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Epidemiological study on poultry coccidiosis: Prevalence, species identification and post mortem lesions in grower chicken in Kombolcha, North-Eastern Ethiopia

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A cross sectional study was conducted in Kombolcha Poultry Farm Enterprise to determine the prevalence, assessment of the risk factors and to identify the pathological abnormalities due to different species of *Eimeria* in 582 chickens of age 21 to 100 days, kept under deep litter management system from December, 2013 to March, 2014. The study involved fecal examination, post mortem examination, gross lesion examination, mucosal scraping examination and identification of *Eimeria* species. A statistically significant difference (p=0.000) was noted among the different age groups of grower chickens with the highest prevalence (73.1%) at 91 to 100 days old age group and the lowest (10.3%) in 21 to 30 days old age group. Out of 90 chicken subjected for post mortem examination, 58% (n=52) have showed gross pathological lesions in different parts of intestine. Gross lesions recorded were higher in Kookook breed (62.1%); however, there was no statistically significant difference among breeds. There was a statistically significant difference (p=0.011) in different age groups for gross lesion and it was high (71.4%) in 81 to 90 days old chickens and lower (22.2%) in 21 to 30 days old. Five *Eimeria* spp. were identified with *Eimeria brunetti* and *Eimeria tenella* which recorded most frequently prevalence of 17.8 and 12.2%, respectively, as single infections. Finally, it is concluded that the risk factors that are associated with coccidiosis should be taken into account in designing the prevention and control regimen. It was advised to design and implement strategic prophylaxis against coccidiosis than therapeutic approach, and conduct continuous coccidiosis monitoring via regular litter oocyst counts and taking appropriate measures accordingly.

Key words: Coccidiosis, *Eimeria* species, Ethiopia, Kombolcha, poultry, prevalence.

INTRODUCTION

Ethiopia has estimated poultry of 42 million (CSA, 2010) accounted to be 60% of the total chicken population of

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East Africa, playing significant role in human nutrition, and as source of income (Mekonnen et al., 1991). Poultry production in Ethiopia is categorized into traditional, small and large-scale orientated sectors, which is based on the objective of the producer, the type of inputs used, and the number and types of chickens kept (Alemu, 1995). The rural poultry sector constitutes about 99% of the total chicken population and managed under the traditional village poultry production systems (Tadelle et al., 2003).

One of the main constraints for the development of commercial poultry production is development of disease conditions (Alamargot, 1987), which can have devastating effects particularly on intensive production. Indeed, commercial poultry consist of exotic birds selected for their capacities in producing eggs and meat; and because of this selection, these animal are much more susceptible to diseases than the traditional backyard poultry (CIRAD, 2005).

Different diseases have been diagnosed or suspected in commercial poultry in Ethiopia, leading to economic loss and these are Newcastle disease, coccidiosis, salmonellosis, chronic respiratory disease and nutritional deficiencies (Alamargot, 1987; Nasser, 1998). But there is little information about the prevalence of these diseases and their direct impact on poultry production (CIRAD, 2005). Coccidiosis is endemic in Ethiopia, causing great economic losses, particularly in young growing birds, in different production systems (FAO/LIRI, 1995). For example, in deep-litter intensive system, prevalence rates of 50.8% (Fessessework, 1990), 48.2% (Methusela, 2001), and 38.34% (Lobago et al., 2005) were reported in Debrezeit and its surroundings, Debrezeit and Addis Ababa, and Kombolcha, respectively. Apart from causing disease and losses, sub-clinical infections (mild infections without showing symptoms) cause reduced feed conversion. Since feed expenses form some 70% of the cost of producing broiler chickens, the economic impact of coccidiosis can be immense (Jordan et al., 2002; Vegad, 2004).

Quantitative losses due to coccidiosis in Ethiopia are not well documented, but Methusela (2001) and Methusela et al. (2004) have reported that coccidiosis contributes to 8.4 and 11.86% loss in profit in large and small scale farms, respectively.

Coccidiosis still continues to be one of the most economically important, but still wide spread disease of poultry in-spite of advances made in prevention and control through immunomodulation, chemotherapy, management, nutrition (Graat et al., 1996; Pangasa and Singla, 2007) and genetic selection (Jordan et al., 2002; Vegad, 2004).

The epidemiology of coccidiosis is a timely issue to be established for determining the potential risk factors and species causing the diseases, and subsequent design of prevention production system, agro-ecology and level of and control regimen, which is suiting the local management (Sandhu et al., 2009).

Kombolcha Poultry Farm Enterprise has introduced Kookook, Isabrown and Lohman breeds for multiplication, evaluation and dissemination to different level of producers in semi-intensive and backyard poultry production systems in Ethiopia. Therefore, this research was conducted to address coccidiosis in Kombolcha Poultry Farm Enterprise with the following objectives.

(1) To determine the prevalence of poultry coccidiosis
(2) To assess the risk factors associated with the disease
(3) To determine different species of *Eimeria* causing coccidiosis in the farm, and
(4) To assess the pathological abnormalities due to different species of *Eimeria* in poultry in Kookook, Isabrown and Lohman breeds.

MATERIALS AND METHODS

Study area

This study was conducted in Kombolcha Poultry Farm Enterprise, Kombolcha, South Wollo, North-Eastern Ethiopia, located 380 km North of Addis Ababa, the capital city. Kombolcha is at an altitude of 1864 m above sea level, situated at 11°7' N latitude and 39°44' E longitudes. The size of the farm is 7.5 hectare. The area has a bimodal rain fall, with a three year annual average rain fall of 1038 mm, annual mean temperature of 18°C and a relative humidity from 23.9 to 79% (ARARI, 2008).

Study population

The study was conducted from December 2013 to March 2014 on three breeds, Kookook, Lohman and Isa brown of chicken in Kombolcha. The first study group was out in dual purpose type Kookook breed having the age of 21 to 100 days. The study animals are grouped into breeds (Kookook, Lohman, and Isa brown) and age groups (21 to 30, 31 to 40, 41 to 50, 51 to 60, 61 to 70, 71 to 80, 81 to 90 and 91 to 100 days).

Housing and birds management

The Kombolcha Poultry Farm Enterprises is a deep litter large scale intensive poultry farm with 7.5 hectare land cover area. Currently, the farm has 13 functional poultry houses from which 6 were used for rearing of 20,212 grower chickens and the remaining 7 houses were for raising of parent stocks, during the study period. Four of the rearing houses have an area of 307 m^2 and the rest two houses have 207 m^2 each. Each of the four parent stock houses has an area of 350 m^2 and the remaining three houses have an area of 307 m^2. The farm has flock sizes ranging from 2180 to 4190 per rearing house and 1170 to 2000 per raising parent stock houses. Standard feeding with commercial available ration were followed.

The health management was based on prevention which comprised of vaccination, medication, bio-security and sanitation (cleaning and disinfection). In these farm vaccines, three types of diseases were given; these are Newcastle disease vaccine, which were given in three rounds at days 1, 18 and 42 of age. Gumboro (Infectious Bursal Disease) vaccination was also given in two rounds at 21st and 28th days of age. The third vaccine which was given in the farm is fowl pox vaccine; given at 56 to 60 days old bird.

Anticoccidial drug (Amprolium 20% powder) was by 3 types of dosage system, 30 g per 100 L of drinking water, 60 g per 100 L of drinking water and 120 g per 100 L of drinking water for prevention, mild outbreak and severe outbreak, respectively, for 5 to 7 consecutive days (carried out twice in this study population). Antibiotics like oxytetracycline 20% powder, at a dosage of 0.5 g per 1 L of drinking water (for prevention) and 1 g per 1 L of drinking water (for treatment) was given for 5 to 7 consecutive days.

Sample size determination

The sample size was determined based on the formula recommended by Thrusfield (1995).

$$n = 1.96^2 \times P_{exp} (1- P_{exp})/d^2$$

where n = sample size required, P_{exp} = expected prevalence, d = desired absolute precision.

Since the prevalence of coccidiosis in these breeds of chicken in Kombolcha Poultry Farm Enterprise had not been studied earlier, 50% expected prevalence rate was assumed. A 95% confidence interval and 5% desired absolute precision was used (Thrusfield, 2005). Though the calculated sample size was 384, to increase precision, it was strived to double the sample size, and a total of 582 birds were included in the study.

Study design and methods

Proportional number of birds from different breeds was randomly selected, and sampled for fecal examination. Birdsat different age groups from each breeds of grower chicken were included in the sample to reach the required size for prevalence estimation. For gross lesion examination and species identification, birds were randomly selected from fecal sample positive birds whereby it was attempted to include different age groups in the samples.

Fecal examination

The selected birds were given identification number by permanent markers and kept separately in selection guard. The fecal samples were collected from the upper surface of the litter immediately after dropping of the feces by the selected bird. Then samples were processed in the laboratory immediately and oocyst examination was done. After fecal examination, there was an observation of chickens found in the selection guard and some selected chickens coprologically positive were subjected for post mortem examination. During sampling for post mortem examination age and breed were considered as factor of interest. Oocysts in each faecal sample of chicken were detected by using flotation technique using saturated Sodium Chloride Solution (MAFF, 1982; Conway and McKenzie, 2007).

Post mortem examination

Post mortem examinations on selected coprologically positive chickens were conducted following the procedure by Long and Reid (1982) and Conway and McKenzie (1991, 2007). After selecting the sample of chickens from coprologically positive chickens, they were transported to postmortem room in the farm. Chickens were sacrificed by cervical dislocation using the technique by Zander (1991). The examination was performed on a daily basis and the finding (major gross lesions associated with coccidiosis) of each age group were registered.

Gross lesion examination

Examination of the serosal surface of unopened intestines for lesions was done after being freed from mesentery. After opening of intestine with scissors, extending from the duodenum to the rectum, including caecum, all intestinal walls were examined for gross pathological changes. The intestinal portions were divided into five sections, the duodenum, jejunum, mid intestine (above and below the yolk sac diverticulum), the lower part (distal ileum and rectum) and caecum. The lesions were considered positive when there was a minor to major abnormalities like (enlargement, petechia, reddening, thickening, ballooning, hemorrhage (bleeding), caecal core, whitish spot), and were considered negative when there were no gross abnormalities.

Mucosal scraping examination

Small scraps were taken from different segments of intestine and put on the slide and diluted with saline then covered with cover slip and examined under microscope first with (10x magnification and proceeded to 40x magnification) appropriate light and recorded oocyst shape and size of oocyst by using micrometer.

Eimeria species identification

Identification of *Eimeria* spp. was based on the combination of observations on the nature of gross pathological lesions, the site of infection, and the size and shape of the oocyst according to Long and Reid (1982), key for coccidia species identification.

Data analysis

Data was entered and managed in Microsoft Excel worksheet and descriptive statistics was utilized to summarize the data. The data was analyzed using the latest version of SPSS 20 statistical software package. Pearson’s Chi square test was used to measure statistical significance of results. In order to consider a result to be statistically significant, 95% CI and p-value < 0.05 was considered.

The point prevalence is calculated for all data by dividing positive samples by total number of examined samples and multiplied by hundred. The association between the prevalence of the disease and risk factors is assessed by Chi-square. A statically significant association between variables is considered to exist if the computed p-value is less than 0.05.

RESULTS

Prevalence of coccidiosis in different breeds, age groups and months of the year

From 582 fecal samples examined from three different breeds, the overall prevalence was 48.5% (n=282). Breed bases prevalence was 52.7, 35.0, and 42.0% in Kookook, Isabrown and Lohman, respectively (Table 1). There was statistically significant difference (p = 0.005) in the

Table 1. Breed and age level prevalence of coccidiosis in grower chickens which were collected in different months of the year.

Risk factor		No. of sample taken	No. of positive cases	Prevalence (%)
Breed	Kookook	402	212	52.7
	Isa brown	80	28	35.0
	Lohman	100	42	42.0
Total		582	282	48.5
Age	21-30	145	15	10.3
	31-40	80	32	40.0
	41-50	80	54	67.5
	51-60	103	65	63.1
	61-70	52	34	65.4
	71-80	45	28	62.2
	81-90	25	16	64.0
	91-100	52	38	73.1
Total		582	282	48.5
Months	December	173	88	50.9
	January	269	128	47.6
	February	60	24	40.0
	March	80	42	52.5
Total		582	282	48.5

In breed: χ^2 = 10.417, df= 2; p value = 0.005; In age: χ^2 = 131.503, df = 7; p value = 0.000; In month: χ^2 =2.726, df = 3; p value = 0.436.

prevalence of coccidiosis among different breeds.

The age level prevalence in 21 to 30, 31 to 40, 41 to 50, 51 to 60, 61 to 70, 71 to 80, 81 to 90, 91 to 100 days were 10.3, 40.0, 67.5, 63.1, 65.4 62.2, 64.0, and 73.1%, respectively. The lowest coccidiosis cases were recorded at the age of 21 to 30 days, 10.3% and the highest numbers of coccidiosis cases (73.1%) were recorded at the age of 51 to 60 days (Table 1). The prevalence in different age groups was found to be statistically significant.

In this study, the samples were collected within four months of the year, from December to March. The highest prevalence of coccidiosis was recorded in March (52.5%) and the lowest prevalence was in February (40%) (Table 1), however, there was no statistically significant difference (p = 0.436) in prevalence among the months of the year.

Gross lesions occurrence in different breeds, age groups and months of the year

Post mortem examination revealed gross lesion in 58% (n = 52) of birds of three different breeds. The gross lesions commonly identified included enlargement, ballooning, hemorrhage, intestinal intussusceptions, petechial hemorrhage, thickening, white spots and core (caecal). On breed bases, 62.1, 40.0 and 54.5% lesions were recorded in Kookook, Isa brown and Lohman, respectively (Table 2). There was no statistically significant difference (p=0.221) on occurrence of gross lesion among different breeds.

The age specific distribution of gross lesions were found to be 22.2, 56.3, 63.6, 58.8, 75, 50, 71.4 and 60% in 21 to 30, 31 to 40, 41 to 50, 51 to 60, 61 to 70, 71 to 80, 81 to 90, and 91 to 100 days of age groups, respectively. There was lowest lesion prevalence (22.2%) in age group 21 to 30 days and the highest (75%) in age group of 61 to 70 days (Table 2). There was no statistically significant difference (p=0.11) in the gross lesion occurrence in different age groups of birds.

The month bases distribution of gross lesions were 50, 65, 50 and 54.5% in December, January, February, and March months, respectively. There was statistically non-significant difference in the occurrence of gross lesion across the months of the year.

Eimeria species identified in different breed, age groups and months of the year

Five *Eimeria* spp.: *Eimeria acervulina, Eimeria maxima,*

Table 2. Occurrence of gross lesion in different breeds, ages and months of the year.

Risk factor		No. of sample taken	No. of lesion positive cases	Prevalence (%)
Breed	Kookook	58	36	62.1
	Isa brown	10	4	40.0
	Lohman	22	12	54.5
Total		90	52	58
Age	21-30	9	2	22.2
	31-40	16	9	56.3
	41-50	22	14	63.6
	51-60	17	10	58.8
	61-70	8	6	75
	71-80	6	3	50
	81-90	7	5	71.4
	91-100	5	3	60
Total		90	52	58
Months	December	20	10	50
	January	40	26	65
	February	8	4	50
	March	22	12	54.5
Total		90	52	58

In breed: χ^2=5.725, df= 4; p value = 0.221; In age: χ^2=28.758, df = 14; p value = 0.11.

Table 3. Distribution of different *Eimeria* species in different breeds.

Species of *Eimeria*	Frequency of *Eimeria* at different breeds			Total
	Kookook	Isa brown	Lohman	
E. acervulina	5 (8.6)	1 (10)	1 (4.5)	7 (7.8)
E. maxima	4 (6.9)	0 (0.0)	2 (9.1)	6 (6.7)
E. necatrix	7 (12.1)	1 (10)	1 (4.5)	9 (9.7)
E. tenella	8 (13.4)	1 (10)	2 (9.1)	11 (12.2)
E. brunette	11 (19)	2 (20)	3 (13.6)	16 (17.8)
E. acervulina + E. maxima	3 (5.2)	0 (0.0)	2 (9.1)	5 (5.6)
E. tenella + E. acervulina	4 (6.9)	1 (10)	2 (9.1)	7 (7.8)
E. tenella + E. brunetti	10 (17.2)	2 (20)	6 (27.3)	18 (20)
E. tenella + E. necatrix	6 (10.3)	2 (20)	3 (13.6)	11 (12.2)
Total	58 (64.4)	10 (11.1)	22 (24.4)	90 (100)

Breed level: χ^2=10.382, df =18; p value = 0.919.

Eimeria necatrix, Eimeria tenella, and *Eimeria brunetti* were identified as single and/or mixed infections, due to *E. tenella* co-infection with other species, with prevalence as shown in Table 3.

Single infection occurrence recorded was 54.4% (n = 49) and mixed infections for 45.6% (n = 41) of the total infection from the total of 90. The distribution of *Eimeria* spp. on different breeds of chickens was found to be statistically non-significant (p=0.919), although the distribution was higher in Kookook breed (64.4%), and lower in Isa brown breed (11.1%) (Table 3).

E. brunetti and *E. tenella* occurred most frequently with prevalence of 17.8 and 12.2%, respectively, as single infections, whereas the prevalence of *E. necatrix, E. acervulina* and *E. maxima* were found to be 9.7, 7.8 and6.7%, respectively, as single infection (Table 3).

DISCUSSION

The results of this study showed that coccidiosis is still a major problem in Kombolcha Poultry Farm Enterprise, with overall prevalence of 48.5% (n = 282) in grower chickens of 21 to 100 days old. The highest numbers of coccidiosis cases (52.7%) were found in Kookook breed. The prevalence of coccidiosis in Kookook breed is significantly higher than the other two breeds (p=0.005). This finding is in line with the finding of Taylor et al. (2007) where the occurrence and incidence of the disease is also to a great extent affected by the type of chickens reared and breeds sensitivities to infection. The finding of this research is in agreement to previous reports in other parts of Ethiopia, where prevalence of 50.8% (Fessessework, 1990) and 48.2% (Methusela, 2001) were recorded in deep litter system of management. In contrast, the 48.5% prevalence of this study is higher as compared to the previous study done in the same area reported; 38.34% (Lobago et al., 2005), 22.3% (Abadi et al., 2012) in age of 1 to 60 days of dead RIR and WLH chickens, respectively.

The high prevalence of coccidiosis in this study may be ascribed mainly to the age group of birds which were sampled. That is, in the previous study, chickens age was from day 1 to 60, in which most of the time, the coccidia populations take time to build clinically significant levels where outbreaks usually occurs when birds are between 3 and 8 weeks of age (Fanatico, 2006). But, the study done by Abadi et al. (2012) included the lower age groups (1 to 20 days) which are rarely infected by coccidiosis; so, may decrease the overall prevalence of coccidiosis. The difference in prevalence in the current and previous study may also be due to breed difference, previous study had been done on WLH breed.

Another important factor which could lead to high prevalence was high stocking density (ranging from 16 to 18 birds/m^2) at the poultry farm; but normally it should be between 10 and 15 birds/m^2 (Hamet et al., 1982). This finding was also in agreement to the production systems operating under high density conditions (that is, greater than 15 birds/m^2) increases the risk of greater competition for feed and water, which there by increases litter contamination, buildup of oocysts and litter moisture (Hamet et al., 1982).

The prevalence of coccidiosis in different age groups was found to be statistically significant ($p<0.05$), with the highest being in 91 to 100 days (13 to 14 weeks). This finding was not in concordance with the findings of the other researchers (Methusela, 2001; Lobago et al., 2005; Abadi et al., 2012). This could be due to the vulnerability of the birds in the age group 91 to 100 days during the study period in the farm, due to irrational use of anticoccidial drugs.

On temporal bases, coccidiosis occurred higher in January (50%) and the lowest lesions were recorded in February (7.7%). This is not in agreement to the survey of chicken coccidiosis; the monthly prevalence of *Eimeria* infection was higher in July (94.4%) compared to other months and June was the lowest (57.9%). This could be due to the local weather conditions and the management practices in the farm, and also, not all months of the year were assessed by the current study.

In the present study, 58% birds showed gross lesions, higher number of lesion being in Kookook breed (69.2%) and lower in Isa brown breed (7.7%), with no statistically significant difference. This variation may be due to high variation of number of sample taken from different breeds despite the fact that there could still be breed level variation. This should be further investigated.

The occurrence of gross lesion was the highest (26.9%) in the age of 41 to 50 days and the lowest (3.8%) in age of 21 to 30 days, with statistically significant difference among age groups. This finding is in agreement to Lobago et al. (2005) and Abadi et al. (2012), who reported that the age group between 41 and 50 days is the age at which the occurrence of coccidiosis is at peak.

In the current study, five *Eimeria* spp., *E. acervulina, E. maxima, E. necatrix, E. tenella,* and *E. brunette* were identified. These species were also investigated by Abadi et al. (2012), in the same area. Age group 41 to 50 days (6 to 7 weeks) was the age group which showed the highest prevalence of overall distribution of *Eimeria* spp. (23.9%). This finding could be attributed to the fact that *E. tenella* infection which generally affects chickens below 10 weeks of age with maximum prevalence in 4 to 8 weeks old chicks (Mc Dougald, 2003); and it is rarely observed in the chickens below 2 weeks of age (Chauhan and Roy, 2008). The infection with *E. acervulina* and *E. maxima* are seen at 3 to 6 weeks of age and then *E. necatrix* at 8 to 18 weeks of age, whereas *E. brunetti* is seen both early and late (Mc Dougald, 2003).

The present study showed that *E. tenella* (34.1%) and *E. brunetti* (24.6%) occurred most frequently, with no statistically significant difference of species distribution with respect to age (p=0.216). This finding is not in agreement to previous reports in Ethiopia. *E. acervulina* was the most prevalent coccidial species (Ashenafi, 2000; Metusela, 2001; Dereje, 2002); and *E. brunetti* (45.3%) and *E. tenella* (40.8%), were found most frequently (Lobago et al., 2005). This variation could be due to the difference in breed of poultry or the different management systems of the study population.

Conclusion and recommendations

Coccidiosis is still a major problem in the Kombolcha Poultry Farm Enterprise, with increasing prevalence in grower chickens. Managerial problems such as high stocking density, poor quality and management of the litter, leaking waterers, inadequate cleaning, the presence of birds of different ages and different breeds in a single house, the absence of vaccines and non-strategic prophylaxis against *Eimeria* were the main reasons and predisposing factors for the higher prevalence of clinical coccidiosis and occurrence of outbreak in the age between 90 and 100 days than the young ones in the farm.

The identified *Eimeria* species, causing coccidiosis in Kombolcha Poultry Farm Enterprise were *E. tenella, E. necatrix, E. brunetti, E. acervulina,* and *E. maxima*. This shows that all economically important *Eimeria* species are present and will continue to be a threat to the farm unless otherwise appropriate measure are taken.

Poultry coccidiosis is a major burden to poultry producers and veterinary health professionals from time to time by changing its mode of occurrence and with variation in the conditions of the different management system and level. Hence, poultry coccidiosis is demanding a lot of interventions and further research, to develop economical and sustainable prevention and control strategies. It has to be worked at least to bring it to acceptable level if not possible to get rid of it in Kombolcha poultry farm and other farms in the country.

Moreover, special attention should be given to the most susceptible age groups and breeds, as these are the potential risk factors associated with poultry coccidiosis, in order to minimize the losses associated with coccidiosis in poultry. The economical incursion by coccidiosis can be minimized through improving management level, which minimizes the predisposing factors at strategic time, will be effective mechanism particularly in intensive production system.

Therefore, based on the above conclusion the following recommendations are forwarded:

(1) Management procedures which limit contamination of litter should be paid with high emphasis, keep litters dry through proper installation and management of watering systems.
(2) Appropriate stocking density should be maintained and raising of multiple age and breeds in the same house should be avoided.
(3) Strategic prophylaxis and treatment against *Eimeria* should be developed and implemented on the bases of the level of management in the farm, probably strategic inclusion of anticoccidials in diets or water should be sought for Kombolcha in specific.
(4) Continuous coccidiosis/iasis monitoring should be conducted via regular monitoring of litter oocyst counts and appropriate measures should be taken accordingly.
(5) Further research has to be conducted to assess natural relative resistance among different breeds in different management system to coccidiosis under natural infection, particularly at the young age groups between Kookook, Isa brown and Lohman breeds.
(6) Vaccination against coccidiosis should be sought for in the future, particularly for highly susceptible breeds and in predisposing management systems with appropriate timing.

Conflict of interest

The authors declare that there is no conflict of interest.

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