Investigation of parasitic and bacterial diseases in pigs with analysis of hematological and serum biochemical profile

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Abstract The present study was undertaken to evaluate various disease conditions prevalent in slaughtered pigs and zoonotic importance. The study was conducted on two hundred non-descript pigs slaughtered at an organized slaughter house, Mumbai. The animals included in the study were randomly selected. Post mortem examination of the animals was performed to note various disease conditions and tissues were collected for histopathology. Direct examination of stool was found negative for parasites. Gross and microscopical examination revealed presence of *Ascarops strongylina*, Sarcocyst, Hydatid cyst, *Cysticercus cellulosae*, *Ascaris suum* and *Cysticercus tenuicollis*, along with bacteria like *Salmonella*, *Pseudomonas*, *Shigella*, *Streptococci*, *Proteus* and *Pasteurella* spp. were isolated. Indirect ELISA was performed for detection of antibody titer in the pig serum against classical swine fever. Studies on hematological and serum biochemical profile revealed decreased total protein concentration and globulin level with leukocytosis and neutrophilia and in parasitic infections eosinophilia was evident.

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Though significant progress has been made in the last few decades in reducing the prevalence of animal diseases, there is still an increasing concern over the losses associated with diseases that cause reduction in production efficiency. Moreover, lesions of several diseases that can affect the market value are common in slaughtered pigs which can be identified by postmortem and various laboratory methods, even if present, at sub-clinical level.

The hematological and clinico-chemical profiles have reflected the degree of inflammation in slaughtered pigs (Odink et al. 1990). Histopathology as an art by itself and one of the oldest and most reliable tool is sufficient enough to diagnosis of any type of alterations in organs and tissues in diseases and disease conditions from the starting to end point if the basics are properly followed (Rajan 2007). Microscopic examination allows the diagnostician to evaluate whether the organisms recovered from the tissues are significant contributors to the lesion and also whether organisms not detected may also be involved (Bruce 1995).

In order to prevent the diseases an understanding of their prevalence at the effect of these is essential. Thus, the present study was undertaken with the objective of evaluating various disease conditions prevalent in slaughtered swine and to evaluate the zoonotic impact.

The present study was conducted on 200 non-descript pigs slaughtered at an organized slaughter house at Mumbai, India. They were brought to the slaughter house from various districts of Maharashtra and Gujarat (India). The animals included in the study were randomly selected and the blood

samples were collected during bleeding (exsanguination) and the specimen were collected during evisceration.

During exsanguination the blood sample were collected in glass vials containing sodium EDTA for complete blood count and blood smear examination and in glass vials without EDTA to separate serum for serum chemistry and ELISA. During evisceration the mesenteric lymph node, kidney, spleen, liver, lung, heart, stomach, intestine and tissues showing lesions were collected in 10% neutral buffer formalin. The entire musculature was checked by incising it at few places to detect any abnormality and cysticerci. In positive cases the location of cysticerci were noted and the intensity of the infection was expressed in terms of number of cysts found per unit area of two square inch.

Both the kidneys attached to the dorsal wall of peritoneal cavity along with the peri-renal fat were checked to detect any parasite and lesions and were collected for histopathology in 10% neutral buffered formalin. The visceral organs were examined for the presence of bladder worm and any other abnormalities. In positive cases for bladder worm size and intensity of the bladder worm were recorded. Spleen, liver and heart were checked properly for any type of lesion and collected in neutral buffered formalin for histopathological study.

Piece of spleen was collected separately and aseptically for bacterial culture and isolation. The entire digestive tract from pharynx to rectum was divided into three parts viz., oesophagus with stomach, small intestine and large intestine with rectum and checked thoroughly and specimens were collected for histopathology. Mesenteric lymph nodes were checked and collected for histopathology. Rectal contents were collected for fecal examination.

The respiratory tract was opened from the trachea to the bronchioles and checked thoroughly for any abnormality and parasite. Mediastinal lymph nodes were checked for lesion and collected in 10% neutral buffer formalin.

Hematological parameters

Hematological parameters were analyzed with hematology automatic cell counter (Abacus-Diatron) by impedance method. Blood smear was taken in clean glass slides and stained with Leishman's stain and examined under microscope for presence of any microorganism and differential leukocyte count which was converted to absolute leukocyte count.

Serum biochemical profile

Serum obtained was separated by centrifugation for 10 min at 1,500 rpm and parameters as mentioned below were

estimated by using serum biochemistry semi-autoanalyzer using commercial reagent kits: Total protein estimation by Biuret method (Tietz 1986), Serum albumin estimation by BCG Dye method (Doumas et al. 1972), Serum alanine aminotransferase (ALT) estimation as per IFCC method (International Federation of Clinical Chemistry) suggested by Tietz (1995), Serum alkaline phosphatase estimation by the method of IFCC (Tietz 1983), Total and direct bilirubin estimation as per method suggested by Pearlman and Lee (1974), BUN (blood urea nitrogen) estimation by GLDH-UREASE method (Tietz 1976) and estimation of Serum Creatinine by Jaffe's method (Jaffe 1986).

Examination of fecal sample

Direct smear examination was performed as described by Chauhan and Agarwal (2006) to detect the presence of parasitic egg and larvae in feces.

Parasitic examination

Small pin like pink colored round worms embedded in gastric mucosa in fundic region were collected and dehydrated in ascending grades of alcohol up to 100% for 20 min in each. In order to ensure complete dehydration, two changes of absolute alcohol were used. The cuticle was cleared in lactophenol and the cut ends were mounted on the glass slides using lactophenol and examined under microscope to identify the parasite. (Soulsby 1982). Intestinal round worms encountered during the total procedure were collected in 10% formalin. Metacestodes of *Taenia solium* (*Cysticercus cellulosae*), *Taenia hydatigena* (*Cysticercous tenuicollis*) and *Echinococcus granulosus* (hydatid cyst) were preserved in 70% alcohol. Fertility of hydatid cyst was determined by aspirating hydatid fluid with pasture pipette and was subjected to centrifugation at 2,000 rpm for 10 min. The sediment was examined for detection of protoscoleces.

Bacterial examination

Organs were collected aseptically and stored in ice and brought to the laboratory for further processing. For isolation of pathogenic bacteria from the organ samples, pre-enrichment was performed in various selective broths followed by plating on selective media as per the method described by Cruickshank et al. (1975). Identification of the bacteria and biochemical tests were done according to the standard procedures given by Cowan and Steel (1993).

Enzyme linked immunosorbent assay (ELISA)

The indirect ELISA was performed for detection of anti-classical swine fever (CSF) antibody titer in the pig serum by using standard method described by Sarma and Sarma (1996). The serum showing titer 1:20 and above were considered positive for presence of antibodies for classical swine fever virus.

Statistical analysis was done by completely randomized design as suggested by Snedecor and Cochran (1994).

Investigation on presence of parasitic diseases

In the present study, fecal examination by direct smear method showed negative results in all cases. The lesions noted during postmortem revealed the presence of parasite infection in 114 animals (57%). These were visible grossly and confirmed microscopically. Among the positive cases, 67 cases revealed the presence of *Ascarops* sp. (58.77%), 12 hydatid cyst (10.53%), five *Ascaris suum* (4.39%), five *Cysticercous cellulosae* (4.39%) and one *Cysticercous tenuicollis* (0.88%). Sarcosyst was confirmed histologically in 24 cases (21.05%). Out of total 33.50, 6.00, 2.50, 2.50, 0.50 and 12.00%, respectively. Hydatid cysts were found in viscera and the organ wise prevalence of the hydatidosis in spleen, lung and liver were 41.67, 41.67 and 16.67%, respectively. Out of total, 2.5, 2.5 and 1% hydatidosis found in spleen, lung and liver, respectively. All the cysts were sterile (Table 1).

Investigation on presence of bacterial diseases

Bacteria could be isolated from twenty-six spleen samples (13.00%). *Salmonella* was isolated from seven samples (26.92%), *Pseudomonas* from five samples (19.23%), *Shigella* from five samples (19.23%), *Streptococci* from four samples (15.39%), *Proteus* from three samples (11.54%) and *Pasteurella* was isolated from two samples (7.69%), respectively.

Investigation on presence of viral disease (classical swine fever)

Indirect ELISA was performed to detect the viral antibody titer present in the pig sera (IgG) against Swine fever. 142 (71.00%) showed presence of antibody. Twenty-six sera samples (18.3%) showed titer 1:20, fifty-four samples (38%) showed titer 1:40, six samples (4.23%) showed titer 1:80, fifty-six samples (39.44%) showed titer above 1:80 in serum and fifty-eight samples (29%) showed no titer. In all

Table 1 Mean hematological and serum biochemical parameters in different groups of pig

Group	1	2	3	4	5	6
SGPT (IU/l)	77.91 ± 14.47 ^{2a}	67.45 ± 23.32 ^{2ab}	44.80 ± 5.23 ^{3bcd}	43.65 ± 2.36 ^{5cd}	51.50 ± 2.83 ^{4bc}	43.450 ± 0.760 ^d
ALP (IU/l)	79.81 ± 13.24 ^{2c}	179.50 ± 31.00 ^{3a}	172.19 ± 13.58 ^{0b}	87.58 ± 22.87 ^{4bc}	107.15 ± 9.34 ^{2b}	81.52 ± 2.85 ^{1c}
TP (gm/dl)	6.60 ± 0.32 ^{3a}	5.30 ± 0.62 ^{5b}	6.19 ± 0.06 ^{1ab}	5.59 ± 0.52 ^{4b}	5.81 ± 0.31 ^{0b}	6.83 ± 0.07 ^{7a}
Albumen (gm/dl)	2.48 ± 0.17 ^{9ab}	1.94 ± 0.28 ^{8bc}	2.05 ± 0.04 ^{3bc}	1.77 ± 0.39 ^{7c}	2.11 ± 0.14 ^{0bc}	2.50 ± 0.04 ^{7a}
Globulin (gm/dl)	4.13 ± 0.27 ^{6ab}	3.36 ± 0.55 ^{5b}	4.14 ± 0.09 ^{2ab}	3.82 ± 0.17 ^{0ab}	3.70 ± 0.23 ^{9b}	4.33 ± 0.06 ^{6a}
TB (mg/dl)	0.39 ± 0.13 ⁴	0.40 ± 0.09 ⁰	0.35 ± 0.03 ³	0.26 ± 0.02 ⁶	0.35 ± 0.03 ⁴	0.33 ± 0.01 ³
DB (mg/dl)	0.13 ± 0.01 ⁷	0.07 ± 0.01 ³	0.11 ± 0.04 ²	0.15 ± 0.02 ⁶	0.11 ± 0.02 ¹	0.11 ± 0.00 ⁷
Hb (gm %)	11.73 ± 0.31 ^{7b}	10.930 ± 0.44 ^{4b}	11.87 ± 0.06 ^{7ab}	12.68 ± 0.89 ^{8a}	12.10 ± 0.25 ^{3ab}	12.62 ± 0.09 ^{8a}
PCV (%)	38.12 ± 1.26 ⁰	35.88 ± 1.99 ⁷	37.33 ± 3.33 ³	36.10 ± 2.13 ⁰	37.80 ± 0.89 ⁸	39.53 ± 0.35 ⁵
A:G	0.64 ± 0.07 ^{0ab}	0.79 ± 0.08 ^{4a}	0.78 ± 0.05 ^{5a}	0.56 ± 0.01 ^{6b}	0.58 ± 0.02 ^{2b}	ND

Values are expressed in mean ± SE. Values in the same row bearing at least same superscript did not differ significantly ($P \leq 0.05$)

$n_1 = 11$, $n_2 = 4$, $n_3 = 3$, $n_4 = 4$, $n_5 = 25$ and $n_6 = 153$

ND not detected

eleven sera samples from live animals from a known farm where regular vaccination was done, antibody titer showed above 1:80.

Study on hematological and serum biochemical findings

Serum alanine aminotransferase (ALT) level (IU/ μ l)

The mean value of serum ALT in groups 1, 2, 3, 4, 5 and 6 were 77.91 ± 14.472 , 67.45 ± 23.322 , 44.80 ± 5.233 , 43.65 ± 2.365 , 51.50 ± 2.834 and 43.450 ± 0.760 , respectively. The mean ALT value in Group 6 was significantly lower than groups 1, 2 and 6. The mean value of group 1 was significantly higher than groups 3, 4, 5 and 6 but did not differ from group B (Table 1).

Serum alkaline phosphatase (ALP) level (IU/ μ l)

The mean value of serum ALP in groups 1, 2, 3, 4, 5 and 6 were 79.81 ± 13.242 , 179.50 ± 31.003 , 172.19 ± 13.580 , 87.58 ± 22.874 , 107.15 ± 9.342 and 81.52 ± 2.851 , respectively. The mean values of groups 2 and 3 were significantly higher. The means of groups 1 and 6 were significantly lower than group 2, 3 and 5 but did not differ from group 4 (Table 1).

Serum total protein (TP) concentration (gm/dl)

The mean serum total protein level in Groups 1,2,3,4,5 and 6 were 6.60 ± 0.323 , 5.30 ± 0.625 , 6.19 ± 0.061 , 5.59 ± 0.524 , 5.81 ± 0.310 and 6.83 ± 0.077 , respectively. The mean TP values in groups 1 and 6 were significantly higher. The values of group 2, 3, 4 and 5 did not differ from one another (Table 1).

Serum albumin concentration (gm/dl)

The mean value of serum albumin in groups 1, 2, 3, 4, 5 and 6 were 2.48 ± 0.179 , 1.94 ± 0.288 , 2.05 ± 0.043 , 1.77 ± 0.397 , 2.11 ± 0.140 and 2.50 ± 0.047 , respectively. Values of Groups A and F did not differ significantly. Value of Group 3 was significantly lower than that of groups 1 and 6. Values of 1, 2, 3 and 5 did not differ significantly (Table 1).

Serum globulin concentration (gm/dl)

The mean value of serum globulin in groups 1, 2, 3, 4, 5 and 6 were 4.13 ± 0.276 , 3.36 ± 0.555 , 4.14 ± 0.092 , 3.82 ± 0.170 , 3.7 ± 0.239 and 4.33 ± 0.066 , respectively. The value of group 6 was significantly higher than group B

and E but not from groups 1, 3 and 4. The mean values in group 1, 2, 3, 4 and 5 did not differ significantly (Table 1).

Serum total bilirubin concentration (mg/dl)

The mean serum total bilirubin level in groups 1, 2, 3, 4, 5 and 6 were 0.39 ± 0.134 , 0.40 ± 0.090 , 0.35 ± 0.033 , 0.26 ± 0.026 , 0.35 ± 0.034 and 0.33 ± 0.013 , respectively. The mean values for total bilirubin did not differ significantly (Table 1).

Serum direct bilirubin concentration (mg/dl)

The mean serum direct bilirubin level in groups 1, 2, 3, 4, 5 and 6 were 0.13 ± 0.017 , 0.07 ± 0.013 , 0.11 ± 0.042 , 0.15 ± 0.026 , 0.11 ± 0.021 and 0.11 ± 0.007 , respectively. There was no significant difference between the values (Table 1).

Hemoglobin concentration (Hb %)

The mean hemoglobin level in groups 1, 2, 3, 4, 5 and 6 were 11.73 ± 0.317 , 10.93 ± 0.444 , 11.87 ± 0.067 , 12.68 ± 0.898 , 12.21 ± 0.253 and 12.62 ± 0.098 , respectively. Values of D and F did not differ significantly and were higher than other groups. The mean of 1, 2, 3 and 5 did not differ and that of group 3 did not differ from that of 4 and 6 (Table 1).

Packed cell volume (PCV) (%)

The mean PCV level in groups 1, 2, 3, 4, 5 and 6 were 38.12 ± 1.26 , 35.88 ± 2.00 , 37.33 ± 3.33 , 36.1 ± 2.13 , 37.80 ± 0.9 and 39.53 ± 0.1 . The values were not significantly different from each other (Table 1).

Serum albumen–globulin (A:G) ratio

The mean serum A: G ratio in groups 1, 2, 3, 4 and 5 were 0.64 ± 0.070 , 0.79 ± 0.084 , 0.78 ± 0.055 , 0.56 ± 0.016 and 0.58 ± 0.022 , respectively. The mean A: G ratio in groups 4 and 5 were significantly lower than groups 2 and 3. Values of group 1, 4 and 5 did not differ significantly. Groups 2 and 3 did not differ. And Groups 4 and 5 did not differ significantly from each other.

In present study, the overall prevalence of parasites, bacteria and classical swine fever in pigs slaughtered at Mumbai was 57.00, 13.00 and 71.00%, respectively. Shastri (1966) reported 42.80% prevalence of helminthic parasite in abattoir. Same type of moderate prevalence was reported by Varma et al. (1977) who surveyed the helminthic parasites in pigs from Bhubaneswar and Hisar in India.

The age of the animals slaughtered probably was also a factor in the low parasitic prevalence noted. In present study evaluation of swine fever was done by estimating the antibody titers against swine fever virus. Since the sub-clinical infections too give a positive titer, the prevalence percentage was high. The parasitic infections are known to be low during these months. It was supported by the findings of D'souza and Hafeez (1998), who reported higher prevalence of cystic infection in pigs above two years of age. Yadav and Tendon (1989) noted highest (73.20%) prevalence of parasites during summer and autumn and lowest (63.0%) in winter.

The prevalence of bacterial infection was evaluated by isolation of bacteria and not by the lesions or clinical symptoms noted. This was probably the reason for recording high percentage of bacteria in the present study.

Varma (1990) found 1.30% of pigs infected with hydatid cyst with highest number in liver. In Bangalore, overall incidence of 3.02% with maximum number of the cysts in liver followed by lung and spleen hydatidosis was reported by Vijayasmitha et al. (1993). Gatne (2001) recorded 1.52% hydatid cyst on liver in pigs slaughtered at Mumbai. 86.73 and 97.14% fertile hydatid cysts were noted in liver by Pednekar et al. (2009) respectively.

Serum biochemistry of this group revealed increased in ALP and this could be attributed to biliary dysfunctions. Benjamin (2005) also observed high ALP concentration in bile duct obstructions.

Parasitic infections cause necrosis resulting in neutrophilia and eosinophils are predominantly noted in parasitic infections which probably led to the above result. Similar observation with regards to the infiltration of eosinophils in parasitic infections has been reported by Coles (1980). Hypoproteinemia probably also resulted due to the affection of glomerular filtration. Parasitic infection leads to increase TLC with neutrophilia and eosinophilia. Serum globulin was significantly lower in this group than normal group and A:G ratio was also higher than normal group. Decreased globulin caused increase A: G ratio which could be attributed to immunosuppression due to bacterial infection. Benjamin (2005) also stated the same regarding decrease A: G ratio. The hematological values in the animal groups were within the normal range similar to the findings of Benjamin (2005).

Rajbonshi (2007) reported that among susceptible breeds of pigs available in the N. E region, free-grazing doom variety and crossbred pigs were found to be affected mostly with CSFV. Nandi (2002) concluded that free grazing and semi intensively reared pigs easily picked up infection from infected pigs as well from feed.

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