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Risk factors and prevalence of coccidiosis in chicken in district Gujarat, Punjab, Pakistan

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Abstract

Coccidiosis is one of most deadly protozoan disease of poultry. This study was designed to ascertain prevalence of coccidiosis, role of critical factors, Identification and relative prevalence of species of *Eimeria*. From February 2013 to October 2017, 5700 gut and 5700 faecal samples of broiler and Layer chickens, suspected for coccidiosis were collected from poultry sale point and poultry farms. There was highest rate of mortality in young birds (age 15-28 days). Highest numbers of positive cases were in august (90.6%) and least in Jun and January (63%). Seven species of *Eimeria* were detected in field isolates. *Eimeria acervulina* was found in highest prevalence and *Eimeria brunette* in least. In summer greatest outbreaks were observed and winter showed least. The incidence of clinical coccidiosis was 30.46% and subclinical was 37.12%. Prevalence of coccidiosis in different management condition was 14.82% good, 20.12% normal, and 32.66% in poor. Prevalence of coccidiosis was 56.02% in broiler and 11.54% in Layer flocks. The prevalence was (36.35%) in rice hull and 31.22% in wood shaving, poultry farms. *Eimeria* was detected in all poultry farms. Over the years, Continue increase in prevalence of disease and reduction in sensitivity of local population of *Eimeria* was observed. Type of litter material, time duration, season, humidity, temperature and disposal of dead birds are other critical factors. Both clinical and sub-clinical coccidiosis retards the growth of flocks and cause huge economic loss to farmers. There is need to develop Epidemiological Database of *Eimeria* for suitable and timely control of coccidiosis.

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Introduction

Poultry is the fastest growing industry in Pakistan; it has an annual growth rate of 2.1%. It is also a source of food and income for many other countries particularly in Africa and Asia (Ayaz *et al.*, 2003; Etuk *et al.*, 2004; Bukar-Kolo *et al.*, 2006; Shah *et al.*, 2009; Györke *et al.*, 2013). *Eimeria* is a protozoan belongs to phylum apicomplexa. It causes disease coccidiosis in all live stocks including poultry birds.

Poultry farming constitutes a major livestock activity in Sri Lanka, Pakistan and other developing countries. It has shown a rapid growth during the past four decades (Wanaslnghe, 1993). The major diseases that affect the industry have been identified as coccidiosis, Newcastle disease, Marek's disease, respiratory diseases and lymphoid leucosis (Fonseka, 1987; Wickramasinghe *et al.*, 1992). Coccidiosis had been diagnosed as an economically important disease in poultry during the past forty years (Seneviratna and Mnhalingam, 1962; Rasiah and Kulasegaram, 1972; Kulasegaram, 1975; Wickramasinghe *et al.*, 1992).

Chickens (Gallus gallus domesticus) are favorite host of *Eimeria*. Chickens have high morbidity and mortality rates of coccidiosis (Abbas *et al.*, 2011; Abbas *et al.*, 2012). It has seven species which cause disease in chickens but in these seven there are three species namely *E. acervulina, E. maxima and E. tenella* which cause disastrous outbreaks in chickens. Minimum two species of *Eimeria* are found in infected chickens at a time (McDougald *et al.*, 1986; Kucera, 1990; Morris *et al.*, 2007). Sometimes the infected chickens have more than two species which cause disease synergistically (Long and Joyner, 1984; Haug *et al.*, 2008; Jenkins *et al.*, 2008).

Oocysts of *Eimeria* species are found in soil, leaf litter and foods of chickens which contain oocysts of these organisms. Chickens are infected from *Eimeria* species by feeding on such foods. The infected birds are source of infection for other bird's residing in their vicinity. These oocysts can survive for long time in chickens and infected things and become infective only in two days (Alqomsan, 2013). Range of prevalence of coccidiosis can vary greatly, has been reported as low as less than 10 % to as high as more than 90 % in broilers and Layers, globally (Morris and Gasser, 2006; Haug *et al.*, 2008; Karaer *et al.*, 2012; Singh *et al.*, 2015).

This study was designed to prevalence of coccidiosis in Gujarat district of Pakistan. Purpose of the study was to collect data from commercial poultry forms of Gujarat in different seasons of year. Identification of *Eimeria* species in diseased chickens was another goal of the study.

Material and methods

Study area

Gujrat is a district found in Punjab province of Pakistan. It is found at 32.6 latitude and 74 longitudes. It is surrounded by Jehlum, Sialkot, Gujranwala and Mandi Bahauddin. On north east of Gujrat beautiful valley of Kashmir and in south east river Chenab are located. It has an area of 3192 sq. kilometer. It has three tehsils namely Gujrat, Kharian and Sarai Alamgir.

Climate

Climate of the study area is moderate type. The average annual rainfall is between 60 cm to 100 cm. In summer season temperature reaches upto 45 ^{o}C while in winter it reaches near freezing point of water. Weather of Gujrat remains pleasant in most part of the year due to mountainous area of Kashmir in its vicinity.

Data collection

Questionnaire was design for data collection about risk factors that contribute to occurrence of coccidiosis. At the time of sample collection, information regarding age, no. of birds per house, no. of houses in the poultry farm, house dimensions, season, use of anticoccidials, ventilation system, genotype of broilers, management practices like watering and feeding methods, condition of drinkers and feeders, nature and condition of litter, frequency of change of litter; were recorded from poultry

farmers.

Sampling

Local chicken industry includes various size flocks and type of poultry farms. Local poultry industry includes 200327 flocks and pullets of chicken. From February 2013 to June October 2017, 5700 gut & 5700 faecal samples of broiler and Layer chickens suspected for coccidiosis were collected from poultry sale point and poultry farms in adjacent areas reported to have signs of coccidiosis. The samples i.e. intestines along with caeca were collected in 2.5% (w/v) potassium dichromate solution and stored in the dark at 4°*C*.

Samples processing

The samples were brought to Microbiology Research Laboratory (MRL), Quaid-i-Azam University, and Islamabad for further analysis. For the isolation of oocysts, each sample was processed using the method described by Eckert *et al*., 1995.

Gut examination

Intestine was freed from mesentery and surface of intestine was carefully observed for the presence of blood clotting, or pitchy patchy or inflamed area. Different species of *Eimeria* infect different part of intestine and have characteristic lesion shape and pattern. Different parts of intestine were carefully open with the help of scissors and observed. Gross lesion score were taken along with other pathological problem ranging from petechi, reddening, thickening, ballooning, hemorrhage (bleeding), caecal core, whitish spot, ladder like appearance, from ceacal destruction to swelling of whole intestine. Depending upon the amount of intestinal destruction, type of species and severity of diseases specific number were allotted ranging from 0-4.

Mucosal scrapping examination

When there is any clue from for the presence of Coccidiosis from fecal samples or from sign and symptom such as bloody diarrhea is due to *Eimeria tenella*, whitish diarrhea is indicator of *Eimeria acervulina*.

Fecal examination

Fecal samples from each poultry farm were collected in plastic zipper bag. Fecal samples were collected in W shape path from each farm. Fresh feces and litter samples from each poultry farm were collected. Litter samples were also taken from places that are wet. Fecal sample were transported to laboratory and stored at 4c till further processing (MAFF, 1986; Conway and McKenzie, 2007).

Eimeria species identification

Combination of different methods was used for species specific identification of Eimeria in poultry. Pathological lesions, Oocysts index, and sporulation time help in differentiation of different species. There is Specific infection site of each *Eimeria* species in the intestine of chicken, criteria for identification of present species was developed by Long and Reid (1982).

Data analysis

Data was summarizing with the help of descriptive statistics by using Microsoft Excel worksheet. Latest version of SPSS statistical software package was used to analyze data. For measuring statistical significance of result Pearson's Chi square test was applied. 95% CI and p-value < 0.05 was used to know the significance. To calculate prevalence number of positive samples was divided by total number of samples and multiplied by hundred. Chi-square helps to draw relationship between prevalence of Coccidiosis and Risk factor of Coccidiosis. If resulting P-value is less than 0.05, association is statistical significant.

Results and discussion

In present study overall prevalence of coccidiosis in fecal and intestinal samples was 67.57% in district Gujarat. Intestinal and fecal dropping samples were collected from 1712 poultry farms and 2000 poultry sale points. Rate of infestation in poultry farm and different flocks was 67.57%. Figure 1 Prevalence of coccidiosis in five years of study (2013-17). It reveal from gut examination and fecal test that prevalence of disease increased over the years. Gut samples

examination proved continue increase in frequencies from 58.90% 2013 to 65.70% 2017 of disease in district Gujarat. Same pattern was observed in fecal samples (Fig 1). Figure 2 Frequency of Coccidiosis in fecal isolates collected from poultry farms. Commonness of disease gent in fecal samples collected from 2013-2017 from poultry industry. Again highest prevalence was found in age group 2. There is highest growth rate in age group 2 birds. Highest numbers of positive cases were noted in august 90.6 % and least positive case was found in Jun and January were 63%. Fecal samples have more percentage prevalence than intestinal samples but revealed same trend of parasite (fig 3).

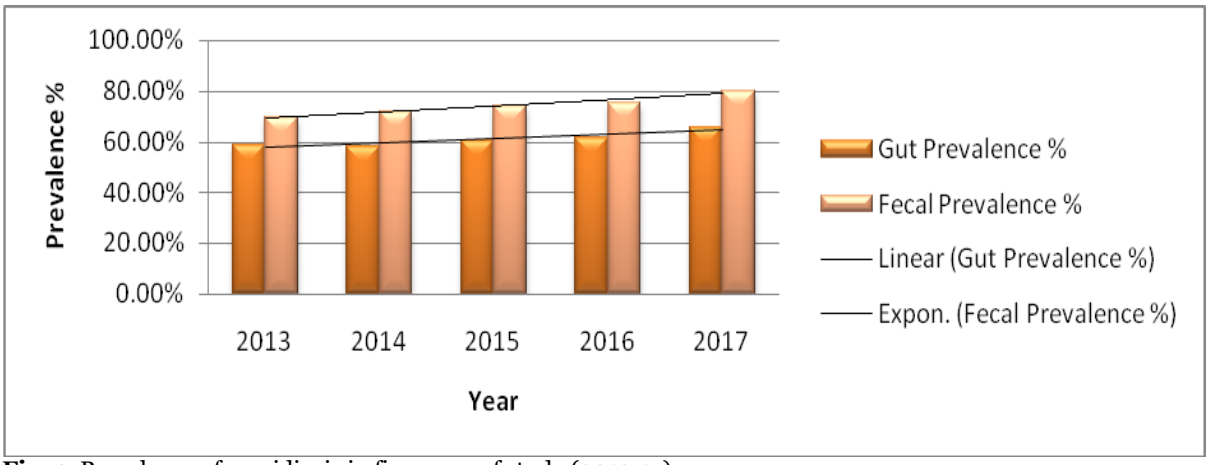

Fig. 1. Prevalence of coccidiosis in five years of study (2013-17).

Prevalence of coccidiosis in our study was moderately high from results of Bachaya *et al.* (2015), 65% rate of coccidiosis in broiler chickens in Muzaffargarh District and 66.7% (Lawal *et al.*, 2016). It was moderately low than Khan *et al.*, (2006) 71.8% prevalence of coccidiosis in broiler in District Rawalpindi. It was much high from the recorded prevalence of 43.89% by Awais *et al.* (2012) and Ahmed *et al.* (2003) 43.9 %.

In Lahore Sultana *et al.* (2009) reported 42.85 % prevalence of Eimeria infection in broiler flocks that was much low than observed in our study. Ayaz *et al.*, (2003) reported the (37.9%) prevalence of coccidiosis in research conducted in 2000-2001 in District Faisalabad-Punjab-Pakistan was disagreement to prevalence in this study.

In disagreement to our study number of studies conducted in different countries reported low prevalence, 39.6% in India (Sharma *et al.,* 2013), 31.7% in India (Nikam *et al.*,2012), 36.7% in Nigeria (Muazu *et al.*, 2008), 38.34% in Ethopia (Lobago *et al.*, 2005), 33.33 % in Jammu (Sood *et al.,* 2009), 54.3% in Turkey (Karaer *et al.*, 2012), 50% in Jordan (Al-Natour *et al.,* 2002), 31.8% in Iran (Gharekhani *et al.,* 2014), 31.8% n northeast Tunisia (Kaboudi *et al.*, 2016), 31.25 % in West Bengal (Bandyopadhyay *et al.* 2006), 17.1% in Sri Lanka (Rasiah and Kulasegaram 1972), 15-25% (Wickramasinghe *et al*, 1992), 64% in Southwest, 55.96% in Northwest, and 38% in Northeast regions in Iran (Nematollahi *et al.*, 2009; Hadipour *et al.*, 2011; Shirzad *et al* 2011).

Number of studies reported higher prevalence of coccidiosis that was not in agreement of our data was 75% in North Iran (Razmi and Kalideri, 2000), 92% in Romania (Györke *et al.*, 2013), 90% in Egypt (Amer *et al.*, 2010), 88.4% in Argentina (Mc-Dougald and Mattiello, 1997), 82.24% by Khelfa (1982), 80% by (Alamargot, 1987), 78.7% by (Lee *et al.*, 2010), 78% in Jordan (Al-Natour *et al.*, 2002) and 70.9% in Ethiopia (Elmira *et al.*, 2012).

Figure 7 Prevalence of coccidiosis in different season in years from 2013-2017 in field isolates. In summer highest outbreaks of coccidiosis were observed and winter shows least commonness of disease.

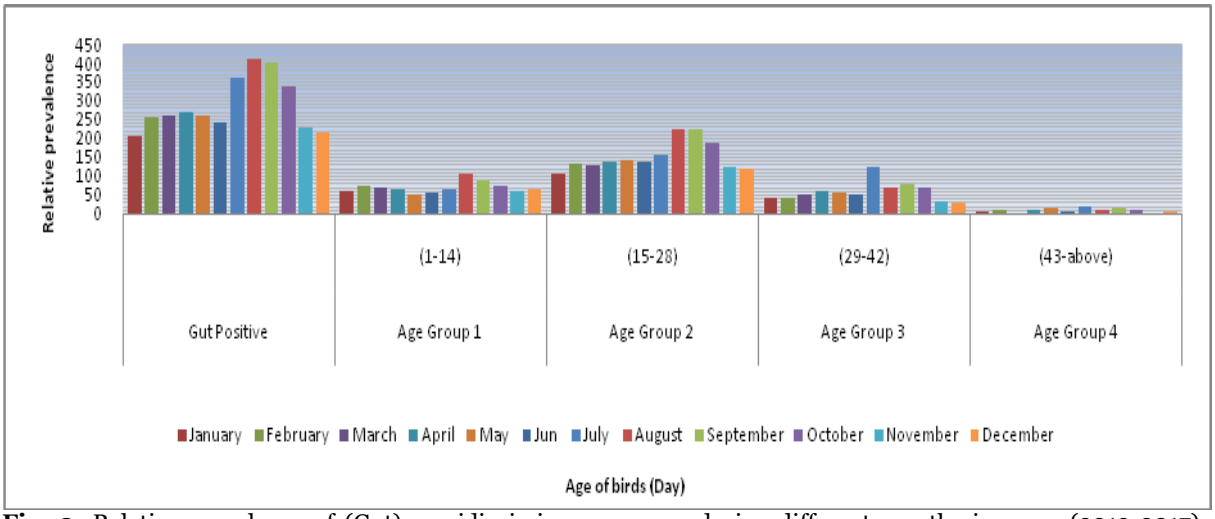

Fig. 2. Relative prevalence of (Gut) coccidiosis in age groups during different months in years (2013-2017).

On average 76.70% of screen samples were positive in summer season. In spring prevalence of *Eimeria* in different types of samples or fields isolate was 53.07% (fig 7). In summer highest outbreaks of coccidiosis were observed and winter shows least commonness of disease. On average 76.70% of screen samples were positive in summer season. In winter prevalence of coccidiosis was 52.23% gut, average 58.46%, 64.69% in fecal samples collected from local farms. Difference rate of infestation of poultry farms in different area could be due to variation in, weather condition in different geographical regions (Al-Natour *et al.*, 2002; Haug *et al.*, 2008; Nikam, *et al.*, 2012; Bachaya *et al.*, 2012; Amin *et al.*, 2014).

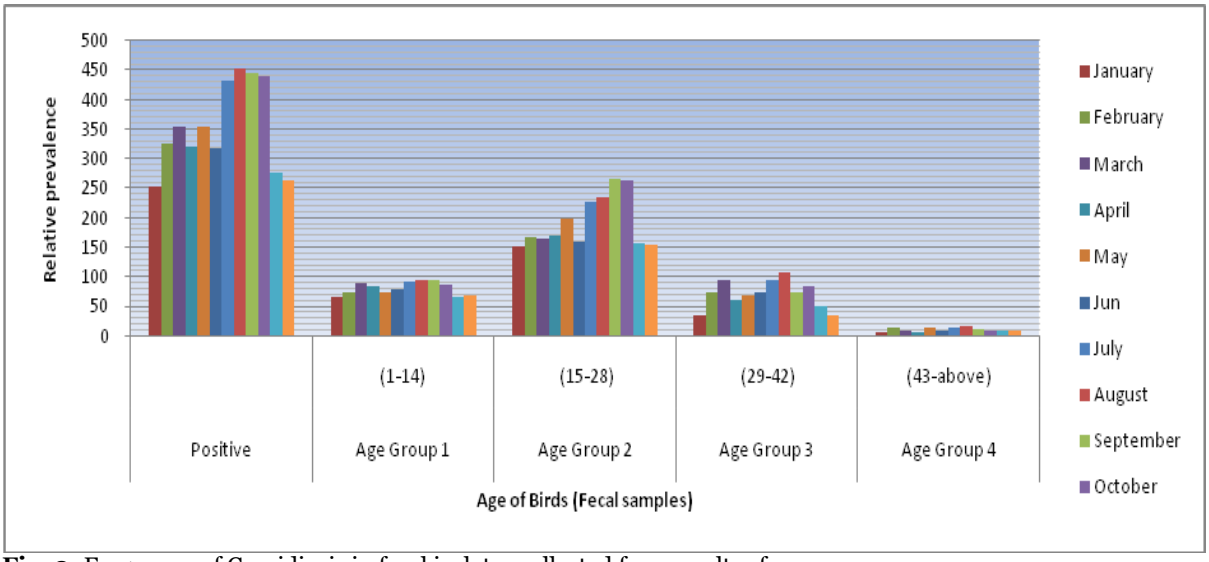

Fig. 3. Frequency of Coccidiosis in fecal isolates collected from poultry farms.

Figure 3 Monthly relative prevalence of coccidiosis in local isolates (2013-17). Highest numbers of positive samples found in august were 82.4% in gut, 90.6% in feces, in average 86.5%. In Jun least number of isolates was positive 49% in gut, in average 57%, 63.6% in feces (fig 6). Highest numbers of positive samples found in august were 82.4% in gut, 90.6% in feces, in average 86.5%. In Jun least number of isolates was positive 49% in gut, in average 57%, 63.6% in feces. Effect of the environment (temperature and moisture) has a great impact on the course and severity of coccidial infection (Shirley, 1992). Temperature, rainfall and humidity are main factor contributing to increase or decrease parasitic burden of different species of *Eimeria*, in intensive broiler industry (Awais *et al.*, 2012). Damp litter

serves as breeding sanctuaries of protozoan parasite that cause coccidiosis (Sultana *et al.*, 2009).

Prevalence of coccidiosis increased gradually in successive year maybe due to development of drug resistance against commonly used anti-coccidial agents. It revealed from gut examination and fecal test that prevalence of disease increased over the years (Abbas *et al.*, 2011; Györke *et al.*, 2013).

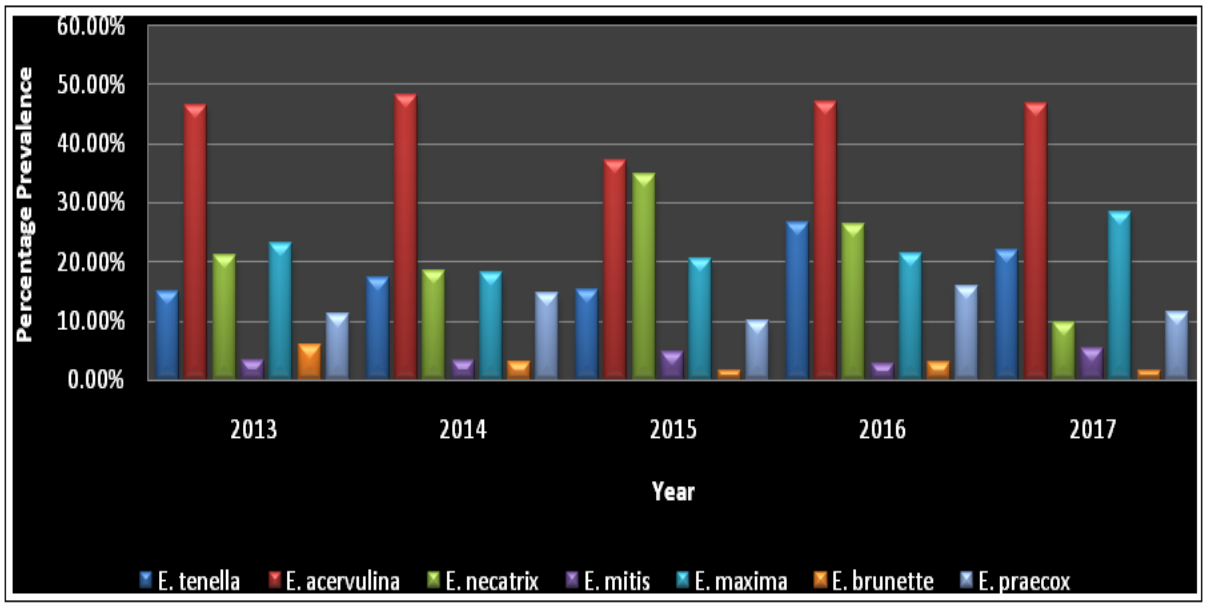

Fig. 4. Percentage prevalence (gut) of different species of Eimeria during study period.

Figure 4 Relative prevalence of (Gut) coccidiosis in age groups during different months in years (2013-2017). Commonness of *Eimeria* in intestinal samples was 60.91%. Chicks were divided into groups on the basis of age. Highest infestation rate was observed in age group 2 and lowest in age group 4.

There is highest rate of mortality in young birds (age 15-28 days). Lowest outbreak 49% was observed in Jun and highest frequency of 82.4 % was noted in august (fig 2). In very young age birds are more prone to disease but there is less load of parasite. With the passage of time age of birds increase, number of oocysts keeps on increasing, cause subclinical or clinical disease in birds that have immunodeficiency and when number of oocysts of *Eimeria* cross the threshold level in the bedding material, cause clinical disease and mortality in birds. Highest infestation rate in intestinal isolates and fecal dropping was observed in age group 2 (15-28 days) and lowest in age group 4(43-above days). Highest susceptibility of coccidiosis was observed in 15-28 days old flocks and least in flocks of 43 or more day old. In age group 3 birds getting older day by day and immune system of birds become stronger as compared to very young bird in age group 1.

When birds develop immunity against the disease, there are oocysts in the poultry house but no clinical sign of diseases. Age group 4 mostly Layers are kept for many weeks, are vaccinated against commonly occurring poultry disease in very early age. Age is one of the most principal factors in coccidiosis (Shirzad *et al.* 2011). Our results of age dependent prevalence are in agreement with William (1996), Mc Dougald *et al.* (1997), Al-Natour *et al.* (2002), Yunus *et al.* (2008), Amin *et al.* (2014) of birds, 3-4 weeks of age were infected with coccidiosis.

Sultana *et al.* (2009) reported highest susceptibility in same age but lowest prevalence was noted in 1-2 week old birds. The higher rate of coccidiosis was determined in >6 weeks' age groups; the significant relationship was observed in agreement with other researchers (Razmi and Kalideri, 2000; Khan *et al.*, 2006; Muazu *et al.*, 2008; Shirzad *et al* 2011; Elmira

et al., 2012). This different may be due to different in management system and breed of chicken (Ashenafi *et al.*, 2004).

Figure 5 Percentage prevalence (gut) of different species of *Eimeria* observed, during study period. Seven different species of *Eimeria* were detected in gut samples of dead bird collected from poultry sale point and poultry farms. *Eimeria acervulina* (44.87%) was found in highest prevalence followed by *E necatrix* and *E maxima*, while *E brunette* (2.88%) was in least frequency (fig 4). Figure 6 Distribution pattern of different species of *Eimeria* detected, in fecal samples (2013-17). Seven species of *Eimeria* were present in field isolates. *Eimeria acervulina* was in highest frequency and *E brunette* was in lowest percentage. Gradual increase in prevalence of some species was observed while other follows zigzag pattern (fig 5). Seven species of *Eimeria* were present in field isolates. *Eimeria acervulina* was in highest frequency and *E brunette* was in lowest percentage.

Eimeria species and their prevalence vary greatly within the different geographical areas (Macpherson, 1978; Chapman, 1997; Györke *et al.*, 2013; Zhang *et al.*, 2013).

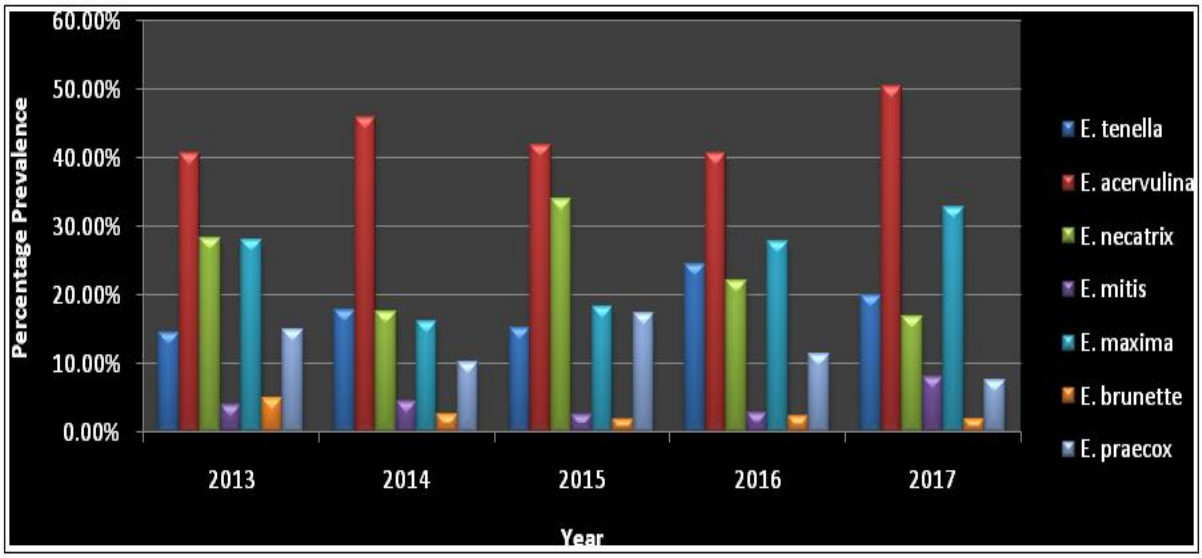

Fig. 5. Distribution pattern of different species of Eimeria in fecal samples (2013-17).

These results are in agreement with number of researchers. These results confirm the presence of all seven recognized species of *Eimeria* in chickens in the District Gujarat, Punjab, Pakistan in agreement with Republic of Argentina (Mattiello *et al.*, 2000).

Our study was in confirmation with (Lee *et al.*, 2010), who reported seven species of *Eimeria* were present and most species was *Eimeria acervulina* and least prevalent species were *E. brunette* and *E mitis*. Simultaneously seven species of *Eimeria* in poultry flocks were detected by Shirley, (1986), Al-Natour *et al.* (2002) and Ayaz *et al.* (2003), was in agreement to our results.

Findings of our research were in disagreement to number of studies in which less number of species was detected. Six species of *Eimeria* was recognized by (McDougald *et al.*, 1997), Williams (1995). Five species of *Eimeria* were reported by (Haug *et al* 2008; Sun *et al.*, 2009; Shirzad *et al.*, 2011; Sharma *et al.*, 2015). Four Species of *Eimeria* was mention in the findings by (Khan *et al.*, 2006; Györke *et al.*, 2013; Jamil *et al.*, 2013).

Figure 8 Relative prevalence of positive cases of disease found in different Management Conditions, (2013-17). Overall prevalence of coccidiosis in local poultry industry during 2013-17 was 67.57%.

The incidence of coccidiosis reveals clinical coccidiosis was 30.46% and 37.12% of subclinical

form of disease was observed. Prevalence of coccidiosis in different management condition was 14.82% good, 20.12% normal, and 32.66% of incidence was in poor managed poultry farms. Prevalence of coccidiosis was 56.02% in broiler and 11.54. % in Layer flocks. The prevalence in 36.35% rice hull and 31.22% in wood shaving poultry farms has been detected (fig 8).

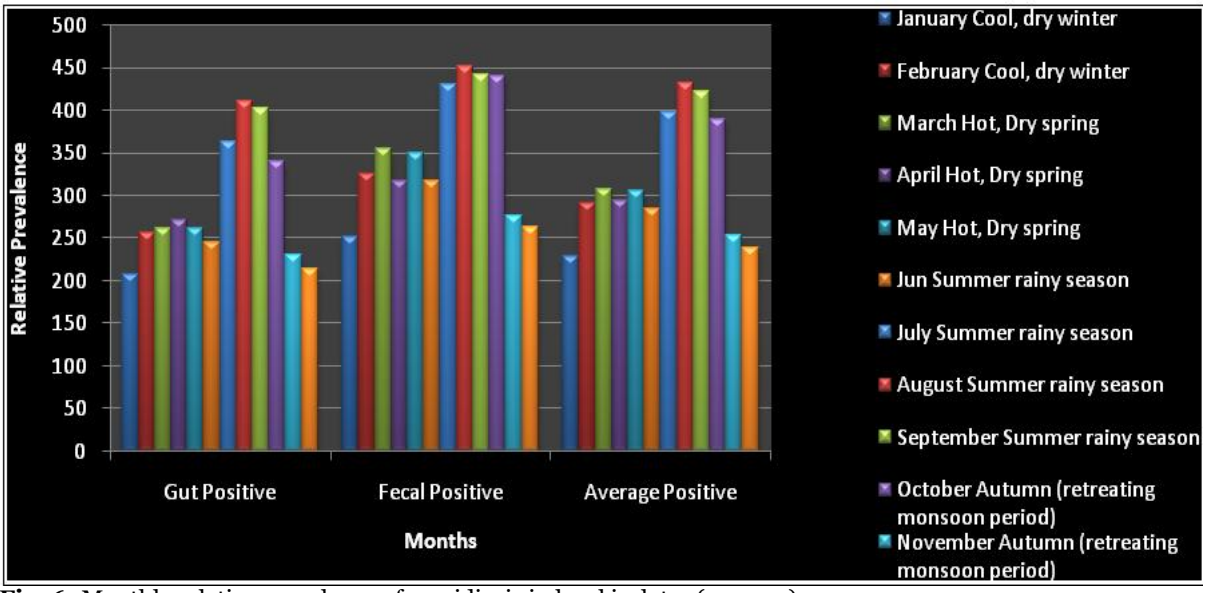

Fig. 6. Monthly relative prevalence of coccidiosis in local isolates (2013-17).

In clinical forms of disease, there is sign and symptom of disease and there is high mortality and mortality. In Subclinical form of disease, oocysts of parasite were present in field but there is no mortality on number of dead bird very low. Sub-Clinical coccidiosis was detected in 37.12% of poultry flocks while 30.46% of flocks have clinical infection. Finding of Shirzad *et al.*, (2011) are in confirmation to our results that large numbers of flocks have coccidiosis infestation in Subclinical farms. Sun *et al.*, (2009) reported subclinical form of coccidiosis in china that supports our findings. Coccidiosis always present in poultry farms because number of studies proved that through cleanout of poultry house during successive flocks cannot eliminate oocysts completely but reduction in outbreaks of coccidiosis (Razmi and Kalideri, 2000; Abbas *et al.*, 2011; Sharma *et al.*, 2013; Bachayha *et al.*, 2015).

Eimeria is omnipresent in each and every type of intensive poultry. Management of poultry farm affects the outbreak, incident and severity of disease. When strict cleanliness is maintained, visitor are not allowed, as in control shed temperature and humidity is auto control, utensil and properly clean, disinfectant are used and large number of bio security measure are followed. In good management condition, farmer tries their level best to reduce risk factors. Incidence observed in all type of poultry farms but as noted in this study that good management condition reduced the outbreak of disease. Prevalence of coccidiosis in different management condition was 14.82% good, 20.12% normal, and 32.66% of incidence was in poor managed poultry farms. Poor management conditions have important role in development of clinical coccidiosis. Contaminated and leaking drinkers, feeders, poor ventilation, overcrowding, wet litter exacerbates mortality and morbidly (Ruff, 1993; Ashenafi *et al.*, 2004).

Prevalence of coccidiosis was different in Broiler and Layer. Broilers are for chicken meat and Layer flocks are kept for egg laying. Life period of broiler is very short as compared to layers. Layers are kept for many weeks while broilers on average are for 34 days. Vaccination against commonly occurring chicken is completed in very early age in layers, while very less

number of poultry farmer use vaccine in broiler. Prevalence of coccidiosis was 56.02% in broiler flocks and 11.54. % in Layer flocks. In our study 56.02% of total visited flocks were infected with coccidiosis.

Only 11.54 % of Layer flocks were infected with coccidiosis. Our results are within range of prevalence of *Eimeria* infection in broiler reported by Oikawa *et al.*, 1979, who reported that worldwide prevalence vary from less than 10% to more than 90% (Oikawa *et al.*, 1979). Value of prevalence of *Eimeria* infection in layer flocks, reported by 33.07% (Sultana *et al.* 2009) are much higher than current results. Coccidiosis was more prevalent in broiler than in layers (Jagadeesh Babu *et al,* 1974; Ghodasara *et al*, 1992).

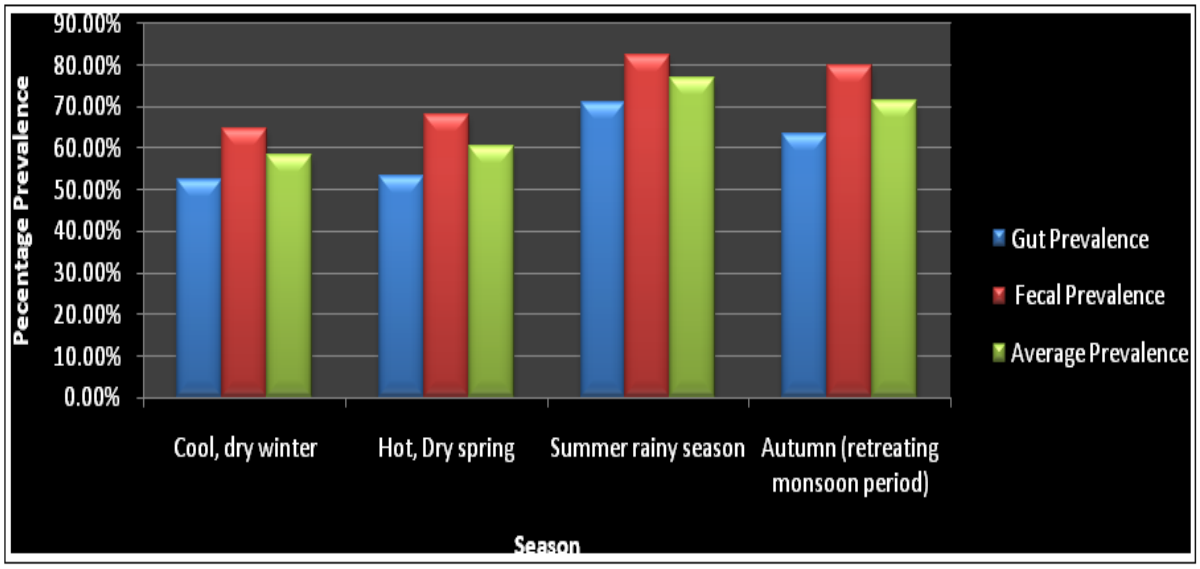

Fig. 7. Prevalence of coccidiosis in different season in years from 2013-2017 in field isolates.

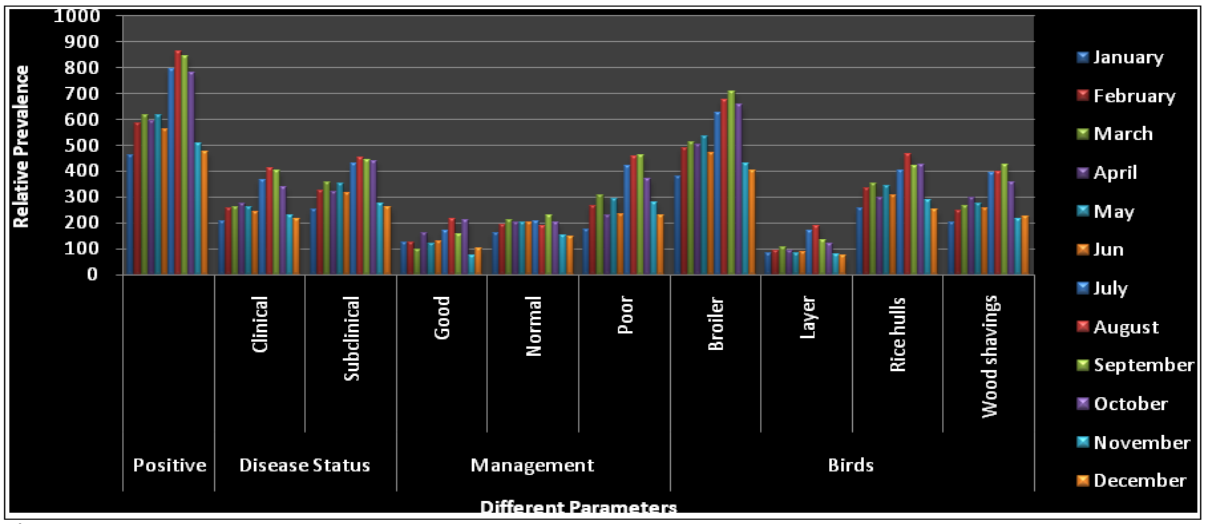

Fig. 8. Relative prevalence of positive cases of disease in different Management Condition (2013-17).

Rice hull and wood shavings are commonly used bedding material used in poultry farm. When results of samples collected from different poultry farms having rice hull bedding and wood shaving was collected, it reveal that there are more incident of coccidiosis in poultry farms having Rice hull as bedding material then poultry farms having wood shavings bedding material. Materials used as bedding material have different properties.

The prevalence in 36.35% rice hull and 31.22% in wood shaving poultry farms have been detected. Poultry farm having different types of floor in have different rate of *Eimeria* infection.

Soiled floor have crack that can retain oocyst, and humidity in bedding material help in sporulation of oocysts, increase infection rate in flocks. Cemented floor are easy to wash and less number of oocyts remain after washing of floor.

We reported that poultry farm using rice hull as bedding material become more rapidly and retain more oocysts, that subsequently become infective and more infection rate was observed in flocks. Our results are in according with number of researchers that littered floors (Baines BS, 1972; Fraser *et al.,* 1998; Marta *et al.,* 2013) provided good breeding grounds for protozoa as the y are mostly damp with the 'excreted' wastes (faeces) of the birds.

Conclusion and Recomendation

More outbreaks of coccidiosis were due to humid litter and due to buildup of oocyts in litters. Significantly higher outbreaks were detected in young age, because of less protection from immune system and buildup of oocysts in litter. More outbreaks of disease and mortality were detected in successive flocks. Type of litter material, time duration, season, humidity, temperature, successive flocks, education level of farmer's, use of same chemical as anti-coccidial and disposal of dead birds are other critical factors. Both clinical and sub-clinical coccidiosis retards the growth of flocks and cause huge economic loss to farmers. More infestation of *Eimeria* was observed in broiler as compared to layer. There is need to develop Epidemiological Database of *Eimeria* for suitable and timely control of coccidiosis. Epidemiology study of coccidiosis help to design model to predict rate of development of resistance against commonly used drugs in local population of *Eimeria*.

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Conflict of Interest

In this study no conflict of interest is found among authors and society.

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