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& Scientific Research
University of Al-Qadisiyah
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Study of intestinal protozoa in buffaloes

A Graduation Project Submitted to the Department Council of the Internal and Preventive Medicine-College of Veterinary Medicine/ University of Al-Qadisiyah in a partial fulfillment of the requirements for the Degree of Bachelor of Science in Veterinary Medicine and Surgery.

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((فَتَعَالَى اللَّهُ الْمَلِكُ الْحَقُّ ۗ وَلَا تَعْجَلْ بِالْقُرْآنِ مِنْ قَبْلِ أَنْ يُقْضَىٰ إِلَيْكَ وَحْيُهُ ۖ وَقُلْ رَبِّ زِدْنِي عِلْمًا))

صدق الله العظيم

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Certificate of Supervisor

I certify that the project entitled (**Study of intestinal protozoa in buffaloes**) was prepared by under my supervision at the College of Veterinary Medicine / University of Al-Qadisiyah. It is part of the requirements for obtaining a bachelor's degree in internal medicine and surgery sciences / internal medicine and preventive veterinary medicine

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/ / 2021

Certificate of Department

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Abstract

This study was conducted to diagnose the spread of stomach and intestinal parasites in buffaloes.

It was also observed that the infection rates were higher in the rainy and cold climates, compared to the warmer climates. Diarrhea was associated with infections.

Most of the cases of infection were accompanied by the emergence of symptoms of diarrhea in buffalo, which affects the health status of the affected animals and the rate of their consumption of feed, and consequently their production, growth rates, and the extent of benefit from the feed that are eaten by the buffalo. Therefore, this study was conducted to attract the attention of educators and veterinarians regarding the importance of infecting parasites.

Stomach and intestine.

Introduction

Buffaloes are an essential part of the economy in many countries and provide sustainable food in addition to being working animals. represent an important economic resource for several regions of the world , The worldwide population of water buffaloes has been estimated as 206 million in 2018, with most populations being found in Asia (97%); buffaloes represent a major contribution to the economy of these regions ,In Europe, buffaloes are mainly found in Italy, Romania, Bulgaria, Greece, Germany, United Kingdom, Macedonia, and Albania [1]. In some areas of southern Iraq, buffalo is one of the most important domestic livestock species as it is a source of dairy and meat for the local population, as well as manure for the Marsh meadows. With a total population of 40,000, the majority of water buffaloes in Iraq are reared in the Marshes of three southern governorates Thi-Qar, Missan, and Basra.Covering energy discombobulate herding many obstacles of facting the economic side for educators such as by intestinal protozoa that cause economic loss and symptoms manifested in poor growth, loss of appetite and digestive symptoms and caused economic losses result of the severity of the pathogenicity is leading to a lack of production of milk ,intestinal protozoan spread through pastures in all countries of the world because presence of carrier and causes heavy losses in the productive animals field [2].

identify the intestinal protozoa like Giardia , cryptosporidium and Eimeria (Coccidia) Which we will deal with in this study , these protozoan infections can cause serious production losses in this animal species[3].

Eimeria(coccidiosis)

Scientific classification	
Clade:	SAR
Infrakingdom:	Alveolata
Phylum:	Apicomplexa
Class:	Conoidasida
Order:	Eucoccidiorida
Family:	Eimeriidae
Genus:	Eimeria

[4]

Type species
E. zuernii and E. bovis.

[5]

Table 1. Prevalence of different Eimeria species in feaces of water

Eimeria are the major cause of diseases that affect the growth and development of buffaloes.

The *Eimeria* life cycle has an exogenous phase, during which the oocysts are excreted into the environment, and an endogenous phase, where parasite development occurs in the host intestine. During the endogenous phase, several rounds of asexual reproduction, or schizogony take place, after which the sexual differentiation of gametes and fertilisation occurs. Parasite transmission occurs via the oral-fecal route. Infections are common in farming environments where many animals are confined in a small space.[6]

Life Cycle of Coccidia

Bovine coccidia have stages both within the host animal as well as outside. The developmental stages in the animal give rise to a microscopic egg (called an oocyst), which is passed out in the manure (Figure 1).[7]

Under proper conditions of temperature, moisture and oxygen, the oocyst develops within three to seven days and is now capable of infecting cattle. At this stage, the oocyst contains eight bodies (called sporozoites), each of which is capable of entering a cell in the animal's intestine after the oocyst is eaten.[7]

When a sporozoite enters a cell, it changes into a meront and divides many times, producing up to 100,000 offspring called merozoites. The numbers produced depend on the species of coccidia involved. Each offspring, in turn, may enter another intestinal cell. This cycle is repeated several times. Because of this multiplication of parasite stages, large numbers of intestinal cells are destroyed.6

Eventually, the cycle stops and sex cells (male and female) are produced. The male fertilizes the female to produce an oocyst, which ruptures from the intestinal cell and is passed in the manure. Thousands of oocysts, each containing eight sporozoites when mature, can be passed in the manure of an infected animal.[7]

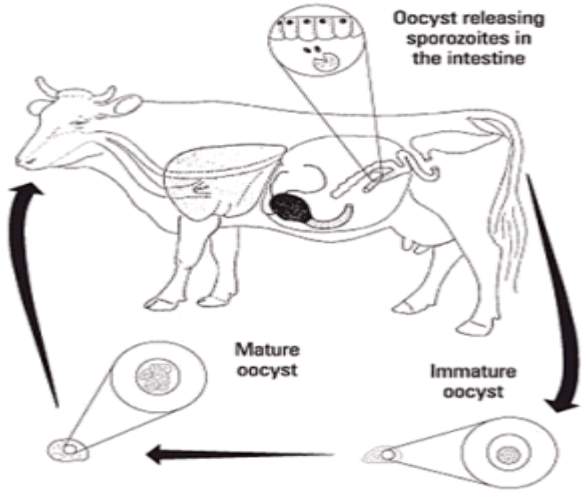

Figure 1. Life cycle of coccidia in cattle.

Mechanism of infection

The parasite enters via contaminated food, water or surfaces contaminated infective (sporulated) oocysts (fecal-oral-route). In the gut environment of the animal the oocysts hatch and releases 8 zoites. The zoites undergo two asexual cycles (schizogony). The first cycle produces many small schizonts inside the cells of the lamina propria, while the second generation of schizonts multiply in the cells of the epithelium. The second generation of schizonts undergo a sexual cycle (gametogony). The multiplication growth of new oocysts cause the destruction of mucosal cells which coincide with diarrhea in the animal. The time from ingestion of the parasite to the first signs of disease (prepatent period) is 16–21 days. The disease usually last 5–15 days (patent period). Oocysts can be observed in a microscope measuring 23–43 µm x 17–23 µm, ovoid shape, having a double-layered wall with a micropyle in the narrower end.[8]

Pathogenesis

The susceptibility of the host to these parasitesdepends on the age, genetic predisposition, innate or acquired immunity, stress level, manipulation, location of the parasite in the intestinal epithelium, and the number of endogenous stages, as well as climatic and other abiotic factors. Among the factors related to coccidia infection that influence the evolution and clinical characteristics of coccidiosis, theEimeria species are associated to the number of cells destroyed by ingested oocysts, which depends on the number of merogonic generations and the number of the merozoites produced by each meront, the infecting dose, the location of the parasite within the tissue cells of the host, the degree of reinfection, the size of the endogenous stages and the viability and virulence of the *Eimeria*species.[9]

clinical signs

The most typical syndrome of coccidiosis is chronic or subclinical disease in groups of growing animals. Buffalos calves may appear unthrifty and have fecal-stained perineal areas. In light infections, cattle appear healthy and oocysts are present in normally formed feces, but feed efficiency is reduced. The most characteristic sign of clinical coccidiosis is watery feces, with little or no blood, and animals show only slight discomfort for a few days. Severe infections are rare.[9]

Diagnosis

Coccidiosis is diagnosed by clinical signs, fecal examination by flotation or smear, and by postmortem examination. Clinical signs usually occur about 17 days after ingestion of oocysts. By the time clinical signs occur, the damage is far advanced, and the coccidia life cycle in the animal is completed. The history frequently includes a preceding stressful event in the animal's life. It must be noted that coccidia can be found in the feces of normal healthy cattle and diagnosis must rule out other diseases such as BVDV, salmonellosis, internal parasites and toxicities.[9]

Treatment

• Treatment of both the above pathogenic species of coccidia is with a sulphonamide, such as sulphadimidine or sulphamethoxypyridazine, given orally or parenterally and repeated at half the initial dose level on each of the next 2 days. • Alternatively, decoquinate or a combination of amprolium and ethopabate may be used.[9]

• Sulphamezathine, mepacrine hydrochloride, amprolium.[10]

Prevention

Focuses on preventing fecal contamination of the buffalo's environment, feed and water. Preventive measures for confined cattle include:

Clean water tanks regularly, with more regular cleaning when new animals are introduced.

Clean feces from feed bunks before each feeding.

Clean and disinfect holding areas between groups of cattle. Drying and exposure to sunlight aids in the die-off of oocysts

Do not overcrowd animals.

Reduce manure buildup (regular scraping of pens).

If in stalls, provide adequate clean bedding.

Utilize coccidiostats in feed, water or salt as recommended by your veterinarian.[11]

Guardia (Giardiasis)

Scientific classification	
Domain:	Eukaryota
(unranked):	Excavata
Phylum:	Metamonada
Order:	Diplomonadida
Family:	Hexamitidae
Subfamily:	Giardiinae
Genus:	Giardia

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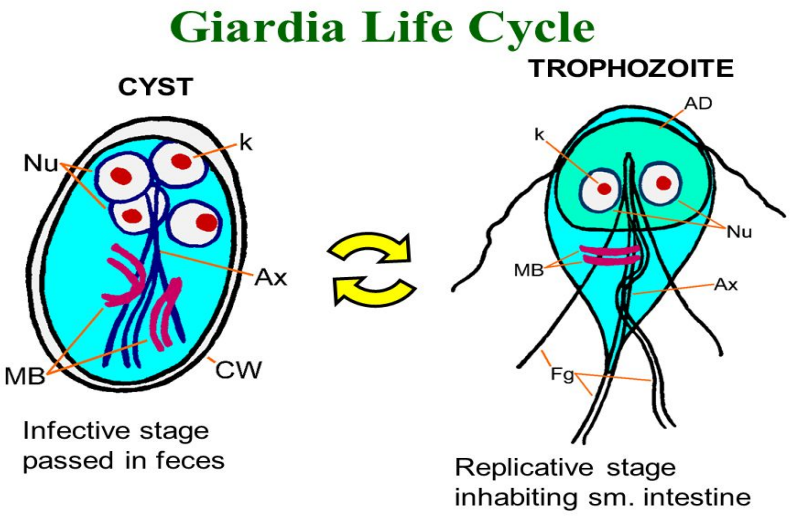
Type species	
species:	G. lamblia
	G. duodenalis

Giardiasis is a parasitic disease caused by *Giardia duodenalis* (also known as *G. lamblia* and *G. intestinalis*), *it's*a flagellated protozoan parasite that inhabits the small intestine in trophozoite and cystic form. There are number of reports of diarrhea and ill health associated with *Giardiasis* in buffalo calves[13].

Life cycle

G. duodenalis takes on two morphologically distinct forms during its life cycle. The replicative form is a motile pear-shaped cell that survives only in host small intestines called a trophozoite. Trophozoites swim through the intestinal mucus until they eventually adhere to the host intestinal epithelium. Adhered trophozoites then divide by binary fission, forming either more trophozoites or the non-replicative cyst stage.Cysts pass through the host large intestine and are shed in the feces.*G. lamblia* cysts are resistant to environment stressors, and can survive in the environment for weeks to months if kept moist. Cysts remain dormant until ingested by a host animal. In the new host, environmental conditions trigger the cyst to produce two trophozoites, which then attach to epithelial cells, starting the cycle anew.[14]



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Mechanism of infection

Giardia usually spreads when *Giardia duodenalis* cysts within feces contaminate food or water, which is later consumed orally. The disease can also spread between people and through other animals. Cysts may survive for nearly three months in cold water. It has also been associated with low weight gain, impaired feed efficiency and reduced carcass weight gain. Studies conducted in different geographical regions of the world have reported a variable prevalence (1-100%) in buffalos. It has been observed that buffalo claves have higher incidence of *Giardiasis* as compared to adult buffalos and intensity of cysts shedding was also higher in young calves.[16]

Pathogenesis

Giardia infections cause an increase in epithelial permeability, increased numbers of intraepithelial lymphocytes, and activation of T lymphocytes. Trophozoite toxins and T-cell activation initiate a diffuse shortening of brush border microvilli and decreased activity of the small-intestinal brush border enzymes, especially lipase, some proteases, and dissacharidases. The diffuse microvillus shortening leads to a decrease in overall absorptive area in the small intestine and an impaired intake of water, electrolytes, and nutrients. The combined effect of this decreased resorption and the brush border enzyme deficiencies results in malabsorptive diarrhea and lower weight gain. The reduced activity of lipase and the increased production of mucin by goblet cells may explain the steatorrhea and mucous diarrhea that has been described in *Giardia*-infected hosts. In particular, calves in resource-poor countries can be severely affected by G. duodenalis infections, which may lead to significant malabsorption, weight loss and growth retardation . The gastrointestinal manifestations include diarrhea, abdominal cramps, greasy stools, flatulence, epigastric tenderness, and steatorrhea accompanied by full-blown malabsorption syndrome. However, infection that is not associated with such overt symptoms occurs frequently, especially in resource-poor countries. These infections may often go unnoticed but epidemiological observations suggest that they are still associated with a malabsorption phenotype[17].

Diagnosis

The motile, piriform trophozoites (12–18 × 7–10 μm) are occasionally seen in saline smears of loose or watery feces. They should not be confused with yeast or with trichomonads, which have a single rather than double nucleus, an undulating membrane, and no concave ventral surface. The oval cysts (9–15 × 7–10 μm) can be detected in feces concentrated by the centrifugation-flotation technique using zinc sulfate (specific gravity 1.18). Sodium chloride, sucrose, or sodium nitrate flotation media may be too hypertonic and distort the cysts. Staining cysts with iodine aids identification. Because *Giardia* cysts are excreted intermittently, several fecal examinations should be performed if giardiasis is suspected (eg, three samples collected throughout 3–5 days). *Giardia* may be underdiagnosed, because the cysts are intermittently shed.[17]

Treatment

Fenbendazole (50 mg/kg/day for 5–10 days) effectively removes *Giardia* cysts from the feces of boffaloes. no adverse effects are reported, and it is safe for pregnant and lactating animals. This dosage is approved to treat *Giardia* infections.[18]

Prevention

Giardia cysts are immediately infective when passed in the feces and survive in the environment. Cysts are a source of infection and reinfection for animals, particularly those in crowded. Feces should be removed as soon as possible (at least daily) and disposed of with municipal waste. Infected buffaloes should be bathed to remove cysts from the hair. Prompt and frequent removal of feces limits environmental contamination. Cysts are inactivated by most quaternary ammonium compounds, steam, and boiling water.[18]

Cryptosporidium

Scientific classification	
(unranked):	Diaphoretickes
Clade:	TSAR
Clade:	SAR
Infrakingdom:	Alveolata
Phylum:	Apicomplexa
Class:	Conoidasida
Subclass:	Coccidia
Order:	Eucoccidiorida
Suborder:	Eimeriorina
Family:	Cryptosporidiidae
Genus:	Cryptosporidium Tyzzer, 1907

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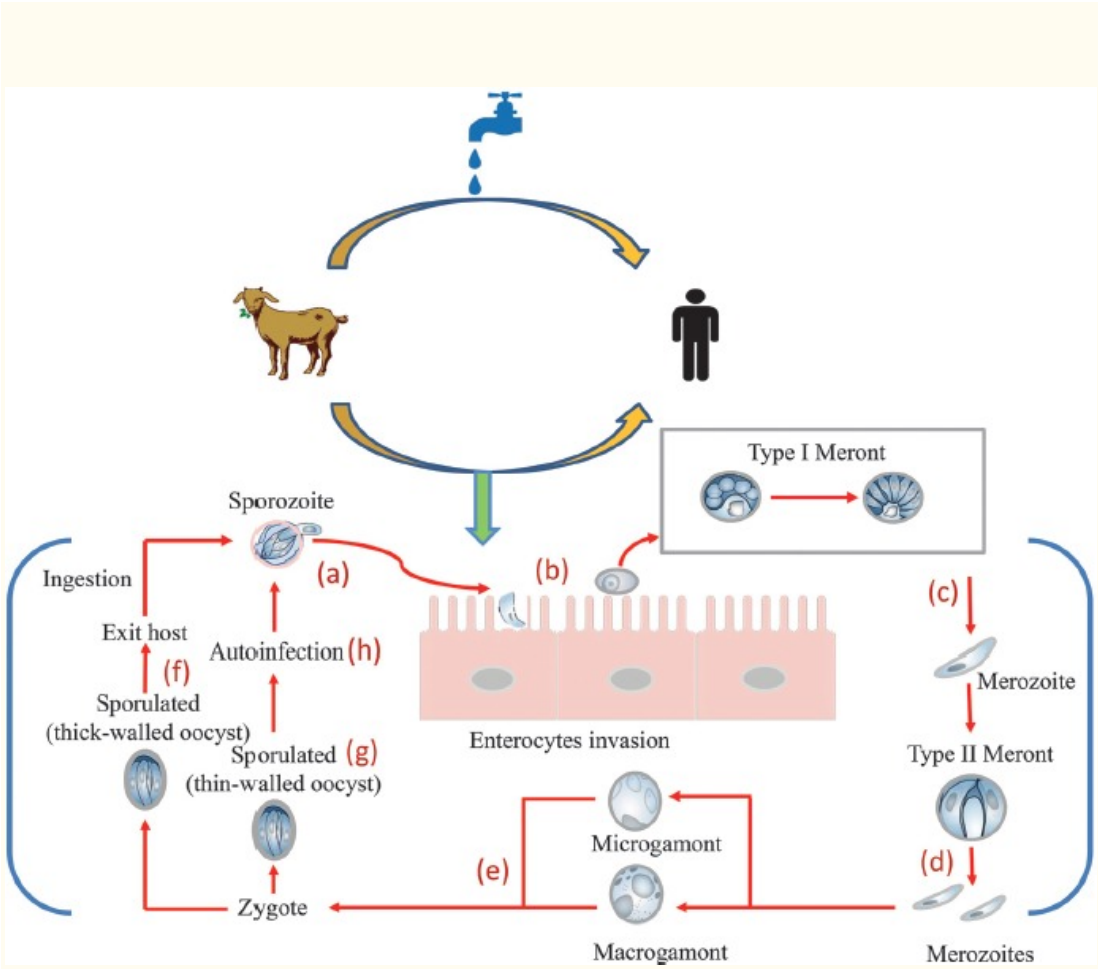
Species
C. parvum C. ryanae C. bovis C. anderson

Cryptosporidium is a microscopic parasite that causes the diarrheal disease cryptosporidiosis. Both the parasite and the disease are commonly known as "Crypto." The parasite is protected by an outer shell that allows it to survive outside the body for long periods of time and makes it very tolerant to chlorine disinfection. While this parasite can be spread in several different ways, water (drinking water and recreational water) is the most common way to spread the parasite. *Cryptosporidium* is a leading cause of waterborne disease among humans in the United States.[20]

There are many species of *Cryptosporidium* that infect animals, some of which also infect humans .protozoans belonging to the genus *Cryptosporidium* are frequent agents of gastrointestinal infection in humans, domestic animals, and other vertebrates. Three species of *Cryptosporidium* have been associated with infection in cattle. Two small-type oocysts, *C. parvum* and *Cryptosporidium bovis* , infect the small intestine. However, the larger type, *Cryptosporidium andersoni* that infects the abomasum has been implicated as a cause of reduced milk production in diary cattle[20].

Life cycle

Infection is initiated by ingestion of oocysts, each of which contains 4 sporozoites that hatch at the intestinal level, releasing infectious sporozoites. After excystation, these sporozoites are ingested into a modified host membrane separated from the cytoplasm by a dense layer; then, the location of parasites within the host is not intracellular but extracytoplasmic. Within the parasitophorous vacuole, the parasite undergoes asexual or schizogony reproduction, leading to the production of 8 merozoites within a type I meront.The merozoites can invade the neighboring epithelial cells and propagate the infection to other sites of the intestines. During this stage, the merozoites can undergo 2 distinct replicative cycles: an asexual stage characterized by multiplication of merozoites (type I meront) and production of thin-walled oocysts that autoinfect the host and/or a sexual stage with formation of type II meront, which, after differentiation in microgametocytes and macrogametocytes, will unite to form the zygote . The diploid zygote, through a process called sporogony, will form 4 sporozoites within thick or thin-walled oocysts. The thick-walled oocyst, protected by a resistant wall, after releasing in the feces is shed into the environment, ready to infect a new individual.[20]



Mechanism of infection

Crypto lives in the gut of infected humans or animals. An infected person or animal sheds Crypto parasites in their poop. An infected person can shed 10,000,000 to 100,000,000 Crypto germs in a single bowel movement. Shedding of Crypto in poop begins when symptoms like diarrhea begin and can last for weeks after symptoms stop. Swallowing as few as 10 Crypto germs can cause infection.[21]

Crypto can be spread by:

- Swallowing recreational water (for example, the water in swimming pools, fountains, lakes, rivers) contaminated with Crypto
    - Crypto's high tolerance to chlorine enables the parasite to survive for long periods of time in chlorinated drinking and swimming pool water
- Drinking untreated water from a lake or river that is contaminated with Crypto
- Swallowing water, ice, or beverages contaminated with poop from infected humans or animals
- Eating undercooked food or drinking unpasteurized/raw apple cider or milk that gets contaminated with Crypto
- Touching your mouth with contaminated hands
    - Hands can become contaminated through a variety of activities, such as touching surfaces or objects (e.g., toys, bathroom fixtures, changing tables, diaper pails) that have been contaminated by poop from an infected person, changing diapers, caring for an infected person, and touching an infected animal
- Exposure to poop from an infected person through oral-anal sexual contact.[21]

Pathogenesis

Cryptosporidia are obligate, intracellular coccidian protozoa that replicate in small-bowel epithelial cells of a vertebrate host.

After *Cryptosporidium* oocysts are ingested, they excyst in the gastrointestinal tract and release sporozoites, which parasitize gastrointestinal epithelial cells. In these cells, the sporozoites transform into trophozoites, replicate, and produce oocysts.

Two types of oocysts are produced:

- Thick-walled oocysts, which are commonly excreted from the host
- Thin-walled oocysts, which are primarily involved in autoinfection

The thick-walled infective oocysts are shed into the lumen and passed in stool by the infected host; they are immediately infective and can be transmitted directly from person to person by the fecal-oral route. Very few oocysts (eg, <100) are required to cause disease, thus increasing risk of person-to-person transmission.

When the infective oocysts are ingested by humans or another vertebrate host, the cycle begins again. Oocysts are resistant to harsh conditions, including chlorine at levels usually used in public water treatment systems and swimming pools despite adherence to recommended residual chlorine levels.

. Ingesting even a relatively small number of oocyts can result in disease. Infections result from the following:

- Ingestion of fecally contaminated food or water (often water in public and residential pools, hot tubs, water parks, lakes, or streams)
- Direct person-to-person contact
- Zoonotic spread[22].

clinical signs

Calves affected with Crypto are usually one to four weeks of age. These calves become weak and lethargic and present with loose to watery stool that may be mild or severe in intensity. Feces can contain mucus, blood, undigested milk, or bile. Tenesmus (straining to defecate) may be seen. Oocyst shedding typically begins with diarrhea and continues for a few days passed resolution of clinical signs. This is important to remember when determining the time to return previously isolated ill calves to a healthy herd.[23]

Treatment

There is no affective or approved treatment for Cryptosporidiosis. Morbidity is high with this disease but mortality is generally low. However, calves do need intensive supportive care. Sick calves should be housed in a clean, warm, and dry environment. They need fluid therapy to counteract and prevent further dehydration as well as electrolytes to replace those lost due to diarrhea. They also need nutritional support to give them energy to fight disease and repair their bodies. A recent study showed no clinical benefit to administering decoquinate as a preventative treatment for cryptosporidiosis.(Moore,D.)[23]

Prevention

Prevention of cryptosporidiosis requires

- Effective public water treatment
- Hygienic food preparation
- Special precautions during international travel
- Appropriate fecal-oral hygiene
- Thorough handwashing after contact with feces of humans and animals
- Not swallowing water when swimming in lakes, rivers, streams, ocean, swimming pools, and hot tubs
- Safer sex practices
- Taking special care when traveling to areas with poor sanitation[24].

Conclusion

Gastrointestinal parasitic infestation is one of the major obstacles for buffalo health in . The GI parasites were very the hot for the helminths to affect the buffalo. The infestation of trematode and nematode was moderate in year round. The protozoal infestation was also recorded moderate in all season. It's an preliminary work, further more study may require for the specific species wise detection of parasites and their virulent effects on the body of the buffaloes by sero-surveillance. It will be helpful for evaluate the economic losses of the farmers due to GI infestation of buffaloes and conduct the fruitful worm control plan.

RECOMMENDATION

1-We recommend using highly effective broad spectrum anthelmintics to the buffalo in every three months interval for proper deworming.

2- Effective public water treatment.

3- buffaloes should be bathed to remove cysts from the hair. Prompt and frequent removal of feces limits environmental contamination.

4- Clean water tanks regularly, with more regular cleaning when new animals are introduced.

Reference

1- FAOinAvailable:http:/www.fao.org/faostat/en/#data/QA.2020,Vol.Availabl e: http:/ www.fao.org/faostat/en/#data/QA. 2020(2020). , Borghese,A.25-28(2010).

2- Abid HS, Fazaa NA. Water Buffalo in the Iraqi Marshes. In Nature Iraq Status Report 2007. Sulaimani, Kurdistan, Iraq. (Accessed in 10 October 2019). http://www.natureiraq.org/ uploads/9/2/7/0/9270858/status_report_buffalothiqar

3- Razmi G.R. Hossini M. and Aslani M.R. (2003).Identification of tick vectors of ovine theilerosis in an endemic region of Iran .Vet .Parasitol .116:1-6.

4- https://en.wikipedia.org/wiki/Eimeria

5- -http://www.fao.org/3/ah847e/ah847e04.pdf

6- ThapaShrestha,U.etal.Effectofdewormingonmilkproductionindairycattleandbuffaloes

7- https://www.thebeefsite.com/articles/1088/coccidiosis-in-cattle/

8- *Lal K; et al. (2009). "Proteomic comparison of four Eimeriatenella life-cycle stages: Unsporulatedoocyst, sporulatedoocyst, sporozoite and second-generation merozoite". Proteomics. **9** (19): 4566–4576. doi:10.1002/pmic.200900305*

9- *Daugschies, A; Najdrowski, M (2005). "Eimeriosis in Cattle: Current Understanding". J. Vet. Med. B. **52** (10): 417–427.* Lima J.D. Coccidiose dos ruminantesdomésticos. *RevistaBrasileira de ParasitologiaVeterinária*, 13(Supl. 1): 9-13, 2004.

10- http://www.ndvsu.org/images/StudyMaterials/Parasitology/Coccidiosis-in-Cattle-and-

*11- Lassen, B; Østergaard, S (2012). "Estimation of the economical effects of Eimeria infections in Estonian dairy herds using a stochastic model". Prev. Vet. Med. **106** (3–4): 258–265.*

12- *https://simple.wikipedia.org/wiki/Giardia_lamblia-*

13- Esch KJ, Petersen CA (January 2013). "Transmission and epidemiology of zoonotic protozoal diseases of companion animals". *Clinical Microbiology Reviews*. **26** (1):58–85

14- Despommier DD, Griffin DO, Gwadz RW, Hotez PJ, Knirsch CA (2019). "Giardia lamblia". *Parasitic Diseases*(7 ed.). Parasites Without Borders.

pp. 11–20. Retrieved 3 June 2019.

15- https://www.google.com/url?sa=i&url=https%3A%2F%2Fslideplayer.com%2Fslide%2F10713565%2F&psig=AOvVaw1MpDW1GQcSIMZudtEjPL3d&ust=1622408962917000&source=images&cd=vfe&ved=0CAIQjRxqFwoTCJjKt4Ce4_ACFQAAAAAdAAAAABAa

16- Deshpande&Shastri, 1981; Oviedo *et al*., 1987; Nikitan*et al.*, 1991; Himonas*et al*., 1998

17- Cacciò SM, Thompson RC, McLauchlin J, Smith HV (2005) Unravelling*Giardia* epidemiology. TrendsParasitol 21:430–437

18- Rinaldi L, Biggeri A, Cringoli G (2006) Geographical information systems and remote sensing in veterinary disease mapping. CAB Rev PerspectAgri Vet SciNutr Nat Resour 1:1–16.

19- https://en.m.wikipedia.org/wiki/Cryptosporidium

20- Rehman, K., Javed, K., Tunio, M.T., and Kuthu, Z. H. (2009) Passive surveillance of Gastrointestinal Parasites in Buffaloes of MandiBahauddin and Gujarat district of the Punjab. The Journal of Animal & Plant Sciences. 19(1):17

21- Aurich J.E., Dobrinski I., Grunert E. Intestinal cryptosporidiosis in calves in a dairy farm. *Veterinary Record.* 1990;127:380–381.

22- https://www.vet.k-state.edu/docs/vhc/farm/ag-practice-updates/Crypto.pdf

23- Bouzid M, Hunter PR, Chalmers RM, Tyler KM. Cryptosporidium pathogenicity and virulence. *ClinMicrobiol Rev*. 2013;26(1):115–34.

24- Gharpure R, Perez A, Miller AD, et al: Cryptosporidiosis Outbreaks—United States, 2009-2017. *MMWR Morb Mortal Wkly Rep* 68:568-72, 2019.