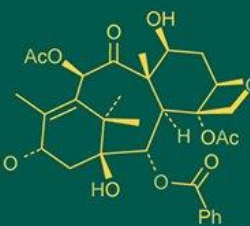
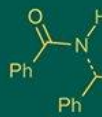
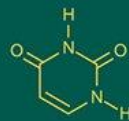
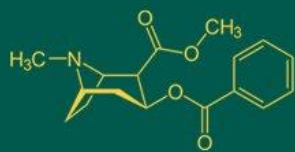


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Cryptosporidiosis in pig farms around Tirupati, Andhra Pradesh

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Abstract

Cryptosporidium is a protozoan parasite infecting pigs, particularly young piglets causing gastrointestinal disturbances such as diarrhoea, reduced growth rate and economic losses in swine production. Due to its zoonotic nature there is an increased risk of transmission from pigs to humans. The present study was aimed to study the farm wise prevalence of *Cryptosporidium* spp. by collecting pig faecal samples among nine farms in Tirupati dist, Andhra Pradesh. Samples were screened using the modified Ziehl-Neelsen staining (mZN) method. Of the 9 farms, 6 farms (66.6%) showed the *Cryptosporidium* spp. infection with prevalence ranging from 0-33.33%. Risk factors like age and sex were also determined. Higher prevalence was observed in younger piglets. While in case of sex majority of the farms showed higher prevalence in females rather than males. So the prevalence of *Cryptosporidium* oocysts in pig's farms indicates the zoonotic risk of transmission.

Keywords: *Cryptosporidium* spp., pigs, pig farms, risk factors, mZN Staining

Introduction

Cryptosporidium is one of the potential zoonotic protozoan parasite belonging to the phylum Apicomplexa and family *Cryptosporidae* responsible for severe gastro intestinal illness in humans and animals. It is an obligate intracellular parasite capable of infecting diverse group of vertebrates (Fayer, 2010) [5]. Ryan *et al.* (2021) [11] reported the presence of 44 species and more than 120 genotypes worldwide, in humans four genotypes and 19 *Cryptosporidium* species were identified. Food and Agricultural Organization ranked *Cryptosporidium* as 5th most important food borne parasite (out of 24 parasites) with public health significance. Infection with different *Cryptosporidium* species in pigs were noticed more frequently especially *C. suis*, which affects younger pigs below 5 weeks old (Ryan *et al.*, 2004) [10] and *C. scrofarum* mostly affects post weaned piglets, aged over 4 weeks (Kvac *et al.*, 2014) [6]. The transmission route is directly through faeco oral route, by contact with infected animal or human or indirectly through contaminated food or water which acts as major vehicles of transport (Pumipuntu Piratae, 2018) [9]. Cryptosporidiosis in humans results in transient diarrhoea with an incubation period of seven days and individuals with weakened immune function experience impaired nutrient absorption, severe diarrhoeal illness accompanied by dehydration with gradual weight loss and extra intestinal infection in pulmonary and biliary tract (CDC, 2024) [1].

In India, only few reports were available with variable prevalence rates and in Andhra Pradesh, prevalence of cryptosporidiosis in pigs has not yet been much reported. Hence the present study was conducted to know the epidemiological prevalence of *Cryptosporidium* spp. in pigs, in and around Tirupati.

Materials and methods: The present research was performed in the Department of Veterinary Public Health and Epidemiology, College of Veterinary Science, Sri Venkateswara Veterinary University, Tirupati.

Sample collection and microscopic examination

Pig faecal samples were collected from nine farms in Tirupati district of Andhra Pradesh each from different location and details of the samples number was mentioned in (Table 1). Freshly

voided faecal samples were collected into 30 ml sterile container by wearing disposable hand gloves from pigs aged between 30 days to 2 years for the detection of *Cryptosporidium* species. These samples were kept in cold

chain conditions during transport to the department of Veterinary Public Health and Epidemiology for laboratory examination.

Table 1: Details of pig faecal samples collected during the study

Farm No.	Location of sample collection (m-mandal)	No. of pig faecal samples collected
1.	Rayalacheruvu (Ramachandrapuram-(m))	38
2.	SVVU Tirupati (Tirupati Urban)	21
3.	Oddimetta Venugopalapuram (Renigunta-(m))	4
4.	Paata Mangalam (Tirupati Urban)	20
5.	Gudimallam (Yerpedu-(m))	20
6.	Musalipedu (Yerpedu-(m))	9
7.	Rompicharla (Chinnagottigallu-(m))	22
8.	Venkatagiri (Venkatagiri-(m))	52
9.	Bondevandlapalle (Yerravaripalem-(m))	26

Immediately after reaching to the lab the samples were processed and staining was performed by using modified Ziehl- Neelsen method. The procedure was carried out as per the protocol described by Fayer and Xiao, (2008) ^[4] with slight alterations in the preparation of smear (Venu *et al.*, 2013) ^[15]. Under 1000x magnification the oocysts appeared

as red/pink spherical bodies against blue-green background showing differences in staining intensity between the oocysts.

Results and Discussion: After mZN staining, the slides were observed under microscope with 1000x magnification. The oocysts of *Cryptosporidium* appear as pink/ dark red spherical bodies with clear halo around them (Figure 1).

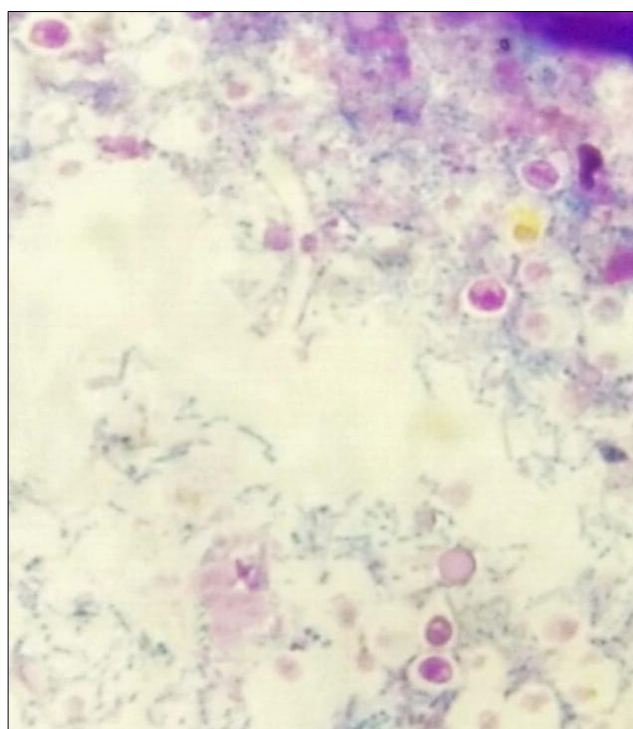


Fig 1: Oocysts of *Cryptosporidium* spp. (1000x)

From the total nine farms, farm 6 showed a highest prevalence of 33.33% (3/9) followed by farm 5 with 25.0% (5/20) and farm 1 with 23.68% (9/38). Farm 8 showed a medium prevalence of 15.38% (8/52), followed by farm 9 with 11.54% (3/26) and farm 7 with 9.09% (2/22). No positives were recorded in farm 2, 3, 4 each with 0% prevalence. The overall farm wise prevalence ranges from 0-33%. (Figure 2). Of the total 9 farms 6 farms showed the infection with 66.66% prevalence. This range was lower between 0% -17.5% reported by De Felice *et al.*, (2020) ^[3]

and recorded prevalence in 85% of farms compared to the current study. In contrast (Schubnell *et al.*, 2016) ^[13] reported 18.9% farms were infected with *Cryptosporidium* which is lower than the present investigation. In similar to the present study almost similar prevalence with 52.9% of farms was reported positive by Nguyen *et al.*, (2013) ^[8]. In Worldwide, the farm prevalence's ranges from 1and 100% and the differences in prevalence's were associated with management system, sample size and diagnostic approach (Zheng *et al.*, 2019) ^[18].

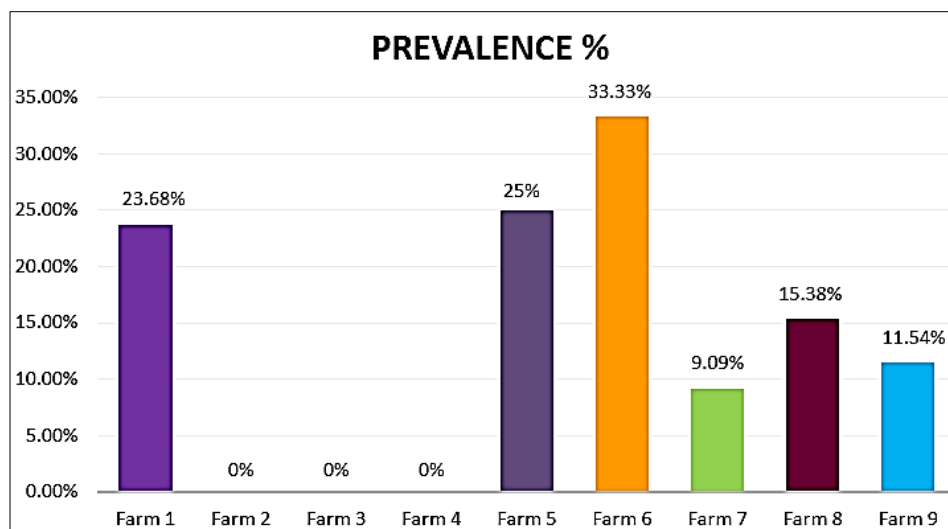


Fig 2: Prevalence of *Cryptosporidium* spp. among different farms by mZN

In current investigation farm no.5, 6 showed highest infection rate while farm no.2, 3, 4 showed no prevalence of infection. The reason for highest prevalence in those farms might be due to the unhygienic conditions in the farm with poor biosecurity measures and higher infection in younger ones was observed in the current study may be due to the transmission from sows to their piglets. The higher number of farms showed more percentage of *Cryptosporidium* spp. infection in females.

Farm 2, 3, 4 showed 0% prevalence as those farms maintained good hygienic conditions and sometimes the animal may be in pre-patent phase or post recovery phase where the oocyst shedding is absent.

Effect of risk factors on farm wise prevalence of *Cryptosporidium*spp. by mZN method: Age wise prevalence

among different farms: The age-wise prevalence of *Cryptosporidium*spp. varied across farms (Table 2). Farm 1 showed infection in all age groups, with higher prevalence in 2-3 and 3-6 months. Farms 2, 3, and 4 showed no infection. In Farm 5, infection was mainly observed in 2-3 and 3-6 months, while in Farm 6 it was predominant in 1-2 months. Farm 7 showed infection in both younger and older (>6 months) animals. Farm 8 had higher prevalence in the 1-2 months group, and Farm 9 mainly in animals older than 6 months. Overall, prevalence tended to be higher in younger animals, though variation existed between farms. In support to this multiple studies reported higher prevalence in younger piglets (Vitovec *et al.*, 2006^[16]; Saurez-Luengas *et al.*, 2007^[14]; Rzezutka *et al.*, 2014^[12]; Yui *et al.*, 2014^[17])

Table 2: Age wise prevalence of *Cryptosporidium* spp. among different farms

Age /Production stage	Farm 1(%)	Farm 2 (%)	Farm 3 (%)	Farm 4 (%)	Farm 5 (%)	Farm 6 (%)	Farm 7 (%)	Farm 8 (%)	Farm 9 (%)
Pre weaned / Weaned piglets)	0% (0/1)	0% (0/21)	0% (0/0)	0% (0/5)	0% (0/0)	33.33% (3/9)	11.11% (1/9)	50% (4/8)	0% (0/6)
Post weaned piglets	35.7% (5/14)	0% (0/0)	0% (0/0)	0% (0/0)	40% (2/5)	0% (0/0)	0% (0/3)	0% (0/8)	0% (0/0)
Growers	23.07% (3/13)	0% (0/0)	0% (0/0)	0% (0/10)	20% (3/15)	0% (0/0)	0% (0/1)	11.53% (3/26)	0% (0/3)
Finishers	10% (1/10)	0% (0/0)	0% (0/4)	0% (0/5)	0% (0/0)	0% (0/0)	11.11% (1/9)	10% (1/10)	17.64% (3/17)

Sex-wise prevalence

Variation in prevalence was observed across farms. The highest prevalence in males was recorded in Farm 6 (50%), followed by Farm 1 (30.76%). In females, the highest prevalence was recorded in Farm 5 (50%), followed by Farm 1 and Farm 6 (20% each). Females showed higher infection than males (Figure 3). Similarly, Maurya *et al.*, (2013)^[7] also reported higher infection in female pigs than in male pigs by mZN staining.

Conclusion

The current investigation reported *Cryptosporidium* prevalence in farm wise across nine farms with overall prevalence ranges from 0-33%. Of the total 9 farms examined

for *Cryptosporidium* oocysts 6 farms showed the infection with prevalence of 66.6%.while higher prevalence is recorded in farm 5, 6 as *Cryptosporidium* has zoonotic potential the chance of getting infection from pigs to humans are higher and oocysts of *Cryptosporidium* are highly resistant to disinfectants and there is no effective treatment available for cryptosporidiosis in pigs and humans. To prevent the contamination in farms, farm hygiene should be maintained thoroughly and biosecurity measures should be followed strictly by pig handlers. The current study is the first research investigation to focus on the prevalence of *Cryptosporidium* spp. in domestic pigs in Andhra Pradesh. Further molecular studies were required to understand the epidemiology and its public health significance.

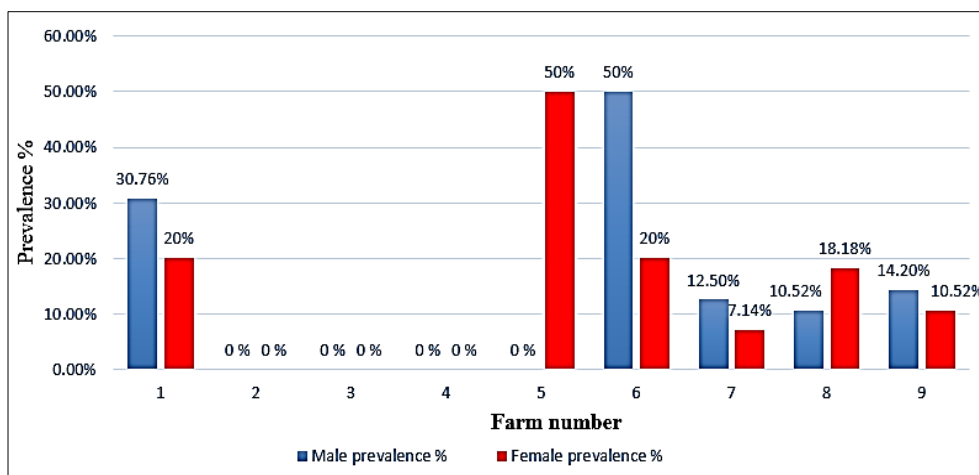


Fig 3: Sex-wise prevalence among different farms

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Conflict of interest

There is no conflict of interest.

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