within approximately 10 days, and mortality may reach 100% (Moennig et al., 2003). Similar to this, in the present study, around ninety pigs out of one hundred and ten pigs of all age groups reared under scavenging system without any vaccination died within two to three days period. The ailing animals showed clinical signs such as high fever, staggering gait, frothy excessive salivation, severe respiratory distress, paddling of legs with convulsions followed by death.

Necropsy findings revealed petechial haemorrhages in the kidney capsule, ileocecal valve, lymphnodes, spleen and pericardial sac. Emphysema and congestion was noticed in lung. The lymphnodes such as retropharngeal and mesenteric were enlarged and haemorrhages were observed. Necrotic ulceration with haemorrhages was observed in the colon. Similar observations were reported by Murphy et al. (1999) and Ravishankar et al. (2011).

Histopathological observations made in the present study such as congestion and depletion of lymphocytes in the paracortex of lymph nodes, depletion of lymphocytes in white pulp of spleen, enlargement of kidney sinuses, haemorrhages in proximal convoluted tubule and degeneration of hepatocytes in the liver were correlated very well with the findings of Govindarajan et al. (2003) and Palanivel et al. (2012).

The reverse transcriptase-polymerase chain reaction (RT-PCR) has been proved to be specific and more sensitive technique for detection of CSF in tissues than fluorescent antibody technique, immunoperoxidase assay using monoclonal antibodies and antigen-capture enzyme-linked immunosorbent assay (Handel et al., 2004). Similar to present study, several authors (Liu et al., 1991;

Singh et al., 2005; Rathnapraba et al., 2012 & 2013) also detected CSF viral nucleic acid in tissue samples such as liver, spleen, kidney and mesenteric lymphnodes by RT-PCR. All the samples were proved to be CSF positive. This established 100 per cent relationship between the suspected tissues and the presence of virus.

Conclusion

In conclusion, based on necropsy findings and by RT- PCR, it was confirmed that the mortality among the indigenous pigs reared under scavenging system was due to swine fever. The detection of CSF in desi pigs in the state is critical to introduction of suitable prevention and control measures. However, control strategies should be planned and activated only after the prevalence of CSF in desi pigs in the state has been fully investigated.

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Research Article | Open Access

Evaluation of Extract from Sweet Flag Rhizome for Biological Activity against House Fly

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Abstract The problem of house fly has become more acute everywhere especially in poultry and piggery farms. They act as potential vector for many viral, bacterial, helminthic and protozoan diseases and as nuisance pests. The control of fly is very much effected by the use of synthetic organic insecticides. These drugs, even though efficacious, can be detrimental to the mankind and environment and also lead to the development of resistance. Hence, in the present study, the larvicidal and adulticidal effect of Vasambu (*Acorus calamus)* were evaluated against the housefly. For larvicidal evaluation, the test drug, Vasambu at different concentrations (1 per cent, 2 per cent, 3 per cent and 4 per cent) was sprayed on the dung sample containing 100 number of 3rd instar larvae of house fly. The larvae were observed daily for the development of pupae and subsequent emergence of adult fly. Lowest fly emergence (Highest mortality of larvae) was observed in 4 per cent and 3 per cent treatment groups and they differed highly significantly ($P<0.01$) with rest of the groups. There is no significant difference between 4 and 3 per cent treated groups and between the 1 per cent, 2 per cent and control groups. For adulticidal effect, Vasambu at different concentrations (2, 4 and 6 per cent) were used as bait (consisting of rice bran, jaggery and Vasambu). Fifty number of house fly pupae were placed in a fly rearing chamber. Immediately after the emergence of the first fly, the Vasambu bait was kept inside the fly rearing chamber and observed daily for the mortality. Highest fly mortality was observed in bait incorporated with 6 per cent concentration and it differed significantly ($P<0.05$) with control and 2 per cent treated treatment groups. However, there is no significant difference between 6 per cent and 4 per cent treatment groups and between 4 per cent, 2 per cent and control groups.

Keywords *Sweet Flag Rhizome; Botanical Insecticides; House Fly*

1. Introduction

House fly, *Musca domestica* is a public health pest that causes annoyance to humans, livestock and Poultry industry resulting in considerable economic loss in livestock business (Zumpt, 1965). Further, they act as vectors for many medical and veterinary pathological organisms. The conventional insecticides are primarily used to reduce the population of house flies (Malic et al., 2007). So, there is

a need to test the new chemical insecticides for vector control in view of the development of physiological resistance to many of the pesticides that were advocated so far. The indiscriminate and continuous use of conventional insecticides may lead to development of resistance and insecticidal residual for humans and the environment (Mohan, 1990). This warrants the search for biologically based alternatives. Plant-derived materials are expected to be a possible alternative insecticide to synthetic chemicals in controlling the flies (Pandian and Manoharan, 1995). Many studies gave good results when evaluated some plant extracts in control of medical and veterinary insects such as *C. albiceps* (Morzy et al., 1998a: El-Shazly, et al., 2000) and *Lucilia sericata* (Mazyad et al., 1999). Several studies have also looked at the possibility of using plant extracts in the control of eggs, larvae, pupae and adults of *Musca domestica* (Issakul et al., 2004; Malik et al., 2007).

The dried rhizome of *Acorus calamus* has been traditionally used by the rural farmers by way of tying it around the neck of the animal as fly repellent. Hence, the present study has been undertaken to evaluate the efficacy of *A. calamus* against *M. domestica*.

2. Materials and Methods

2.1. Plant Material

Acorus calamus belongs to the family Aroideae, Araceae. The dried rhizome of the plants is used as aromatic, stimulant, carminative, diuretic, astringent, anthelmintic, mild laxative and aphrodisiac.

Rhizomes of sweet flag procured from Siddha medical store and made into bits and shade dried for a week. After a week Rhizomes were then ground into fine powder with the help of mixer grinder and used.

This fine powder of rhizome and water was mixed in equal volume (1:1) in required quantity. Then such a mixer was kept overnight for soaking to obtain extract. In the morning soaked material was squeezed using fine muslin cloth and extract was obtained. This was considered as pure extract. To get one per cent concentration of sweet flag rhizome extract, one ml of pure extract was mixed with 99 ml of water to get two per cent sweet flag extract. The same procedure was followed for two, three and four per cent extract solutions and used for seed treatment.

2.2. Larval Culture

Thirty five gram of calf feed was mixed with 120 ml of water. To this little jiggery and yeast was added. This medium was kept in a fly breeding chamber and the house flies were allowed to lay eggs. The larvae and pupae developed in the medium were used in the trial.

For larvicidal evaluation, 100 g of cow dung each were sprayed with 1 per cent, 2 per cent, 3 per cent and 4 per cent of test drug of *A. calamus* extract and then they were dispensed into four containers separately. A container with 100 g of dung was maintained as control. Approximately, 100 numbers of third stage larvae of *M. domestica* each were seeded into all the containers and kept in a fly rearing chamber. The larvae were monitored daily for 15 days and fly emergence was observed.

For adulticidal evaluation, *A. calamus* at different concentrations (2, 4 and 6 per cent) was used as bait consisting of rice bran, jiggery and vasambu). Fifty numbers of *M. domestica* pupae were placed in a fly rearing chamber. Immediately after the emergence of first fly, the vasambu bait was kept inside the chamber and observed daily for the fly mortality. Bait containing rice bran and jiggery was maintained as control.

2.3. Statistical Analysis

Analysis of data was done by suitable statistical methods as described by Snedecor and as per Snedecor and Cochran (1989).

3. Results and Discussion

Results computed in Table 1 clearly indicated that lowest fly emergence was recorded with sweet flag rhizome formulations at 4 per cent concentration (21.30 %) followed by sweet flag rhizome powder extract at 3 per cent (26.0 %) as against 85.0 per cent in untreated control. The emergence of fly observed with 4 and 3 per cent groups differed highly significantly ($p<0.01$) with rest of the groups. However, there is no significant difference between 4 and 3 per cent treatment groups and between 1 and 3 per cent and control groups. The results of the present work cannot be compared with any other results as studies pertaining in this type of work is lacking in the literature.

Table 1: *Effect of Sweet Flag Rhizome Extracts on House Fly Larvae*

Larvicidal Effect	1%	2%	3%	4%	Control
Percentage of fly emergence	85.17 ± 4.45 (6)	76.5 ± 6.69 (6)	26.0 ± 7.69 (6)	21.3 ± 5.39 (6)	85.0 ± 5.72 (6)

The highest fly mortality was observed in *A. calamus* bait incorporated with 6 per cent concentration (Table 2) and they differed significantly ($p<0.05$) with control and 2 per cent treatment groups. However, there is no significant difference between 6, 4 per cent treatment groups and between 4, 3 per cent and control groups.

Table 2: *Effect of Sweet Flag Rhizome Extracts on House Fly Pupae*

Adulticidal Effect	2%	4%	6%	Control
Percentage of fly mortality	41.83 ± 5.67	39.16 ± 5.08	34.16 ± 6.01	43.5 ± 2.345

Many studies have been carried out to demonstrate the toxic effects of plant extracts on related diptera (Dhar et al., 1996; Cao et al., 2004; Malic et al., 2007). The basis for toxicity by topical application of plant extracts to houseflies has been fairly documented (Malik et al., 2007). Repellents and attractants properties of natural oils and various plant extracts on *M. domestica* have been documented by Braverman and Hogsette (2001). The toxic effects of Eucalyptol (Sukontason et al., 2004); crude extracts and essential oils of *C. anisata* and *H. spicigera* on *M. domestica* (Dongdem, 1997) has been reported. Issakul et al. (2004) reported the insecticidal effect of *Mammea siamensis* and *G. simplicifolia* crude extracts on the eggs of housefly and inferred that seed and root extracts of *G. simplicifolia* were the most active against the housefly when incorporated into the growth medium.

The results revealed that the extract obtained from the powder of dried rhizome of *A. calamus* applied topically to various instars was toxic to the flies when incorporated into the growth medium. *Acorus calamus* may act as contact poison to the larvae thereby retarding further development of the fly. Further, the potency of the drug might also be due to stomach poison to the fly by disrupting digestive processes and nutrient assimilation as stated by Zhusalzman et al. (1998) thereby leading to affect growth and development as recorded in the present study with of high mortality of fly. Although the insecticidal property of sweet flays rhizome powder against storage pests were proved by Kalasagonda (1998); Sunilkumar (2003); Hampanna (2004), the findings of this study cannot be compared as there is no such finding in the literature related to effect of sweet flag rhizome powder

against housefly. Nevertheless, Sophia and Pandian (2009) recorded that biting activity of *Cx. quinquefasciatus* was very much prevented by the repellent activity of *Acorus calamus*.

The significance of the study is that the extract of *A. calamus* could be used to control houseflies without polluting the ecosystem by suitable bait feeding techniques. However, further assays regarding the effect of *A. calamus* under field condition should be followed.

4. Conclusion

In conclusion, the extracts of Sweet flag rhizome (*Acorus calamus*) offer an alternative source for the control of house flies without polluting the environment by adopting suitable techniques.

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Research Article | Open Access

Preliminary Genetic Diversity Study on Different Isolates of *Eimeria tenella* from South India

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Abstract Coccidiosis is an intestinal disease of chickens caused by more than six species of the protozoan parasites of the Genus *Eimeria* and has a major economic impact on the poultry industry throughout the world. Various studies revealed that the diversity of the parasites results in the pathogenicity and in the host pathogen relationship. So, in the present study we analyzed the genetic diversity of *Eimeria tenella* using the small subunit of 18S rRNA from different poultry managements of South India. About 23 variations were observed among the four isolates of *Eimeria tenella* with the similarity ranged from 99.3%–95.6% and 28–39 variations observed when compared the four isolates with the reference sequences obtained from the public domain. More than 90% of the similarity was observed between the study and the reference sequences. A mean distance of 0.03 was observed between the *E. tenella* isolates.
Keywords *Chicken Coccidiosis; E. tenella; 18S rRNA; Genetic Diversity*

1. Introduction

Chicken coccidiosis is a major cause of acute disease in the poultry caused by the protozoan, *Eimeria*. Coccidiosis remained the focus of anxiety in the commercial poultry producers not only due to the losses incurred as a result of mortality in acute infections or lowered production, but also due to the cost input required for effective chemoprophylaxis and immunoprophylaxis (Fayer, 1980).

Seven species of *Eimeria* have been recognized to infect chicken. Three species *E. acervulina, E. maxima* and *E. tenella* are highly recognized as most economically significant species of *Eimeria*. Coccidial infection in chicken normally found to have a minimum of two, or more, of these species at any given time (McDougald et al., 1986; Kucera, 1990; Morris et al., 2007), and it has been shown that individual chickens can be concurrently infected by multiple species of *Eimeria* (Long and Joyner, 1984; Haug et al., 2008; Jenkins et al., 2008). Recently, the correlation between specific species composition and genetic diversity of *Eimeria* species has been addressed in the United States (Scharwz et al., 2009) and Europe (Haug et al., 2008).

Recent reports revealed that the antigenic diversity in the parasites results in the pathogenicity and the host pathogen relationship (Smith et al., 2002; Blake et al., 2006). Hence it is implicit that the identification of the genetic variation in *Eimeria* species as a crucial factor in order to understand the pathogenicity and epidemiology of chicken coccidiosis (Morris and Gasser, 2006). Though there are many reports on the antigenic diversity, parasite genetics and host parasite relationship (Blake et al., 2006; Beck et al., 2009; Smith et al., 2002), there are no studies on the intra specific variation of the *Eimeria* species. Hence, the present investigation was attempted to study the diversity of different isolates of *Eimeria tenella* from South India using the small subunit of 18S rRNA.

2. Materials and Methods

2.1. Sampling

Poultry fecal samples were collected from Commercial Broiler (CB), Commercial Broiler Breeder (CBB), Commercial Layer (CL) and Backyard Poultry systems. The samples were collected in a 50 ml centrifuge tubes containing 1 gm of 1.0 mm glass beads and 4 ml of 2.5% potassium dichromate. Fresh droppings of the chicken from every two to five places was collected depending on the size of the unit until the tube was filled to the 10 ml mark in a 15 ml falcon tube. Each tube was then properly capped, labeled, transported to the laboratory and refrigerated at 4ºC until further process.

2.2. Identification of *Eimeria* Species

The fecal samples collected were processed as described by Eckert et al., (1995) for the identification of *Eimeria* parasite. The enumeration of OPG was done using a 3-chambered McMaster chamber as described by Haug et al., (2008) and the *Eimeria* species were identified using COCCIMORPH, the online diagnostic tool as described in previous studies (Kumar et al., 2014).

2.3. Amplification and Sequencing of 18s rRNA of *Eimeria tenella*

DNA was extracted from the fecal samples was carried out using QIAamp stool DNA isolation kit (Qiagen, Germany). The small subunit of the 18S rRNA of the isolated DNA was amplified and sequenced using published primers (Schwarz et al., 2009) and then sequenced by Sanger sequencing method.

2.4. Phylogenetic Analysis of Small Subunit of 18s rRNA Sequences of *Eimeria tenella*

The sequence alignment was performed using ClustalW program and phylogenetic and molecular evolutionary analyses were conducted using *MEGA* program version 6 (Tamura et al., 2007). A maximum parsimony tree was constructed using the 18S rRNA sequences from Indian isolates with publically available sequence as reference (FJ236372). Pair wise percentage identity was calculated using GeneDoc multiple sequence alignment editor version 2.6.002.

3. Results and Discussion

3.1. Distribution of *Eimeria* species

Based on the curvature characterization, size, symmetry and internal structural characterization for *Eimeria* species using COCCIMORPH identification revealed the presence of *E. acervulina* (79.49%), *E. tenella* (72.88%), *E. mitis* (50.62%), *E. maxima* (35.52%) *and E. necatrix* (10.83%) in the farms screened. *E. brunette* was not recorded in any of the farms screened.

The incidences of the *Eimeria* species were greatly varied between each management. Highest incidence of *Eimeria* infection were observed in the commercial broiler breeder (88.475%), followed by commercial broiler (62.856%). Whereas the incidence observed in commercial layer, backyard

poultry and the colour broiler poultry of managements were 44.69%, 44.44% and 42.81% respectively. Multi species infection was found highly in the commercial broiler breeder, commercial broiler and colour broiler managements with minimum of 4 species in a single farm.

3.2. Phylogenetic and Genetic Diversity Analysis of *Eimeria tenella*

E. tenella isolates of four different poultry managements were amplified and ~1790 bp of small subunit of 18s rRNA of the field *Eimeria* isolated were amplified using specific primers (forward: acctggttgatcctgccag, reverse: cttccgcaggttcacctacgg) and the amplicon (Figure 1) was sequenced and deposited in the NCBI DNA Data Bank with accession nos. JX312812 (CBB), JX093900 (BP), JX093899 (CL) and JX093898 (CB). Overall 48 variations are observed in the study sequences when aligned with 18S rRNA reference sequence (FJ236372). About 29 variations in the *E. tenella* isolate from CBB (JX312812), whereas 34 variations in BP (JX093900), 39 variations in CL (JX093899), and 28 variations in CB (JX093898) were observed. A total of 23 distinct variations were observed in the four *E. tenella* isolates.

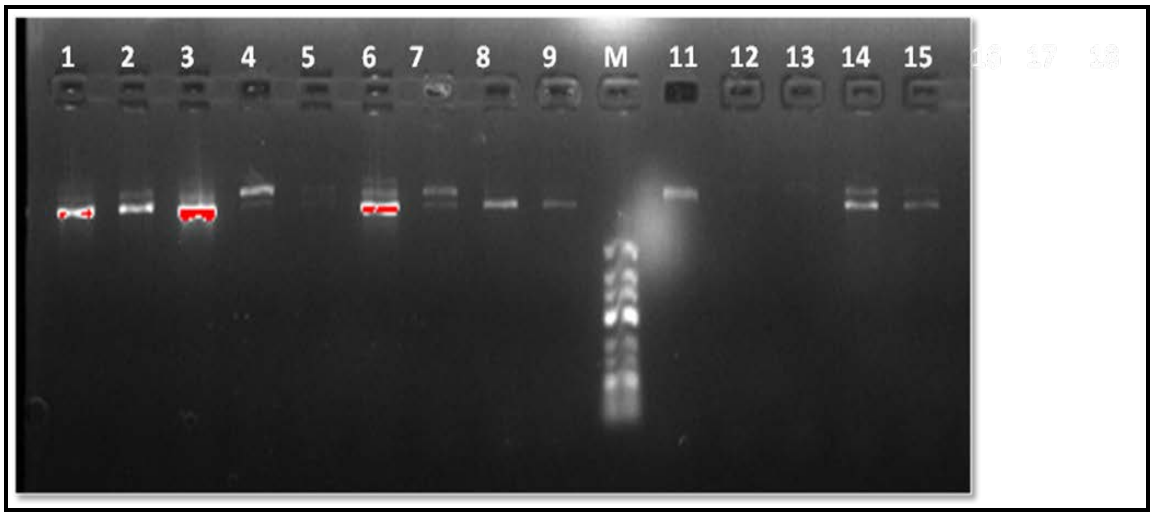

Figure 1: *Agarose Gel Picture Showing Amplification of 18S rRNA Gene of Field Eimeria isolates*

18s rRNA amplicons: ~1790bp (Lane 1,2,3,4,6,7,8,9,11,14,15), Lane M : 100bp-1000bp

Pair wise similarities of the sequences were ranged from 95.3% to 99.3%. The highest sequence homology 99.3% observed between the *E. tenella* isolate from Backyard Poultry and Commercial Broiler (JX093900 andJX093898) and Commercial Layer and Commercial Broiler (JX093899 and JX093898) and the lowest homology 95.6% was observed in the *E. tenella* isolate from Commercial broiler breeder and Commercial Layer (JX312812 - JX093899).

Sequence variation was relatively high between reference sequence and the isolates. The sequence similarity of between reference sequence and JX312812, JX093900, JX093899, and JX093898 were 94.5%, 96%, 94.3% and 96.3% respectively.

Phylogenetic analysis of small subunit of 18S rDNA sequences of four *Eimeria* species isolates showed two different clades (Figure 2). Except Commercial Broiler (JX093898), other three isolates were clustered in monophyletic clades (groups). The clades of JX093898 consisted to be phylogenetically distant from the other isolates. Cluster one contained of JX312812 (CBB), JX093899 (CL), and JX093900 (BP). The second cluster contained of JX093898 and the reference sequence. The overall mean distance of the isolates is 0.03. The tree demonstrates the close relationship of JX312812, JX093899, and JX093900 isolates.

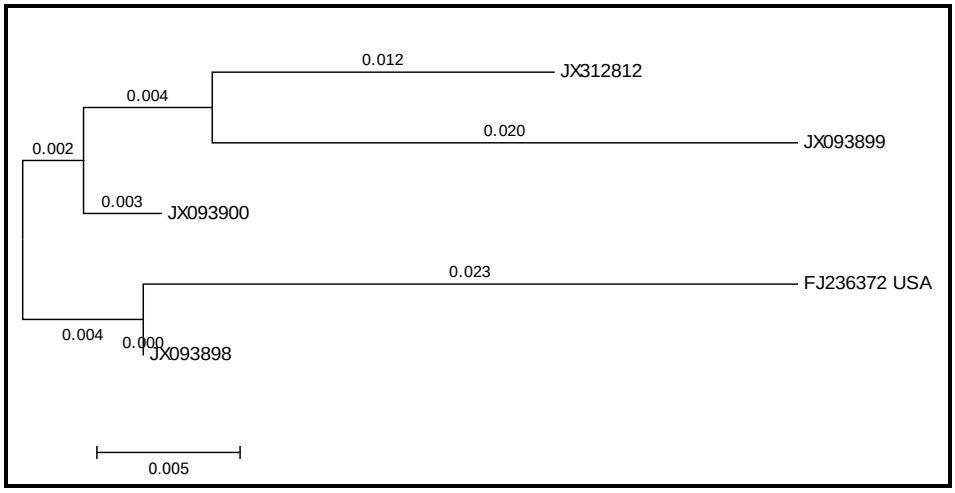

Figure 2: *Phylogetic Analyses of 18S rRNA Sequences of E. tenella isolates*

4. Conclusion

In the present investigation COCCIMORPH is found to be highly effective in the diagnosis of *Eimeria* species. As the measurement of oocysts undergo variations due to changes in metabolism of parasites or birds and even in the value of the shape morphometric indices that overlap, the use of COCCIMORPH can only be used with a sort of limitations to be used as a single tool for diagnosis of *Eimeria* species (Kumar et al., 2014). The present study showed the varying degree of divergence of *Eimeria tenella* isolates from different managements of chicken farms. *E. tenella* isolated from Commercial broilers observed with the highest diversity among the isolates. This study using small subunit of 18S rRNA in screening the genetic diversity of *Eimeria* species is a preliminary study to see the diverse effect of the *Eimeria* among various management system and it is also necessary to study the genetic diversity in the other potential vaccine targets genes and protein coding genes in larger sample size to study the inter and intra specific diversity of *Eimeria* species in different host and different management to understand the evaluation as well as the control of the diseases by the selection of appropriate method.

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Research Article | Open Access

Serum Biochemical Levels of Repeat Breeder Cross Bred Cows under Rural Condition of Satara District of Maharashtra

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Abstract Present investigation was carried out on 28 repeat breeding cross bred cows during their post-partum period in their second to seventh lactation from different villages of Satara district. Serum was separated from collected blood samples and analyzed for certain biochemical parameters viz. protein, albumin, globulin, cholesterol, calcium, and phosphorus and blood urea nitrogen. These serum biochemical constituents were compared with serum levels in normal cyclical and repeat breeding cattle reported by various researchers.
Keywords *Blood Biochemical; Field Condition; Repeat Breeder Cows*

1. Introduction

Minerals play an intermediate role in the promotion of action of hormones and enzymes at sub cellular levels in an integrated fashion and thus regulate functions of reproduction and production of domestic animals. Minerals like calcium, phosphorus, magnesium influence the ability of animal to utilise other micro minerals. Nutritional deficiencies in animals result into depletion of minerals and deranged enzymatic activity affecting the normal reproductive behaviour. Lactation however places a heavy drain on an animal already deficient especially in certain minerals and in the general level of feeding [1]. Biochemical profile can indicate the nutritional status of the minerals and other constituents, and help in diagnosis and management of infertility in animals. The repeat breeding condition in dairy cows not only affects the fertility of the cattle to a considerable extent but also incurs great economic loss to the farmer. Mineral imbalances or deficiency may be a factor responsible for repeat breeding condition in animals.

Certain biochemical constituents in blood serum during estrus period have found to be associated with the fertility status of cows and their reproductive behaviour. The findings of many authors [2, 3, 4] suggest that normal blood levels of various biochemicals constituent are indispensable for normal function of various systems of body including reproductive system. The present study was undertaken

to elucidate the relationship between certain serum biochemical including mineral status and repeat breeding condition in dairy cows. Changes in the levels of these metabolites have been associated with reproductive failures and might be having a potential to be used for diagnostic purpose.

The attempts have been made to investigate the levels of serum calcium, phosphorus, blood urea nitrogen, cholesterol and protein in repeat breeding cattle so as to ascertain their possible involvement and usefulness as a tool for clinical diagnosis of repeat breeding.

2. Materials and Methods

Present investigation was carried out on 28 repeat breeding cross bred cows during their post-partum period attended during infertility camps at different villages in Satara district. Repeat breeder cows chosen for the study were in their second to seventh lactation selected on the basis of history. Health status was ascertained through clinical examination. All the animals selected were devoid of genital abnormalities. The animals were maintained by the farmers in rural areas under the traditional animal husbandry practices. The animals were fed some concentrate mixture, paddy straw and allowed to graze in the grazing field, mostly dominated by carpet grass.

About 10 ml blood was collected aseptically from jugular vein of each of experimental animal. Serum was separated with help of pasture pipette and collected in small sterilized plastic vials and stored at -20^{0}C temperature till analysis. The serum biochemical constituents like protein, albumin, globulin, cholesterol, calcium, phosphorus and blood urea nitrogen (BUN) were estimated by standard methods [5]. These serum biochemical constituents were compared with serum levels in normal cyclical and repeat breeding cattle reported by various researchers.

3. Results and Discussion

The average circulating levels of serum Total Proteins, Albumin, Globulin, Cholesterol, Calcium, Phosphorus and BUN with standard error recorded in 28 repeat breeding and 8 normal cyclic cross bred cows have been presented in Table 1.

Table 1: *Serum Biochemical Constituents (Mean ± S.E.) in Normal Cyclic and Repeat Breeder Crossbred Cows*

S. N.	Name of the Serum Biochemical Parameter	Normal Cyclic Crossbred Cows (n=08)	Repeat Breeder Crossbred Cows (n=28)
1.	Calcium (mg/dl)	$10.39^{a} \pm 0.31$	$8.49^{b} \pm 0.30$**
2.	Inorganic Phosphorus (mg/dl)	$5.40^{a} \pm 0.30$	$4.44^{b} \pm 0.17$*
3.	Blood Urea Nitrogen (BUN) (mg/dl)	$13.08^{a} \pm 1.76$	$13.44^{a} \pm 1.15$
4.	Total Cholesterol (mg/dl)	$201.46^{a} \pm 16.34$	$142.60^{b} \pm 8.28$**
5.	Total proteins (g/dl)	$6.57^{a} \pm 0.37$	$5.46^{b} \pm 0.17$**
6.	Albumin (g/dl)	$3.84^{a} \pm 0.26$	$3.37^{b} \pm 0.06$**
7.	Globulin (g/dl)	$2.73^{a} \pm 0.12$	$2.09^{a} \pm 0.18$
8.	A:G ratio	$1.40^{a} \pm 0.06$	$1.99^{a} \pm 0.20$

Note: Values with similar superscripts in a row indicate no significant difference.
Values with dissimilar superscript in a row indicate significant difference.
In column two, (*) indicate significant at 5% and (**) indicate significant at 1% level within a row.

Repeat breeding is among reproductive disorders which hinder favourable productivity in buffaloes [6]. In the present study, the concentrations of calcium (Ca) in Normal cyclic (NC) and Repeat breeder (RB) Groups were found to vary significantly at (p<0.01). The concentrations of Ca were found to be lower in Repeat breeders than normal cyclic crossbred cows. These findings are in agreement with the results of many other workers [7, 8, 9, 10, 14]. However, lower serum calcium level in normal cyclical cows and higher level in repeat breeder cross bred cows have also been

reported [15]. Calcium plays a key part in improving the number and size of ovarian preovulatory follicles, and the ovulation rate [11].

In comparison with NC crossbred cows, the concentrations of serum inorganic phosphorus were significantly lower (P<0.05) in RB crossbred cows. Lower inorganic phosphorus concentration in repeat breeder animals has also been reported in many other studies [7, 10, 12, 13].

Synthesis of ovarian steroids is under the control of gonadotropic hormone regulation in which the Ca plays a pivotal role [16]. Regulation of the membrane potential of oocytes is also controlled by the Ca. Further, it is also suggested that Ca is involved in regulation of gap junctions with respect to their numbers between cumulus cells resulting in disruption of cohesiveness of cumulus cells [17], which contributes to the process of ovulation. Disturbances in ovulation along with pituitary-ovarian axis could be caused by marginal deficiency of phosphorus [18]. Moreover, the process of ovulation is inhibited through putting the breaks on the pituitary gland function as a result of disturbed calcium-phosphorus ratio [19].

There was no significant difference between NC and RB crossbred cows in present study with respect to the levels of BUN, Globulin and Albumin/Globulin ratio. However, the reported values of BUN and A/G ratio were lower in NC than RB crossbred cows.

Significantly lower (P<0.01) concentration of serum total protein in the RB crossbred cows in comparison with the NC crossbred cows is comparable to the findings of many workers [20, 12, 21]. However certain others observed no significant variation in the protein levels between normally cycling and repeat breeding cows [22, 23]. Low level of plasma protein resulted in the deficiency of certain amino acids required for the biosynthesis of gonadotropins and gonadal hormones [24, 25] might cause reproductive hormonal disturbances in animals leading to inactive ovaries [26].

The RB crossbred cows showed significantly lower (P<0.01) concentration of albumin when compared to NC crossbred cows. Similar finding is reported [23, 28]. This high level of albumin in normally cycling cows revealed increased demand for amino acids and protein for the biosynthesis of GnRH and LH to initiate ovulation [28].

The total serum cholesterol concentrations in RB crossbred cows were lower (P<0.01) when compared to NC crossbred cows. This is in agreement with the findings of many workers [27, 12, 23, 8].

4. Conclusion

It can be inferred from this study that Blood Urea Nitrogen, Globulin and Albumin/Globulin ratio do not play a significant role in causing Repeat Breeding problem in crossbred cows and underlines the importance of other serum biochemical parameters like protein, albumin, cholesterol, calcium, and phosphorus in diagnosing the cases of RB crossbred cows. However, more detail study of these parameters along with trace minerals like Iron, Copper, Zinc, and Manganese, Selenium etc. in Satara district on organized and non organized farms is needed.

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Research Article | Open Access

Four Different Therapeutic Regimens for Management of Repeat Breeder Dairy Cattle in Theni District of Tamilnadu

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Abstract The objective of this study was to evaluate the efficacy of four different treatment regimens for repeat breeder dairy cattle with antibiotic and hormonal. The study was conducted in 50 apparently healthy crossbred repeat breeding dairy cows and divided in to five groups with 10 animals each. Animals of group 1 were administered 2 ml of hydroxyl progesterone i/m administered on 4th day of estrum after 1st insemination. Animal in group 2, were administered 60 ml of diluted cephalixen intra uternally immediately after the first time artificial insemination. Animals in group 3 and 4 were administered i/m 1500 IU human chronic gonadotrophin and i/m 10 mg GnRH analogue respectively, immediately after first insemination. All the 5 groups' animals were re-inseminated at interval of 12 hrs after the first insemination. The group 5 animals were inseminated without any hormone or antibiotic therapy and were kept as control for the various treatments. Pregnancy diagnosis was carried out in all groups at 60 days after AI by per rectal examination. Analysis of data showed that there was a significant lower in duration of estrum in the treatment compared to control group except group 2. The conception rate in groups 1 to 5 was 40; 40; 50; 30 and 30 per cent respectively. A maximum of 50 per cent conception rate was observed in group 3 repeat breeder dairy cows. All groups were re-inseminated at 12 hrs interval for increasing the conception rate in repeat breeder dairy cows with prolonged oestrum.

Keywords *Repeat Breeder Dairy Cattle; Four Different Treatment Regimens; Theni District of Tamilnadu*

1. Introduction

Repeat breeding has long been considered as one of the important reproductive disorders in dairy cattle and its incidences are varies among different management systems, environments and regions. GnRH or human Chorionic gonadotrophin (hCG) administration at the time of insemination favours LH surge, which results improved conception rate. Progesterone or intrauterine antibiotic administration prevents the early embryonic mortality, which results in improved conception rate. In this paper, four therapeutic approaches viz, progesterone, intrauterine antibiotic, human chorionic

gonadotrophin and GnRH analogue were used, following artificial insemination in repeat breeding dairy cattle and their efficacy and pregnancy rate were compared.

2. Materials and Methods

The study was conducted in 50 apparently healthy crossbred repeat breeding dairy cows. The dairy cows showing estrual discharge for more than 36 hours were grouped under prolonged Oestrum. They were selected at random and divided in to five groups with 10 animals each. Animals of group 1 were administered 2 ml of hydroxyl progesterone (Duroprogen–Vetcare Pvt Ltd) i/m administered on 4^{th} day of estrum after 1^{st} insemination. Animal in group 2, were administered 60 ml of diluted cephalixen (Lixen IU-KAPL Bangalore) intra uternally immediately after the first time artificial insemination. Animals in group 3 and 4 were administered i/m 1500 IU human chronic gonadotrophin (Chorulon-Intervet Pvt Ltd) and i/m 10 mg GnRH analogue (Buserelin Acetate – Receptal - Intervet Pvt Ltd) respectively, immediately after first insemination. All the 5 groups' animals were re-inseminated at interval of 12 hrs after the first insemination. The group 5 animals were inseminated without any hormone or antibiotic therapy and were kept as control for the various treatment regimens. Pregnancy diagnosis was carried out in all groups at 60 days after AI by per rectal examination and the obtained data were subjected to statistical analysis.

3. Results and Discussion

Groups 1, 2, 3, 4 and 5 the mean duration of estrum before treatment were 75.00+3.17; 71.50+2.16; 75.12+2.76; 70.00+1.76 and 74.13+3.10 hours respectively and mean duration of estrum after treatment were 48.21+3.12; 70.00+1.76; 48.13+3.12; 49.12+2.81 and 73.36+3.14 hours respectively. Analysis of data showed that there was a significant lower in duration of estrum in the treatment compared to control group except group 2. Mathew et al. (2013) reported that treatment with hCG or GnRH analogue at the time of insemination affected the duration of estrum. Tanabe et al. (1994) did not find any difference on treatment with GnRH.

On 11^{th} day of cycle, palpation of ovary indicated functional CL were present in all treatment groups where as in control group animals, only 7 out of 10 (70%) indicated presence of functional CL. Among a total of 47 ovulatory animals both treatment and control group, 30 animals (63.8%) exhibited presence of functional CL on right ovary and remaining 17 animals (36.2%) exhibited presence of functional CL on left ovary. This indicates that right ovary was more active than left ovary during induced and normal cycles in cattle.

The conception rate in groups 1 to 5 was 40; 40; 50; 30 and 30 per cent respectively. A maximum of 50 per cent conception rate was observed in group 3 repeat breeder dairy cows.

The conception rate of group 1 was 40 per cent. Ferguson et al. (2012) reported 40 to 50 per cent conception rate in progesterone supplemented repeat breeder dairy cows.

The conception rate of group 2 was 40. Awasthi and Nema (1995) reported that 90 per cent conception rate in 1.5 g and 750 mg of cephalexin treated repeat breeder dairy cows.

The conception rate of group 3 was 50. Patel et al. (2010) reported 83.3 per cent conception rate in 1500 IU hCG treated repeat breeder. Mathew et al. (2013) reported that 75 per cent conception rate in 1500 IU hCG treated repeat breeders with two insemination.

The conception rate of group 4 were 30 and 40 per cent when treatment with 10 mg GnRH analogue. Kharche and Srivastara (2007) reported high conception rate of 58 per cent when 10 mg GnRH analogue was administered in cows at the time of insemination. Patel et al. (2010) reported a lower

conception rate 28 per cent in 10 mg Buserelin treatment. Mathew et al. (2013) quoted 12.5 per cent and 25 per cent conception rate in first AI and subsequent AI in repeat breeder dairy cows treated with 10 mg Buserelin.

The conception rate of control group 30 per cent was obtained with double insemination at 12 hrs interval without any hormonal treatment. Hernandez Ceron et al. (1993) observed that conception rate of repeat breeder were 34.6 per cent with double insemination.

4. Conclusion

The study was carried out to find out the efficacy of progesterone, intra uterine antibiotic, hCG and GnRH analogue for increasing the conception rate in 50 apparently healthy crossbred dairy cows with prolonged oestrum. A higher overall conception rate of 50 per cent was obtained in repeat breeder dairy cows treated with hCG compared to progesterone on 4th day after insemination (40%); intra uterine antibiotic (40%) GnRH analogue (30%) after immediately insemination and control (30%) respectively. All groups were re-inseminated at 12 hrs interval for increasing the conception rate in repeat breeder dairy cows with prolonged oestrum.

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Analysis of Economic Loss Due to Equine Herpes Viral Infection

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Abstract Equine herpes virus 1 and 4 is considered to be cause of rhinopneumonitis abortion in horses which can cause severe economic loss to the equine industry. Outbreaks of EHV-1 and 4 result in poor racing performance, cancellation of race entries, decreased revenue to the race tracks, mortality, closure or quarantine of stud farms and restrictions on the movement of horses. In the present study an attempt has been made to quantify the loss due to loss of work function among the equids in Tamilnadu. The models used in evaluation of economic impact of animal disease on production can be grouped under two headings namely statistical/epidemiological models and economic models. Out of the data collected from 184 respondents, 67.50 per cent (27 out of 40) and 32.50 percent (13 out of 40) of horses were utilized for anti-snake venom production and patrol respectively. 95.45 per cent (84 out of 88) and 4.45 per cent (4 out of 88) of ponies were utilized for transport and riding respectively. 100 per cent (17 out of 17) of mules were utilized for anti-snake venom production, 100 per cent (30 out of 30) of donkeys were kept as pack animals. One donkey and eight foals were kept without any work. It has been found that income loss due to loss of work function was 95.45 per cent (Rs. 1,47,000) in case of equids utilised for anti-snake venom production, 74.13 per cent (Rs. 1,200) in case of equids used for riding, 62.50 per cent (Rs. 200) in pack animals and 50.80 per cent (Rs. 1,200) in case of equids used for transport purpose (Table 3). The economic loss on account of equine herpes viral infection among the equids was quite high. The loss due to the loss of work function ranged from Rs. 200 to Rs. 1,47,000.

Keywords *Equine Herpes Virus 1&4; Respiratory Disease; Abortion; Equine Industry; Economic Loss*

1. Introduction

Equine rhinopneumonitis is caused by two closely related herpes viruses, equine herpes virus 1 and 4 (EHV-1 and EHV-4). Infection by either one of the two viruses is characterized by a primary respiratory tract disease of varying severity that is related to age and immune status of the infected animal. Infection with equine herpes virus 1 cause not only restricted to respiratory mucosa but also to cause more serious disease manifestations as abortion, perinatal foal death, or neurological dysfunction (OIE, 2000).

ER can cause severe economic loss to the equine industry. Outbreaks of EHV-1 and 4 result in poor racing performance, cancellation of race entries, decreased revenue to the race tracks, mortality, closure or quarantine of stud farms and restrictions on the movement of horses.

In India outbreaks of EHV-1 was first recorded in 1965 and serosurveys conducted have proved the prevalence and endemicity. EHV-1 abortion is a significant cause of economic losses to the horse industry, while the losses associated with respiratory disease due to lost training time and poor race performance are unquantified (Gilkerson *et al*., 1999). In the present study an attempt has been made to quantify the loss due to loss of work function among the equids in Tamilnadu.

2. Materials and Methods

2.1. Analytical Tools for Estimating Disease Losses

Ngategize and Kaneene, 1985, developed models for evaluation of economic impact of animal disease on production can be grouped under two headings namely statistical/epidemiological models and economic models. These techniques could be used to identify the risk factors that contribute to the development of disease conditions, the magnitude and direction of the contribution and association between factors and diseases.

2.2. Methodology to Study the Economic Losses Due to Equine Herpes Viral (Ehv 1 and 4) Infections

The data required for analysis was collected from the owners of horses, ponies, mules & donkeys of six selected districts of Tamilnadu (Table 1)

Based on utility the equids were classified into five categories:

(1) Anti snake venom production (2) Transport (3) Riding (4) Patrol (5) Pack animals

- Simple random sampling was used to select the sample from the population.
- The data relating to prices of different inputs were also collected
  Capital Investment: Cost of the animal, equipments (Brush, Rope, Harness, Coach)
  Variable Costs: Feeding cost, labour cost, shoeing cost, veterinary costs
- The data collected include morbidity and mortality losses (if any)
- The morbidity losses include
  - i) Loss in production/ work function
  - ii) Loss incurred upon disease control measure
  - iii) Miscellaneous losses as per (Ngategize and Kaneene, 1985)
- The mortality losses included foal loss

The data collected on the questionnaire specially prepared for this purpose (Appendix-I)

3. Results and Discussion

3.1. Economical Losses Due To Equine Herpes Viral Infections

3.1.1. Data Analysis

Utility

Out of the data collected from 184 respondents, 67.50 per cent (27 out of 40) and 32.50 percent (13 out of 40) of horses were utilized for anti-snake venom production and patrol respectively. 95.45 per cent (84 out of 88) and 4.45 per cent (4 out of 88) of ponies were utilized for transport and riding respectively. 100 per cent (17 out of 17) of mules were utilized for anti-snake venom production, 100 per cent (30 out of 30) of donkeys were kept as pack animals. One donkey and eight foals were kept without any work (Table 2).

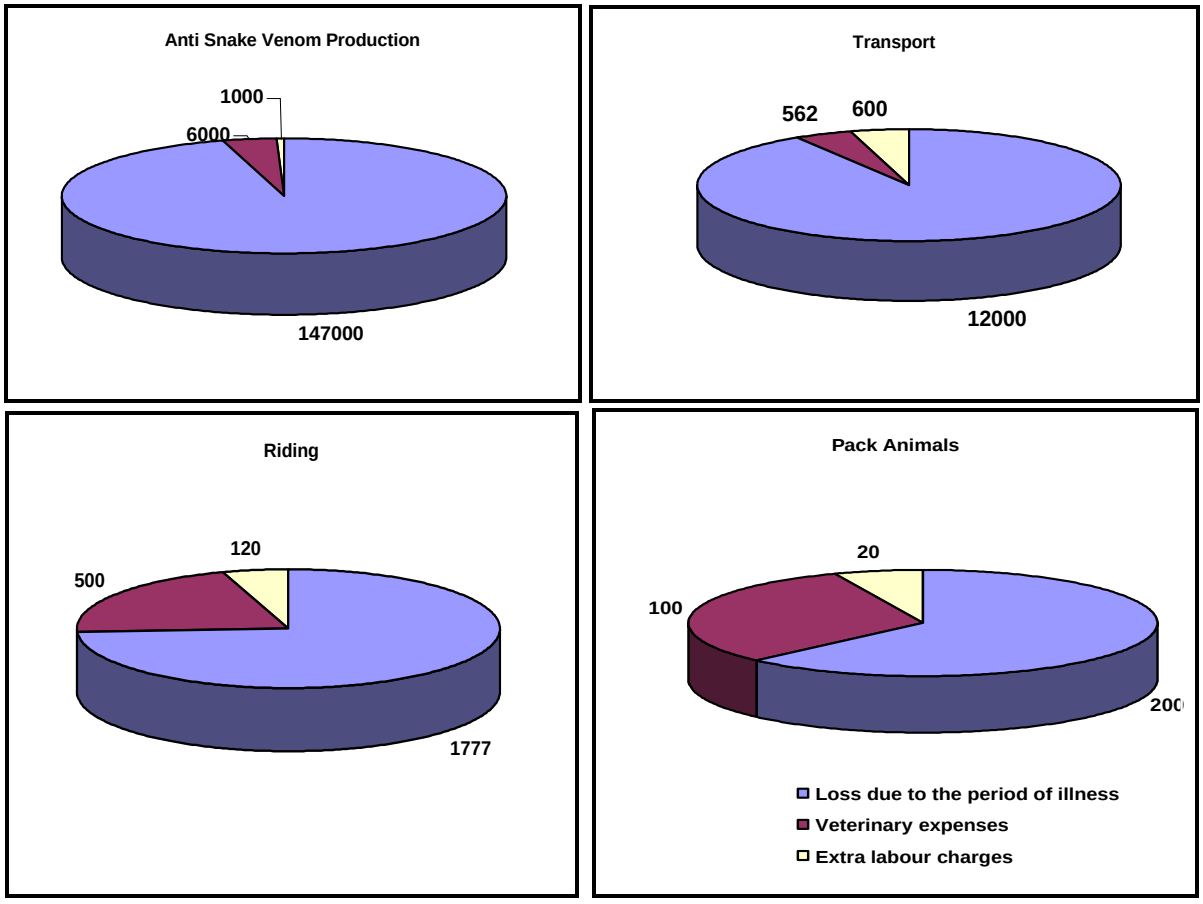

Figure 1: *Average Annual Economic Loss Due to Equine Herpesviral Infection (Per Animal/ Annum in Rupees)*

Table 1: *Economic Data Collected from Equid Owners/Respondents in Six Districts of Tamilnadu*

Sl. No.	District	Species			Total
		Horses and Ponies	Mules	Donkeys	
1.	Chennai	36	17	1	54
2.	Madurai	13	0	0	13
3.	Tiruvannamalai	16	0	0	16
4.	Theni	11	0	30	41
5.	Dindigul	50	0	0	50

6.	The Nilgiris	10	0	0	10
	Total	**136**	**17**	**31**	**184**

Table 2: *Utility of Equids in Selected Regions of Tamilnadu*

Utility	Species			
	Horses	Ponies	Mules	Donkey
Anti-snake venom production	27 (67.50)	0	17	0
Transport	0	84 (95.45)	0	0
Riding	0	4 (4.45)	0	0
Patrol	13 (32.50)	0	0	0
Pack animals	0	0	0	30 (100)
Total	**40**	**88**	**17**	**30**

Note: One donkey and eight foals kept idle were not shown in the Table

Table 3: *Average Annual Economic Loss Due to Equine Herpesviral Infection (Per Animal/ Annum in Indian Rupees)*

Components of Economic Loss	Anti-snake venom Production	Transport	Riding	Pack Animals
Loss due to the period of illness (Rs.)	1,47,000 (95.45)	12000 (50.80)	1777 (74.13)	200 (62.50)
Veterinary expenses (Rs.)	6000 (3.89)	562 (23.79)	500 (20.85)	100 (31.25)
Extra labor charges (Rs.)	1000 (6.49)	600 (25.40)	120 (5.00)	20 (6.25)
Total economic loss	**1,54,000**	**2,362**	**2,397**	**320**

(Figures in parentheses indicates the percentage to the total)

3.2. Economic Losses Due to Equine Herpes Viral Infection

Majority of the equines (96-97 per cent) in India are owned by landless, small and marginal farmers belonging to socio economically deprived communities in rural and semi urban area (ICAR, 2000).

Equine herpes viral infection poses major threat to the equids worldwide causing severe economic losses and most of the authors have recorded the loss due to abortion (Bryans, 1981; Galosi *et al.,* 1989 and Gilkerson *et al.,* 1999) and quantification of the economic loss due to loss of work function was not available. EHV-1 and 4 are substantial because of abortion and respiratory disease of racehorses in training (McGee, 1970).

Retrospective study conducted by Bryans (1981) from 1940 to 1979 in Kentucky, USA, identified 1,524 herpes viral abortions, an average of 39 per year which represented an annual loss of 0.4 to 2.6 per cent of fetuses.

The mortality losses from uncomplicated respiratory infection of EHV-1 in track horses are negligible, but such infections result in substantial indirect financial losses from costs of treatment, cancellation of race entries, decreased revenue to the race tracks and interrupted training schedule (Bryans, 1981).

Galosi *et al*. (1989) reported that infection by the viruses EHV-1 and EHV-4 is a serious economic problem in the Argentina horse industry due to abortion in breeding farms.

Outbreaks of EHV-1 causes economic loss to the racing and breeding industries as they result in poor racing performance, mortality, closure or quarantine of stud farms following cases of abortion and restrictions on the movement of horses (Mumford, 2000).

In the present study an attempt has been made to quantify the loss due to loss of work function among the equids. It has been found that income loss due to loss of work function was 95.45 per cent (Rs. 1,47,000) in case of equids utilised for anti-snake venom production, 74.13 per cent (Rs. 1,200) in case of equids used for riding, 62.50 per cent (Rs. 200) in pack animals and 50.80 per cent (Rs. 1,200) in case of equids used for transport purpose (Table 3).

The economic loss on account of equine herpes viral infection among the equids was quite high. The loss due to the loss of work function ranged from Rs. 200 to Rs. 1,47,000.

Though, the loss stated above in the anti-snake venom production was high the owner (King Institute of preventive medicine) can bear the loss. But loss among the other categories namely riding, transport and pack animals cannot be borne by the owners because; most of their livelihood entirely depends upon the income from the equids.

4. Conclusion

Equine herpes virus 1 and 4 not only cause respiratory infection but also involves reproductive and muscular system which cause economic loss by abortion, reduced productivity in case of snake venom production and also animals which are involved in riding and transport will affect livelihood of the persons. It is necessary to screen the animals and vaccinate against the disease.

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Appendix I

Seroprevalence of Ehv-1 and 4 in Equids of Tamilnadu

Owners Name: Date of collection:
Address: Sample No.

Equid Species: Horse / Pony / Donkey
Breed: Sex: Age: Colour:
Source: Bred / from breeders if breeders specify

Identification

Markings: Face: Leg: Dentition:
Purpose/Utility: Breeding / Racing / Transport / Show / Patrol / Others
Hours of Work:

Purpose	Horses	Donkey / Mule	Pony
Training			
Racing			
Transport			
Show / Riding			

Housing: Stable / Loose Box / Open Area

Feeding Schedule: Time: Roughage :
Concentrate:

Past History: Vaccination: Deworming:
Foaling: Shoeing
Treatment:

Present History: CMM: Pale / Pink / Icteric / Others
Temp: H/R:
R/R: Nasal Discharge:

Samples collected: Dung / Blood smear / EDTA blood / Whole blood

Symptoms: Respiratory: Skin:
Digestive: M. Skeletal:
Reproductive: CNS:

Economics

Earning / Day	Horses	Donkeys / Mules	Ponies
Riding			
Racing			
Transport			

Duration of Previous Illness : Loss of No. of Working Days :
Loss per Day : Cost of a Foal :

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Case Study | Open Access

Diseases of Pigeons Reared on Small-Holdings in Bangladesh

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Abstract A cross-sectional survey was undertaken on dead pigeons to explore the diseases and conditions associated with the death of pigeons reared at smallholdings and sold at live-bird markets in Bangladesh. 108 dead pigeons were collected from 2 live-bird markets-one in Dhaka and other based at Chittagong districts, 38 smallholdings, of which 20, 3, 3, 5 and 7 were located in Jessore, Dhaka, Chittagong, Magura and Jhenaidah districts respectively. Initially the collected dead pigeons were preserved at the households' refrigerators and then transferred to the department of Microbiology, Chittagong Veterinary and Animal Sciences University, where they were further frozen at -84°C until investigated. All the collected dead pigeons were thoroughly examined postmortem. From each of the dead pigeon (except decomposed carcasses) bacteriological examination done through taking samples from the liver with sterile cotton swabs, and inoculated onto at least two media- McConkey and Mueller-Hinton agar (supplemented with 5% defrinated sheep blood). To identify the organism for any bacterial growths on these two media were examined using the standard procedures. The 4 major disease/conditions associated with the deaths of the pigeons were cervical disruption & related haemorrhages (17.6%), colibacillosis (13.89%), external injuries (8.33%) and morbus cordis (10.18%).
Keywords *Columba Livia; Pigeon Mortality; Smallholdings' Pigeon*

1. Introduction

Pigeons (*Columba livia)* are widely distributed in urban and rural areas of Bangladesh (Figures 13 & 14) and come into close contact with humans in smallholdings, parks, temples, shrines, public gardens and rail road stations. Pigeons are considered one of the most important poultry birds in Bangladesh. Bangladesh is an agricultural based country. Most of the village people of this country are engaged in agriculture, livestock and poultry rearing. A great section of this people is poor and they can hardly rear large domestic animals such as cattle. Instead of the domestic animals they prefer to raise poultry birds like backyard chickens and pigeons because they need a little space and feed supplementation. Due to inadequate supply of standard feed and lack of proper health care

services of the village level, pigeons are more vulnerable to diseases. Unfortunately, there is scanty published information on the population of pigeons in our country and their common diseases. There might be some published information on the management aspects and breed of pigeons found in Bangladesh but epidemiological studies regarding the prevalence of diseases of pigeons or gross pathological findings encountered in Bangladesh have never been performed.

Although there was no published report on the total population of pigeons raised in Bangladesh but one might guess, by assessing the number of pigeons and squabs sold in almost every live bird market that pigeons contribute significantly to the poultry meat sector in the country. If the common disease of pigeons is controlled, their production might be increased. To control and prevent pigeon's diseases the first scientific approach should be the identification of diseases and pathologies associated with them. This short period study was carried out to explore gross pathological changes in some common pigeon diseases in Bangladesh.

2. Materials and Methods

This study was carried out during my internship programme period between August, 2007 to April, 2008. During this period 108 pigeons were dead (Figures 9 & 10). The pigeons were collected from 28 pigeon smallholdings in Chittagong, Dhaka, Magura, Jessore and Jhenaidah districts. There were also 2 live bird markets as the sources of the dead birds. Initially the locally collected dead pigeons were preserved at the households' refrigerators. The collected samples were transferred in the air-tight jar containing ice to the department of Microbiology, Chittagong Veterinary and Animal Sciences University. In the laboratory refrigerator, they were further frozen at -84°C until investigated. All the dead pigeons were thoroughly examined and post mortem were performed according to FAO Animal Health Manual 4 (1998). The entire post mortem examination procedure is briefly described below:

1) General body condition was examined through palpation for symmetry, crepitation, ascites etc.
2) The plumage, skin, legs, feet etc. were examined.
3) Special attention was done in cloaca region for enteritis, salpingitis, uraemia etc.
4) Mucosa of openings (conjunctiva, oral cavity & cloaca) was examined for the presence of lesions of anemia, inflammation, avitaminosis-A.
5) The bird was placed on its right side with the head pointed towards the examiner.
6) Sinus infraorbitalis were opened and examined for Mycoplasma infections, *Haemophilus paragallinarum*, NDV, ILT, etc.
7) The beak was opened and the blunt part of the scissors inserted into the oral cavity which was cut open. This process was continued down the neck to open the esophagus and the crop (Figure 8), Oral cavity, pharynx and esophagus for inflammation, avitaminosis-A, pigeon pox, ILT, etc.
8) Larynx and trachea were subsequently opened and examined for NDV, ILT, Syngamus *trachea* etc.
9) The thymus was incised and examined.
10) The bird was placed on its back with the legs towards the examiner.
11) Skin was incised transversely behind processus xiphoideus and s/c incisions towards both knees were being done to enable the removal of skin over the pectoral muscles to be pulled in cranial direction.
12) Bursa praesternalis were inspected for inflammations associated with immobile birds and Mycoplasma and Reovirus infections.
13) Pectoral muscles were incised to inspect tumors (MD, bleedings as seen in septicaemic carcasses, deep pectoral necrosis).
14) Legs and hips were bent outwards until each head of femur was dislocated (luxated) from its acetabulum.

15) Transverse incision was given behind processus xiphoideus to open into the abdominal cavity.
16) Incisions were being made on both sides of the thorax up to the brachial region / shoulders apertura thoracis cranialis.
17) Sternum with the pectoral muscles were then rotated at 180° upwards and cranially to expose the abdominal and the thoracic cavities, but before this was done sternum was lifted slightly to see if there were indications of bacteriological / virological infections such (swollen organs, bleedings, exudates etc.) In that cases tissue samples were removed or inocula taken as sterile as possible.
18) All air sacs were inspected in situ for the presence of inflammation.
19) Other visible organs were subsequently inspected in situ.
20) The heart with the pericardial sac was removed and examined. The necessary incisions were made to examine all parts (form and colour were also evaluated).
21) The liver and the gall bladder were removed and examined.
22) The spleen was removed and examined for the presence of septicemia.
23) A transverse incision cranially to the proventriculus was made and the whole intestinal tract was removed in a caudal direction. To remove the whole intestinal tract a transverse incision was made 1-2 cm cranial to the cloaca. The oesophagus and the crop were removed by separation from the other tissue and pulled in a cranial direction.
24) The intestinal tract was examined from the serosal surface and opened in a caudal direction starting from the proventriculus. During this procedure pancreas was investigated. When examined for endoparasites the whole intestine was placed in a tray.
25) The entire GIT was examined for the presence of nematodes, cestodes, trematodes and coccidia oocysts.
26) The intestinal mucosa was examined for the presence of necrotic enteritis, salmonellosis, NDV, bleedings and ulcerations.
27) An incision was given through the cloaca in the midline in cranial direction to inspect the whole abdominal cavity including reproductive tract, testicles, kidneys and air sacs.
28) In female birds the reproductive tract was being cut free of its ligaments in a caudal direction and subsequently opened through infundibulum, magnum, isthmus, uterus and vagina which were inspected both from serosal and mucosal surface.
29) In young birds bursa fabricius was opened through its opening to the cloaca and inspected for swelling and oedema of the organ or bleedings.
30) The kidneys were subsequently examined for clostridial infections-pale due to toxins, nephropathies of other causes.
31) Plexus lumbosacralis was examined.
32) Lungs were removed and examined.
33) The brachial plexus was examined.
34) Nervus ischiadicus was examined on both sides (beneath musculus gracilis)
35) Knees and hock joints were opened and inspected and tendons and tendon sheets were being examined during the same procedure. All other joints were palpated and opened in case of swellings or asymmetry for Staphylococcus *aureus, reoviruses, and Mycoplasma synoviae, E. coli, P. multocida and S. gallinarum/pullorum.*
36) Ossification was examined in young birds. Attention was paid to the ribs and their junction to the spine and the cartilaginous part for swelling, avitaminosis A-D.
37) Parallel incisions to the bones were being made on tibia/tarsus to examine for TB.
38) The brain was examined upon indication. (Avian encephalitis, avitaminosis A-E).
39) Lesions found were summarized and the pathogenesis / connection between lesions were evaluated. Finally a tentative diagnosis of a disease/pathological condition of the pigeon was being made based upon anamnesis (disease history), symptoms and lesions demonstrated.

2.1. Bacteriological Examination

Sterile inocula taken from liver were inoculated into McConkey agar surface. Colibacillosis was diagnosed by having large pink colonies on the agar surface after 48 hrs of incubation at 37ºC. If the colonies were very minute and whitish or colorless Salmonellosis was diagnosed for the dead pigeon.

2.2. Virological Examination

No virological sample was collected or assured for the presence of any virus in any of the dead pigeons. However, if there was no colony developed on the McConkey agar surface from inocula taken from liver but had the evidence of splenomegaly and or hepatomegaly and general vascular congestion, the death of the pigeon was considered to be of any kind of viral pathogens.

2.3. Mycological Examination

Any nodules found in the lung, liver or air sacs were examined for the presence of fungi. Wet mount preparations were made from these lesions and examined under microscope with a drop of lactophenol cotton blue. The presence of any hyphal segments or reproductive structures in the sample revealed the presence of fungal disease.

3. Results

Table 1: *Disease and Conditions Associated with the Deaths of 108 Pigeons Investigated having collected them from Chittagong, Dhaka, Magura, Jhenaidah and Jessore Districts*

Disease/Condition	No. (%)	Comments
Massive Haemorrhages in the neck region associated with cervical disruption	19(17.6)	1 was a cachectic carcass and E. coli recovered from 1 carcass, 1 was with haemorrhagic enteritis
Cachexia	6(5.56)	
Colibacillosis	15(13.89)	4 were cachectic and 1 had morbus cordis
Staphylococcosis	3(2.78)	
Granulomatous lesions	2(1.85)	1 had lesion in the thoracic air sac and the other on the liver
Pigeon pox	3(2.78)	
External injuries	9(8.33)	
Haemorrhagic & fatty liver syndrome	3(2.78)	
Morbus cordis	11(10.18)	
salmonellosis	4(3.7)	2 were cachectic
New castle disease	4(3.7)	
Vascular congestion	6(5.56)	
Tuberculosis	3(2.78)	
Red hepatization of lung	2(1.85)	
Others	7(6.48)	7 had 7 different conditions: anal gland abscess, egg peritonitis, enteritis, omphalitis, snake bite mark(possibly, pneumonia and suffocation
Undiagnosed	11(10.18)	5 were decomposed; 2 had the evidence of greenish diarrhoea.

3.1. Bacterial Diseases

Bacterial infections (Figures 1, 2 & 5) included 4 cases of Salmonellosis, 15 cases of Colibacillosis and 3 cases of Staphylococcosis. Salmonellosis was recorded in case no, 28, 69, 77 and 108.

Salmonellosis was associated with diarrhoea, soiled vent, white/green colour feces, yellowish necrotic foci on liver, lung, heart, enlargement of liver, catarrhal enteritis, and yellowish mass in caeca, discoloured (ova), and bronze discolouration of liver. There were 3 cases of Tuberculosis (case no. 62, 92 and 93)

6 cases were diagnosed with septicemia due to the presence of generalized vascular congestion. There was an abscess in the anal gland of this dead pigeon. The organism could enter through this abscess to invade the blood circulation and other tissues causing septicemia.

3.2. Viral Diseases

Three pigeons were supposed to die attributable to viral pathogen ND (case no. 74, 83, 96 and 100). The findings associated with these viral diseases included greenish diarrhoea, hemorrhages at the tips of the glands of the proventriculus, hemorrhages at iliocecal junction, edema, catarrhal inflammation in nasal and conjunctival mucosa, consolidated lung, enteritis, hemorrhage in liver, pale kidney and hemorrhage found in the trachea. 3 cases of the samples were supposed to have the infections with pigeon pox (Figures 6 & 12) (case no. 2, 12 and 89)

3.3. Fungal Diseases

Two cases of fungal infections were recorded (Case no. 1 and 99); the birds were cachectic. In addition, pin head size yellowish nodules in lung were observed, air sacs were found with cloudy and thickened yellowish plaques, in one pigeon necrotic foci were found in liver, spleen, kidneys and proventriculus. In other bird hemorrhages were recorded in the lungs and the kidneys were pale.

3.4. Other Conditions

Massive haemorrhages in the neck region (Figure 7) associated with cervical disruption were found in 19 cases. Morbus cordis (Figure 3) were found in 11 cases and other external injuries (Figure 11) were in 9 cases, Hemorrhagic & fatty liver syndrome were found in 3 cases. Red hepatization of lung was observed in 2 cases.

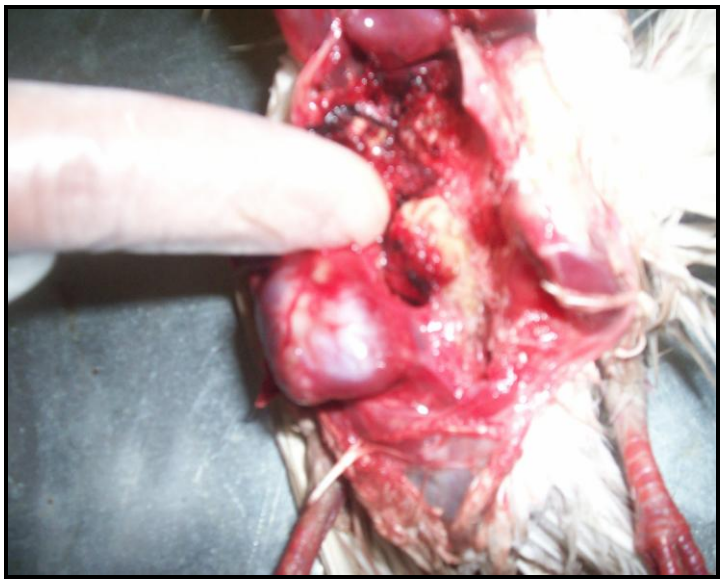

Figure 1: *A Case of Granulomatous Inflammation in a Dead Pigeon*

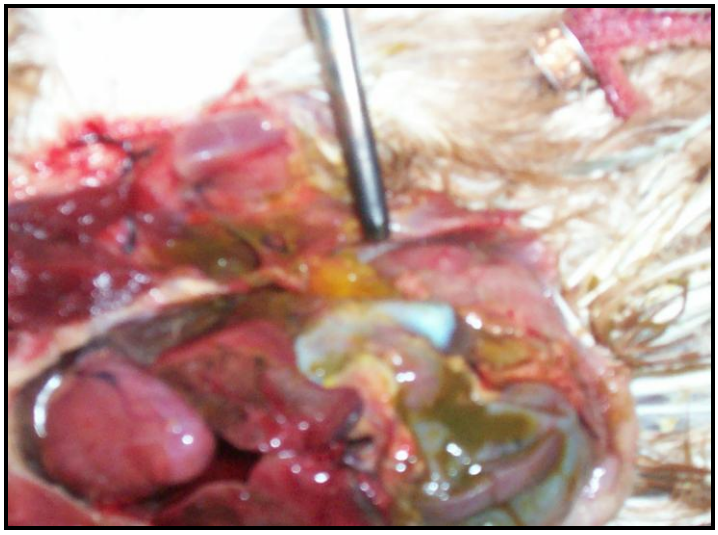

Figure 2: *A Case of Egg Peritonitis in a Dead Pigeon*

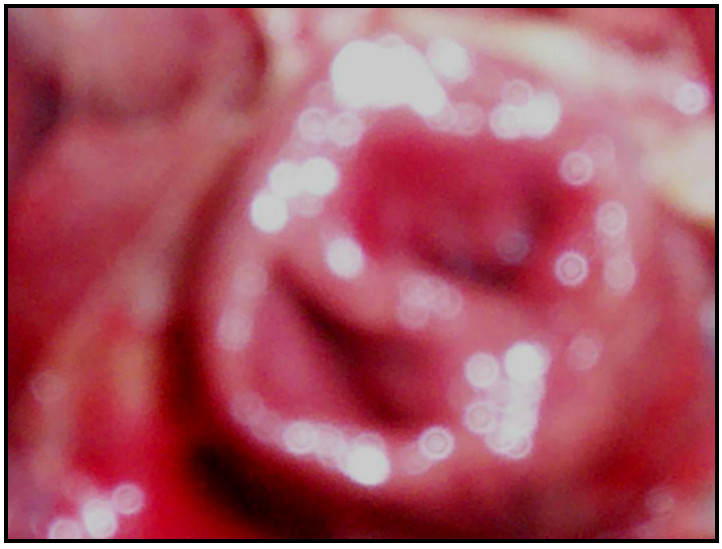

Figure 3: *A Case of Morbus Cordis in a Dead Pigeon Showing Dilatation of the Heart Chambers*

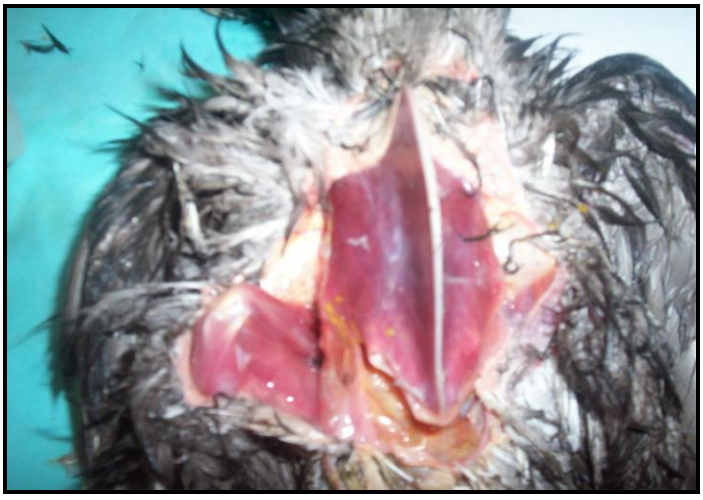

Figure 4: *Cachexia of the Breast Muscle of a Dead Pigeon*

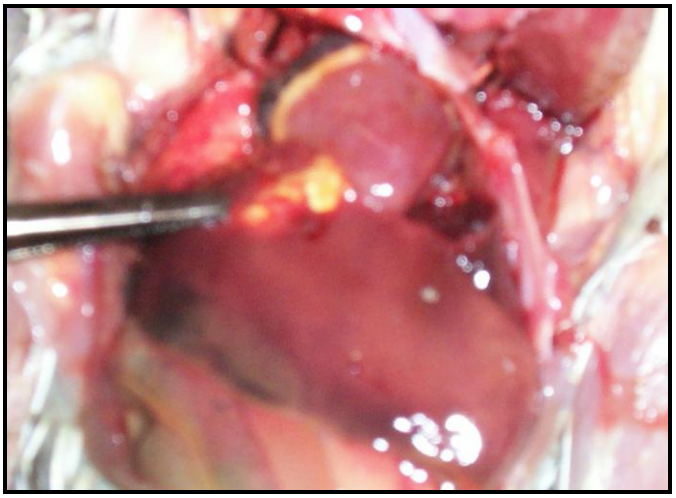

Figure 5: *A Case of Tuberculosis in the Liver in a Dead Pigeon*

Figure 6: *A Case of Pigeon Pox in a Dead Pigeon*

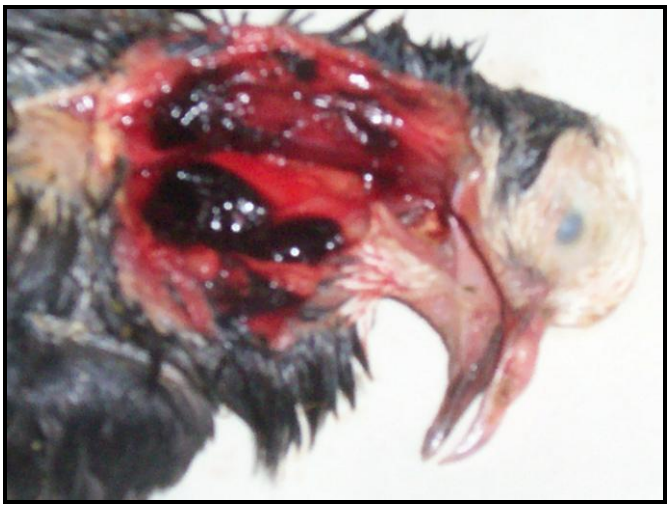

Figure 7: *A Case of Cervical Dislocation in a Dead Pigeon Showing Massive Haemorrhage*

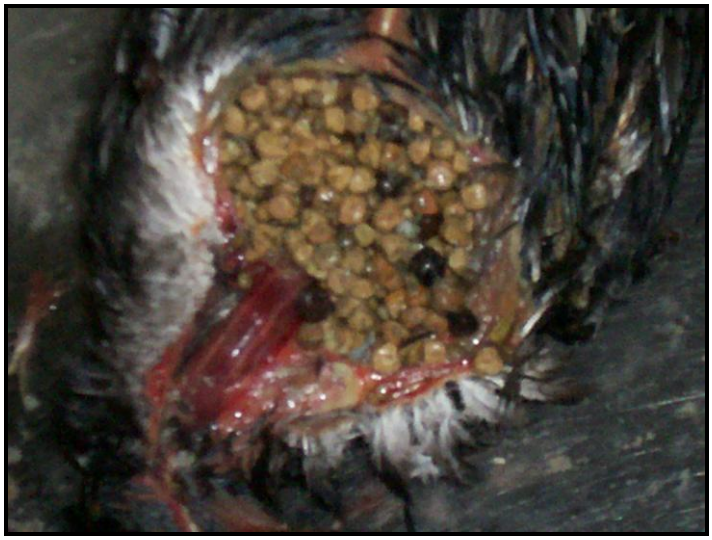

Figure 8: *Crop Content (Gram & Stone) of a Dead Pigeon*

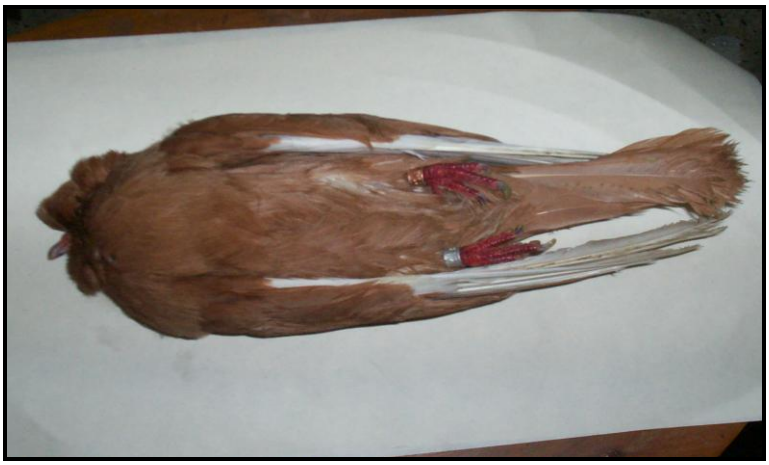

Figure 9: *A Moyurponkhi Pigeon Prior to Post Mortem*

Figure 10: *A Group of Squabs Arranged Prior to Post Mortem*

Figure 11: *A Case of Snake Bite in the Breast Region*

Figure 12: *A Case of Pigeon Pox in a Farm of Chittagong*

4. Discussion

This study shows (Table 1) that 45.63% pigeons were affected with infectious disease and 54.37% was affected with other than infectious diseases; among them 37.3% pigeons were affected with bacterial disease, 6.48% with viral disease, 1.85% with fungal disease, 8.33% pigeons with nutritional disease (Figure 4), 36.78% with external injuries or cervical disruption. About 11.11% cases were undiagnosed.

Bacterial-Abiodun et al., 1998; Abd El-Aziz et al., 2002; Albuquerque et al., 2013; Tanaka et al., 2005; Pasmans et al., 2003; Fukata et al., 1986; Kimpe et al., 2002; Monita Vereecken et al., 2000; Abeera Mubarak and Farzana Rizvi, 2002; Marlier et al., 1997; Medina et al., 2004; Wakamatsu et al., 2006 and Roy et al., 2003 shown the viral infection in pigeon.

Fukushi et al., 1983; Pal et al., 1992 and Prukner-Radovcić et al., 2005 shown the fungal infection in pigeon.

This study seems to be the first report on the gross pathological findings in pigeons reared on smallholdings in Bangladesh. The findings of this study remained uncorrelated due to insufficient published information on this issue in Bangladesh.

Like chicken diseases pigeons of Bangladesh are affected with Salmonellosis and Colibacillosis. Due to insufficient laboratory facilities and time constraints this study failed to identify the serotypes of Salmonella causing disease problems in pigeons. Similarly, the serotypes of *E.coli* prevalent in pigeons need to be investigated further. Because *E.coli* is opportunistic pathogens and is generally mobilized during malnourishment or impairment of the immunity of birds, it is apprehended that the inappropriate feed supplementation to the pigeons in Bangladesh might make them more vulnerable to opportunistic infections such as Colibacillosis.

The viral diseases were presumed based on only post mortem examination findings. More laboratory evidence had to be generated to confirm the involvement of viral pathogens. However, in some countries Newcastle disease was found to cause significant disease problems in pigeons (Marlier et al., 2006). Pigeon pox is another important viral disease affecting pigeons. Based on its characteristic lesions-nodules on the head region, the disease can easily be diagnosed. However, in this study no pigeon pox disease was found, this might be due to the reason that the sample size was poor to find out all of the diseases affecting pigeons in Bangladesh.

Cervical dislocation and haemorrhage, morbus cordis and external injury were found in the post-mortem. It indicates that cannibalism and mishandling of pigeons is one of the major causes for pigeon mortality.

Figure 13: *A Cage Reared Farming of Pigeon*

Figure 14: *A Backyard Farming of Pigeon*

5. Conclusion

This could be the first report on diseases affecting domestic pigeons on smallholding level in Chittagong district. It gives valuable information that most of the domestic pigeons are affected with infectious diseases such as Salmonellosis, Colibacillosis, Staphylococcosis and granulomatous inflammation. During clinical manifestations of those diseases if proper antibiotics are applied the survivability of this poultry birds can be increased, bringing more economic benefits to the farmers/owners and by introduction of vaccines the important viral diseases can be controlled. However, an intensive study should be undertaken encompassing the pigeon population to unveil the overall scenario of pigeon diseases in Bangladesh before a control measure is advocated.

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