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(Official Journal of Association of Public Health Veterinarians)

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Indian Journal of Veterinary Public Health accepts original research articles, short communications and reviews in Veterinary, Animal, Dairy and Fishery Sciences with special emphasis on Public Health, Microbiology, Parasitology, Pathology, Food Safety, Food Hygiene, Health Policy etc. An **original researcharticle** covers results of the primary research with precise description of methodology so that it can be reproduced in other laboratories and clear interpretation of the work, which has not been published elsewhere. A **short communication** is intended to communicate novel ideas and results in new and developing areas of veterinary, animal and fishery sciences, but is limited in the level of performed investigations and is thus insufficient to fulfil the requirements of a full paper. A **review** aims to provide an overview or a meta-analysis for an issue of great public health interest written by experts in the domain.

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Original Research article should be written within 8 to 15 manuscript pages (or 2,500 to 4,500 words), including tables and figures. Research articles should include a title page, an abstract with maximum 250 words, up to five keywords. The main text should include the following sections: introduction, materials and methods, results and discussion, conclusion, conflict of interest, data availability, ethical statement, author's contribution, acknowledgments, references, tables and figures.

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Acknowledgments: When it is considered necessary, acknowledgements should be made to the people, centers or bodies that have collaborated or supported to carry out the work in any form including fund support. Contributors who did not meet any one of the criteria for authorship should not be listed as authors, but they should be acknowledged. The source of funding for the study, if any, should be disclosed.

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Books (Vancouver style): Samanta I. Veterinary mycology. Springer India; 2015.

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Indian Journal of Veterinary Public Health
Department of Veterinary Parasitology
West Bengal University of Animal & Fishery Sciences
37, Kshudiram Bose Sarani, Belgachia,
Kolkata – 700 037. West Bengal, India.
Mobile: +91-9831147627/+91-8145882227.
E-mail: ijvphaphv@gmail.com

CONTENTS

Environmental Degradation, Farm Intensification and the Emergence of Zoonoses

Shekhar Pokhrel

Department of Veterinary Medicine and Public Health, Faculty of Animal Science, Veterinary Science and Fisheries, Agriculture and Forestry University, Rampur, Nepal

Abstract

Zoonotic diseases are the infections transmitted between vertebrate animals and humans naturally that have also become a significant threat to global public health. As humans and animals share ecosystems and habitats, the potential for the transmission of pathogens between species is a constant concern. The root cause for most of the emerging zoonoses is a change in pathogen and or host ecology. The increase in human and livestock population; global travel and trade; expansion of agricultural land; wildlife encroachment; intensification of farming systems; and non-therapeutic use of antimicrobials in the global food production system have brought a lot of changes in natural ecosystems that led to the emerging zoonoses. The threat of zoonotic diseases is a worldwide challenge that demands urgent attention and efforts from the global community. The interconnection among human health, animal health, environmental health and societal changes necessitates a holistic approach of 'One Health' to disease prevention and control.

Keywords: Zoonoses, Environment, Farm intensification, One Health

Introduction:

Human beings are always in a threat of infectious diseases that constitute more than half of zoonoses (Magouras et al., 2020). Zoonoses are diseases or infections that can be naturally transmissible between vertebrate animals and humans and are caused by either bacteria, virus, or parasites (Rahman et al., 2020). Zoonotic diseases are of several types. Some zoonotic pathogens are confined to animal reservoirs and human cases are infrequent like anthrax, rabies, and Nipah virus; whereas some are well adapted to both animal and human hosts like bovine TB, salmonellosis (Magouras et al., 2020). There are also some pathogens with animals as the main host and occasional outbreaks occur in humans but with the transmission chain that leads to extinction like monkey pox and hantavirus (Pastula and Tyler, 2022). Microbes are the most flexible lives on earth that can exist in extreme temperatures, acidity, and alkalinity. They can modify their genes according to the new environmental conditions and easily adapt to it resulting in a large number of hosts (Drew et al., 2021). Whenever a microbe is introduced in a new host or within a new geographical area and causes infection, it is termed as "disease emergence". An emerging disease has appeared in a population of a geographical area for the first time, or that may have existed previously but is rapidly increasing in incidence or geographic range (Magouras et al., 2020). The root cause for most of the emerging infectious diseases (EID) is a change in pathogen and / or host ecology. The increase of human and livestock population; global travel and trade; expansion of agricultural land; wildlife encroachment; and intensification of farming

systems have brought a lot of changes in natural ecosystems that led to the EIDs. About two-thirds of EIDs of humans are zoonoses such as Ebola virus, Nipah virus, avian influenza, severe acute respiratory syndrome (SARS) coronavirus, and Bovine Spongiform Encephalitis (Bloom and Cadarette, 2019). This study will give a short overview of the factors that have a role in the emergence of zoonotic diseases and their transmission.

Figure 1 shows the movement of harmful microbes between wildlife, domesticated animals and human beings where the arrows indicate the direction of flow (Jones et al., 2013). The disease can be transmitted from wildlife to peri-domesticated wildlife, and then spread to livestock or humans. It can also be transmitted directly from wildlife to humans or livestock. The direction and frequency of microbial flow depend upon the types or closeness of interrelationship between wildlife, livestock and human.
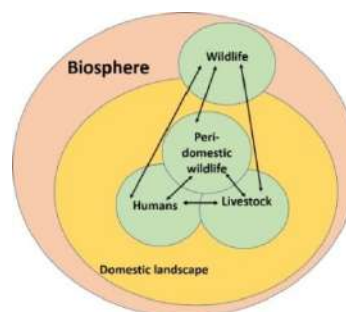
Figure 1: Flow of microbes at wildlife-livestock-human interface

The United Nations Department of Economic and Social Affairs have reported the current world population to be around 7.6 billion and predicted it to reach 8.6 billion by 2030;9.8 billion in 2050 and 11.2 billion in 2100 (United Nations, 2018). Increased human population and high demand for food have resulted in the expansion of agricultural land that promotes encroachment into wildlife habitats. This, in turn, may influence vector-borne disease transmission through altered vegetation, the introduction of livestock, development of human settlements, and loss of biodiversity. Studies have shown that most of the recently emerged zoonotic diseases like Nipah virus and human African trypanosomosis were originated in wildlife and the intensive interaction between human, livestock, and wildlife was the major cause of its transmission to humans (Magouras et al., 2020; Narkar, 2020; Pastula and Tyler, 2022).

Deforestation and wildlife encroachment:

Wildlife encroachment and the changes in the ecosystem have created many opportunities for genetic changes, pathogen spillover, and adaptation to new hosts. For example clearing of the rainforest in Amazon has driven many animals and insects out of the forest into human settlements (Guégan et al., 2020). The close interaction between wild animals and human has helped the pathogen to cross the barriers and enter the human host (Tajudeen et al., 2022).The emergence of bat related viruses in Australia like Hendra virus, Australian bat lyssa virus, and Menangle virus occurred due to the deforestation and loss of bat habitat as a result of agricultural expansion (Zohaib et al., 2023). Logging and mining in forest areas were related to the increase in exposure to the vectors of leishmaniosis, yellow fever, and malaria (Douine et al., 2022). Deforestation brings a change in the ecosystem and creates ecological niches favoring the multiplication and development of vectors as well as parasites. As for an example, the stagnant water puddles in forested areas have higher salinity and acidity as compared to puddles of deforested area which is restricting the growth of mosquitoes. However, the deforested water puddles favor the growth and development of certain *Anopheles* mosquito (Kahamba et al., 2022).

Agricultural technology:

Besides the encroachment of agriculture into wildlife, the rapid development of agricultural technology has also brought changes in the ecosystem resulting in the emergence and re-emergence of infectious diseases (Rohr et al., 2019; Waage et al., 2022). As for example, the Green Revolution of Asia during the sixties and seventies had introduced new technologies in agriculture like improved hybrid seeds, fertilizers, pesticides, irrigation systems and several other inputs that led to increased crop productivity (Sileshi and Gebeyehu, 2021). However, it also created enough opportunities for vector and pathogens to modify themselves and adapt to the new environment and cause new diseases. The Rift valley fever epidemic of 1977/79 occurred after the construction of Aswan High Dam and irrigation channels along the Nile River since it increased the density of breeding sites for mosquitoes resulting in their increased population (Drake et al., 2013). Increased incidence of human fascioliasis in Egypt and Peru occurred due to the adaptation of snail (intermediate hosts) into new irrigation system of Nile Delta (Ahmad et al., 2022; Mas-Coma et al., 2022). Use of fertilizers in intensive agricultural practices increases the level of environmental nutrient enrichment (ENE) resulting in increased population density of trematodes and their survival of infection is also high in the nutrient soil (Rohr et al., 2019). Trematodes are the intermediate hosts of many zoonotic diseases like fascioliasis, fasciolopsiasis, and gastrodiscoidiasis. In addition to this, use of livestock manure as fertilizer has increased the transmission of food-borne pathogens like verotoxigenic *E. coli* and *Salmonella* (Black et al., 2021; Mohamed and Habib, 2023).

Intensification of livestock farming:

Another factor related to emerging zoonotic diseases is the intensification of livestock farming as a result of high demand for animal food (Ferreira et al., 2021; Gilbert et al., 2021; Hayek, 2022). The emergence, re-emergence and spread of certain food-borne pathogens like *Cryptosporidium pavum* (Guo et al., 2022)*,* diarrheagenic *E.coli, Listeria monocytogenes* and *Campylobacter jejuni* have been associated with intensive farming systems (Guo et al., 2022; Leahy et al., 2022). Farmers are increasing the stocking density of animals and birds and feed them with concentrate ration that is fortified with vitamins, minerals, and antibiotics to increase their productivity (Godwin et al., 2022). Pig and poultry farming around the world are mostly intensified by increasing the population size and density. The first outbreak of Nipah virus that occurred in 1998-99 in Malaysia was the result of intensive pig farming around fruit bat habitats that were populated with Nipah virus-infected bats (Soman Pillai et al., 2020). The virus-infected bats got attracted to fruit trees planted around pig farms that created an opportunity for the virus to spill over the pigs. Pigs were infected after consumption of fruits contaminated with bat saliva and / or urine. Infected pigs were sold to markets with a large number of smaller intensive farms and became the amplifier host for human infection causing the rapid spread of the virus (Epstein et al., 2006).

Development of rice paddies combined with free grazing duck farming has also increased the risk of transmission of vector-borne disease like Japanese encephalitis [JE] (Paulraj et al., 2022; Walsh et al., 2022). The rice paddies are vector breeding sites and they also attract wild waterbirds that are natural reservoirs of JE virus and influenza virus. The wild birds come into close proximity of domestic poultry and transmit the virus to them. Influenza A viruses are emerging constantly by mutation and reassortment to develop a new strain with different virulence and host range including pigs, poultry, and human (Kessler et al., 2021). The biodiversity of wild bird population around poultry farms has increased the risk of pathogen spillover to domestic birds. The high density and low genetic diversity of domestic birds in intensive poultry farms also promoted the rapid spread of infection within domestic birds. Intensive poultry production with modern housing and ventilation system can trigger the transmission of pathogens to other species. The ventilation system in poultry sheds not only expels foul air but also the pathogens like *Campylobacter* and influenza virus into the environment that can be transmitted to wild and domestic animals (Hakeem and Lu, 2021; Liang et al., 2020). In addition to this, global trade in poultry, as well as the concentration of markets in food processing and distribution facilities, are also the driving factors for spreading emerging zoonoses (Rohr et al., 2019).

Non-therapeutic use of antibiotics:

Intensification of livestock farming has also promoted the non-medicinal use of antibiotics in livestock and poultry feeds to promote their growth and improve feed conversion rate. Studies have shown that the global use of antimicrobials in food animal production was approximately (63,151±1560) tons in 2010, and it is anticipated to surge by 67% to (105,596±3605) tons by 2030 (Ibrahim et al., 2020). The study reported the growth is largely attributed to the rising number of animals raised for food, accounting for two-thirds (66%) of the total increase. The remaining one-third (34%) is attributed to changes in farming methods, with an expectation that a substantial portion of animals in 2030 will be raised through intensive farming. Approximately 46% of the projected antimicrobial consumption rate in Asia by 2030 is linked to shifts in food production systems. Antimicrobial use in Asia is projected to reach 51,851 tons, by 2030. In 2010, the leading countries in global antimicrobial consumption for food animal production were China (23%), the United States (13%), Brazil (9%), Germany (3%), and India (3%). By 2030, it is projected that China (30%), the United States (10%), Brazil (8%), India (4%), and Mexico (2%) will hold the top positions. The countries expected to experience the greatest percentage increases in antimicrobial

consumption by 2030 are Myanmar (205%), Indonesia (202%), Nigeria (163%), Peru (160%), and Vietnam (157%). The anticipated growth in antimicrobial consumption for livestock in the BRICS (Brazil, Russia, India, China and South Africa) countries is estimated to be 99% by the year 2030 (Ibrahim et al., 2020). The non-therapeutic use of antibiotics in livestock and poultry production may lead to (1) emergence of new resistant microbes, (2) development of multi-resistant microbes, and (3) rapid multiplication of circulating microbes. The prolonged use of low level of antibiotics enables the microbes to develop the resistance against it (Gilbert et al., 2021). Antimicrobial resistance is the ability of a microorganism to tolerate any medicines taken against it. When the microorganisms become resistant to most of the antimicrobials, they are often referred as "superbugs".

Figure 2 shows the development of antimicrobial resistance in bacteria. Extended use of low level of antimicrobials not only kills harmful bacteria but also kill some useful bacteria (blue colour rods). If some of the bacteria have developed resistance against antimicrobials (red rods), they will survive and go on multiplying rapidly. The population of resistant bacteria increases and they can also transfer their resistance power to other non-resistant bacteria (brown rods) making them resistant too.
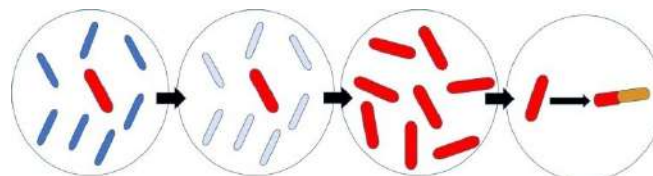
Figure 2: Mechanism of development of antimicrobial resistance

The most common examples of resistant microbes are methicillin-resistant *Staphylococcus aureus* (MRSA), multidrug resistant *Salmonella enterica*, sulfadoxine–pyrimethamine-resistant *Plasmodium falciparum*, tetracycline and ciprofloxacin-resistant *Campylobacter*, and *Enterobacteriaceae* producing extended-spectrum-β-lactamase (ESBL). Studies have shown that 70,000 people in the world die every year due to antimicrobial resistance in illnesses such as bacterial infections, malaria, HIV/AIDS or tuberculosis and it is predicted to peak to 10 million deaths in 2050 (Murray et al., 2022). Most of the resistant microorganisms in humans were transferred from food producing animals. An example is the vancomycin - resistant enterococci that was seen extensively in livestock of Europe in 1970 as the result of extended use of Avoparcin (antibiotic) as growth promoters (Acar et al., 2000; Hammerum et al., 2010). Similarly, after the introduction of streptothricin antibiotic as growth promoters in pig industry of former East Germany in 1983, the streptothricin-resistance gene

was reported in *E. coli* from pigs (Witte, 2007). Later, resistant genes were found in *E. coli* from farmers, family members and from the urinary tract infection of urban citizens. Studies have shown that MRSA can transmit easily from animal to humans and vice versa (Algammal et al., 2020). It was also reported that handling or consumption of contaminated poultry meat transferred resistant *E. coli* causing urinary tract infections in women of United States (Murray et al., 2022).Similarly, after the control of long-term *Campylobacter* epidemics of New Zealand in 2008, it re-emerged in 2014 with added hazard of rapidly emergent antimicrobial resistance (Greening et al., 2021). It was reported that a new strain *C. jejuni* ST - 6964 that is resistant to tetracycline and fluoroquinolones was detected in the sentinel site in the Manawatu region of New Zealand.

Environmental pollution:

In addition to the ecosystem change and farm intensification, the release of environmental pollutants like carbon dioxide, carbon monoxide, nitrous oxide, chemical fertilizers, pesticides, and insecticides have been associated with EIDs (Manisalidis et al., 2020; Rohr et al., 2019). Human activities leading to global warming and climate change have also promoted the emergence of new diseases (Baker et al., 2022; Caminade et al., 2019; Ellwanger et al., 2020). Developed countries are more responsible for this as compared to developing countries since they use more luxurious household items releasing the pollutants to the environment. Besides this, the plans and policies of any country can also affect the environment and consequently lead to the emergence of infectious diseases (Shearer et al., 2020; Tisdell, 2020). But it depends upon the authority that has got the ultimate powers to develop, modify and implement the environmental policies in his country (Neira et al., 2023).

Conclusion:

In conclusion, we can say that environmental changes and intensification of farming are highly associated with theincreased emergence and transmission of zoonotic pathogens. So, these factors need to be controlled wisely by implementing the sustainable agriculture and livestock production without disturbing the wildlife habitat. In addition to this, the non-medicinal use of antibiotics in agriculture / livestock industry should be discouraged by regulating laws. All these can be achieved only if we work collaboratively through "One Health approach" acting on all the three components- humans, animals, and the environment. If we do not act now to safeguard the ecosystem, there will be more emergences of infectious diseases and we may lose the battle with microbes.

Conflict of interest:

The author declare that he has no known competing financial interests or any personal relationships that could have appeared to affect the work reported in this paper.

Data availability:

Not applicable

Authors' contribution:

In crafting this article, SP, as the sole author, undertook a comprehensive and solitary journey from conceptualization to completion. SP drafted the manuscript, structuring it according to the requirements of IJVPH, and ensured clarity, coherence, and the logical flow of arguments throughout the article. This work aims to make a valuable and original contribution to the existing body of knowledge in the factors causing the emergence of zoonosis.

Ethical approval:

Not applicable

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Diagnosis of PPR Virus in Goats through Histological, Immuno-histochemical and Molecular Application

Debasish Niyogi [(1)*], Dharam Prkash Shrivastava [(1)], Satyavrat Singh [(2)], Rajesh Kumar Joshi [(3)]

[(1)] Department of Veterinary Pathology, [(2)] Department of Veterinary Medicine, [(3)] Department of Veterinary Microbiology, College of Veterinary Science & Animal Husbandry, ANDUA&T, Kumarganj – 224229, Ayodhya, Uttar Pradesh

Abstract

Peste des petits ruminants (PPR) are an acute, highly contagious, and economically important transboundary disease. PPR represents an economically important plague of small ruminants. PPR is endemic across much of Africa and Asia with its geographical distribution seemingly expanding. Infection most commonly leads to a profound immunosuppression that allows opportunistic secondary infections to develop. Increasing the morbidity and mortality rates observed. The pathogenesis associated with PPR virus (PPRV) has largely been assumed from that established for closely related viruses such as rinderpest virus (RPV), measles virus, and canine distemper virus. For its diagnosis specific antibodies cannot be missed with classical and validated diagnostic assay (ELISA), an alternative testing method to the conventional virus neutralization test, allowing for monitoring vaccination or locating disease outbreaks. Here, we overview the current thought on the diagnosis of PPR in small ruminants.

Keywords: Peste des petits ruminants (PPR), Goat, Histochemical and histopathological investigation, Immuno histology

Introduction:

Peste des petits ruminants (PPR) are an economically devastating disease of small ruminants caused by the PPR virus (PPRV), a non-segmented negative-strand RNA virus belonging to the morbillivirus genus, within the family Paramyxoviridae. PPR infection is often associated with significant losses due to high morbidity. Alongside this, the highly contagious nature of the virus and uncontrolled movement of animals through trade mean that PPRV is considered to be a serious transboundary problem. Following a PPRV outbreak, the disease is generally controlled through the subcutaneous administration of a live attenuated PPRV vaccine. Although the vaccine provides long-term immunity, the generation virus neutralizing antibodies does not occur rapidly enough to prevent the rapid Spread of the disease among in-contact animals. Therefore, for disease control, a clear understanding of the mechanisms behind the development of disease needs to be defined. The pathology of a viral infection generally encompasses three distinct components of disease: (i) etiology (ii) structural alterations of cells (morphologic changes) (iii) and the consequences of changes (clinical manifestations). The current understanding of PPRV pathology has been heavily assumed from the closely related rinderpest virus (RPV) (Wohlsein et al., 1993; 1995) and other morbillivirus infections, and there have been very few studies performed that have focused specifically on the pathology of PPRV. As such, little is known about the mechanisms underlying the establishment of the disease (pathogenesis) in susceptible animals. We describe what is currently understood regarding PPRV-driven pathology insusceptible hosts.

Clinical manifestation of PPR:

Depending upon the predisposing factors and the virulence of the infecting virus clinical manifestation PPR can be seen in Per-acute acute, subacute, and subclinical forms. However, PPR in sheep and goats is generally observed as an acute disease. The per acute form of the disease has a short incubation period with rapid development of pyrexia with body temperature rising to 40-42⁰C. Depression congestion of mucous membranes, oculo-nasal discharge may become mucopurulent and can occlude the nostrils, dehydration and ultimately death of the animal within 4-5 days (Munir et al., 2013). In the acute form of the disease, a 3- to 4-day incubation period precedes the pyrexia and the onset of other clinical disease signs, including watery oculo-nasal discharge, congestion of the mucous membranes of the buccal cavity, conjunctiva of the eye and the vulva (Abubakar et al., 2008). A diarrhoeic phase follows, often resulting in the generation of bloody faecal matter leading to dehydration and ultimately death of animals. As the disease progresses, the watery development of watery oculo-nasal discharge becomes mucopurulent and can occlude the nostril predisposing to dyspnoea.

In the subacute form of disease, the animals do not develop severe clinical disease and low mortality rates are seen. With this form of infection, the animals may

develop temperatures ranging from 39 to 40°C but do not develop the characteristic clinical signs normally associated with PPRV infection. Animals usually recover from the disease within 10-14 days, A subclinical form of the disease is also seen in large ruminants (buffalo and cattle), where the infected animals are able to clear the virus in the complete absence of clinical disease, but seroconvert to PPRV, often generating strong neutralizing antibody responses.

Stepwise Clinical Manifestations in PPRV-infected Goats:

Our current understanding of PPR pathology has been mainly based on reports detailing field infection. As such, the assessment of pathomorphological disease progression has generally been studied during the later stages of acute disease. As a result, there is a gap in knowledge of events that occur very early on following infection. A few experimental studies have been reported that have looked at earlier time points, although several obstacles have precluded a thorough assessment of very early time points following infection (Couacy-Hymann et al., 2007; EI Harrak et al., 2012; Hammouchi et al., 2012; Pope et al., 2013). Based on both published and unpublished data available to us, we describe here the disease course during both the early and later phases of PPR infection. As detailed above, PPR in susceptible small ruminants occurs most commonly as an acute infection. The severity of the disease may be influenced by a number of factors including, but through knowledge gaps, not limited to: the genetics of the infecting virus strain; the infectious dose of the virus; the route of infection; the species and breed of infected animal; and the immunological and nutritional status of the infected animal. A key factor influencing disease progression, given the profound immunosuppression seen following PPR infection, is the presence of pathogenic organisms, as a result of pre-existing pathological processes, or environmental exposure. Evaluations of the virulence of different PPRV strains have been reported (Couacy-Hymann et al., 2007; El Harrak et al., 2012; Emikpe et al., 2013; Pope et al., 2013; Rajak et al., 2005; Jagtap et al., 2012). Couacy-Hymann and colleagues performed in vivo studies utilizing representative strains of PPRV from each of the 4 defined lineages of PPRV in African dwarf goats via the subcutaneous route. Interestingly, infection with the different isolates led to a variation in the onset of clinical signs, ranging from mild infection to severe disease with some mortality. Although all the virus lineages caused clinical disease, virus isolates from lineage I and lineage IV appeared more virulent in this experimental model. Further to this study, Emikpe and colleagues infected African dwarf goats with a virulent form of the Nigeria 75/I strain through intra-tracheal route and observed severe disease. Although they demonstrated disease by all three routes, it was concluded that the intranasal route was the most suitable to mimic natural infection for pathological and immunological studies. The progression of disease during the acute form generally results in the following sequential order:

i.   The infected animals incubate the disease for 2-7 days before the development of pyrexia ranging from 39.5 to 41°C, which lasts from 3 to 10 days.

ii. 2 to 3 days after the onset of hyperthermia, the conjunctival and oro-facial mucosa becomes congested.

iii. Ocular and nasal discharge occurs from days 4 to 7 post-infectionand lasts between 2 and 4 days; the serous clear discharge gradually becomes mucoid/mucopurulent towards the later phase; the congested oro-facial mucosa concomitantly shows the appearance of lesions on the gum (dental pad), tongue, soft palate, and nasal mucosa.

iv. In severe cases, oral lesions are observed on the hard palate with oral ulcerations and necrotic lesions appearing between days 5 and 9 post-infection.

v.   These necrotic lesions progress with the appearance of a caseous deposit of fibrin on the tongue, and at this point, halitosis is often evident due to these buccal lesions.

vi. Finally, diarrhoea begins 4-10 days post-infection, sometimes becoming projectile, and the animal may become dyspnoeic within 8-12 days, suffering progressive weight loss and emaciation that ultimately leads to death. In some cases, particularly in mild infection, animals may convalesce, returning to a pre-infection health status within 10-15 days of infection.

Clinical Scoring:

Clinical scoring to assess the severity of disease was not clearly defined earlier in the literature. A clinical scoring method for PPRV has been developed to help assess the severity of disease following infection and to guide the human euthanasia of animals exhibiting clear clinical disease during in vivo experimentation (El Harrak et al., 2012; Pope et al., 2013). Both published clinical scoring systems recommend sacrificing animals when a defined score is reached during a defined time period. The decision to euthanize is made on ethical grounds (Hecker, 1983; Smith and Sherman, 2009) if the following criteria are fulfilled, in the system proposed by Pope et al. (2013).

1. A maximal grading of 4/4 is allocated to the general clinical appearance of the animal (severe obtundation, lack of mobility, and dehydration), an indication of severe morbidity.

2. A score of 3/4 is achieved for the above general signs for 2 complete consecutive days, and a score of 10 or greater is achieved in other categories.

3. A score of two is achieved in the above general signs for 2 complete consecutive days, and a score of 15 or greater is achieved in other categories.

4. A cumulative score of 20 across all categories is reached. Not only will the utilization of such clinical score sheets aid the assessment of experimental infection, but it will also aid consistency of reporting when applied to field infections. Of course, the assessment of infection based on clinical observations is insufficient for diagnosis, as several other pathogens can cause clinical diseases similar to those seen with PPRV. Clearly, confirmatory laboratory diagnosis satisfactorily concludes PPRV infection. The differential required to diagnose small ruminant disease is dealt with elsewhere within this publication.

Gross Pathology:

Many pathological characteristics are common at post-mortem examination following PPRV infection. Ulcerative to necrotic lesions are clearly evident throughout the buccal cavity in PPRV-infected sheep and goats at post-mortem. The buccal papillae, dental pad gum, and dorsal surface of the tongue, palatine tonsil and hard palate are mainly affected. Congestion of the digestive tract, particularly the duodenum, abomasum, ileum, caecum, and colon, is often seen. Extensive congestion along the longitudinal folds of the caecum colon and rectum may be evident as zebra striping. The ileocecal valve can also demonstrate extensive mucosal haemorrhage. In severe cases, hyperaemic, oedematous, and ulcerative mucosas are also seen throughout the intestines (Munir et al., 2013). An interesting observation in a study (Pope et al., 2013) was the relative difficulty in the detection of Peyer's patches in the ileum of virus-infected animals in the absence of obvious necrosis or haemorrhage in these areas. Previous studies have reported the association of PPRV with these lymphoid structures and extensive necrosis and collapse of the Peyer's patches have been observed in both natural and experimental infections (Taylor, 1984; Kul et al,. 2007; Kumar et al., 2004). In contrast to infected animals, the Peyer's patches were readily detected in uninfected tissues of control animals, which may imply that during infection, a redistribution of lymphocytes from these aggregates to sites of infection occurs as suggested following CDV infection (von Messling et al., 2006). The enlargement of lymph nodes accompanied with necrosis and haemorrhage, particularly the mesenteric lymph nodes and atrophied congested spleen are also sometimes seen in PPRV-infected goats in the field (Khan et al., 2008). Similar to the buccal mucosa, the nasal mucosa becomes hyperaemic. The caudal part of the trachea and bronchus may contain froth (Emikpe et al., 2013). Pulmonary congestion and edema with varying degrees of red and grey consolidations can be seen with severe infections (Ugochukwu and Agwu, 1991). The pleural surface of the consolidated lobes often shows patchy fibrin depositions (Emikpe et al., 2013).Congestion of lungs and bronchopneumonia was also seen in PPR-infected West African dwarf goats and was associated with bacterial infection (Couacy-Hymann et al., 2007).

Histopathology:

Histopathological assessment of PPRV-infected tissues demonstrates many of the common characteristics of morbillivirus infection, including syncytial formation and extensive necrosis. However, observations are clearly linked to the extent of the disease as despite being seen during the late stage of the disease, inclusion bodies were not detected when assessing sections from animals exhibiting a mild form of the disease (Pope et al., 2013). Despite this, even where the mild disease is seen, large numbers of syncytial cells, ranging in viability and size, can be detected in the paracortex of lymph nodes from day 5 and in the cortex and follicles of lymph nodes, splenic white pulp and gastrointestinal submucosal lymphoid tissue from day 7 of infection (Pope et al., 2013). In this study, necrosis/apoptosis of syncytia and individual cells was marked in paracortical areas on day 5, but declined after this point, indicating that nuclear debris is degraded very quickly within the lymph nodes. Lymph nodes can also become oedematous with mild lymphoid depletion. Moderate-to-severe lymphoid depletion is seen in the germinal centers of the spleen. Cellular necrosis, as manifested by nuclear debris, was not as prominent in tonsillar and splenic tissues, as that seen in the lymph nodes. Squamous epithelial syncytia were also observed in tonsillar, facial, and digestive tract epithelial tissues (Pope et al., 2013). Large alveolar macrophages with intranuclear and intracytoplasmic inclusion bodies, numerous neutrophils, fibrin exudates, and multinuclear giant cells have also been reported in severe PPRV infection (Emikpe et al., 2013), although again of these histopathological changes may result from secondary bacterial or parasitic infections. The alveolar lining cells become cuboidal and the interstitial is infiltrated by lymphocytes and neutrophils. Desquamation of bronchiolar epithelial lining cells is common, and the bronchiolar lumen contains purulent exudates.

Occasionally coagulative necrosis of the lung parenchyma is also seen. Intestinal villi may become

atrophied, mucosal glands may become necrotic, and, depending on the severity of the disease, the lamina propria is infiltrated by lymphocytes and plasma cells. Furthermore, throughout the intestines and in the abomasum, marked diffuse lymphoid infiltration and edema, causing varying levels of crypt disruption, can be observed (Pope et al., 2013). Lymphoid syncytial formation is often seen within the lamina propria with associated cellular necrosis.

Immunohistochemical Localization of Viral Antigen:

The study that examines the distribution of PPRV antigen in field cases at staggered time points following infection is that described by Pope et al. (2013). Here, the conducted study where goats were suspected of a virulent PPRV isolate and died at strategically defined time points to enable pre- and post-mortem sampling. This approach enabled precise monitoring of the progress and distribution of the virus throughout the infection from the time of challenge, through peak viremia and into a period of convalescence (Pope et al., 2013).

In this study, the lymphotropic nature of the virus was clearly evident from the distribution of virus antigens in lymphoid tissues at both early and late stages of infection. Examples of antigen detection within lymphoid tissues at different times of infection by immunohistochemistry (IHC) are presented. The earliest detection of PPRV by IHC was seen in regions of lymphnodes and tonsillar tissue sampled at day 5 post-infection, with the paracortical areas of lymph nodes and diffuse lymphoid tissue of tonsils affected most significantly, before infection, as judged by the degree of immunolabelling spreads to the cortical/follicular areas of nodes/tonsils.

Often, virus antigen was present in the paracortex, medullary cords, and some regions of the non-follicular cortex and subcapsular area of lymph nodes, with large numbers of necrotic and apoptotic cells. Positive immunolabelling was seen in abundance in all sampled lymph nodes and tonsillar sections by IHC from day 7 post-infection. Syncytia formation is evident within paracortical areas of the left pre-scapular lymph node at 5 days post-infection and at day 7 post-infection. Virus antigens can also be seen within the pharyngeal tonsil, advancing from day 5 to 7 days post-infection. Virus antigen was detected most readily within the retropharyngeal lymph node, taken as a representative of the facial lymph nodes, with the tonsil also exhibiting high levels of virus antigen, especially at 7-9 days post-infection. No virus antigen was detected in any of the facial tissues taken on day 2 and day 5 post-infection by histochemical techniques. Mucosal erosions in nasal sections were seen at 7 days post-infection, with mild epidermal cellular swelling and increased numbers of lymphocytes and reticular-type cells seen migrating through the lamina propria and mucosa. IHC detected viral antigens within the epithelium and lymphoid cells of the lamina propria in nasal skin/mucosal samples at 7 days post-infection (Pope et al., 2013). A greater antigen burden was detected in nasal, labial, and conjunctival mucosal cell types at 9 days post-infection with antigens also being detected in the epithelium and lymphoid tissues of the tongue (Pope et al., 2013). Detection of antigen throughout the intestinal tract did not occur until a late stage of infection from day 9. A full immuno-histochemical analysis of the distribution of virus antigen throughout infected animals can be found in Pope et al. (2013).

Various ELISA Formats Based on Whole PPRV:

From the early 1990s, different ELISA formats, Indirect ELISA as well as mAb-based ELISA was developed for the detection of PPR antibodies in the serums of Sheep and goats. The conventional antigen used in these different formats is prepared from the whole PPRV extracted from infected Vero cells. The indirect ELISA format developed by Balamuugan et al. (2007), in which the serum is incubated with a solid-phase PPRV antigen, induces colour development when antiserum enzyme conjugate and substrate chromogen are added. The test is not in principle discriminating between morbillivirus cross-reacting antibodies and is therefore recommended to be used as a screening test, followed by a confirmatory test in case of positive results. In the blocking format b-ELISA, (Saliki et al., 2013), the serum is pre-incubated with a solid-phase PPRV antigen, and then, a specific mAb is added. In competitive format c-ELISA (Anderson and McKay, 1994; Singh et al., 2004) a variant of the previous format where the two reagents are added simultaneously. Formats are developed using the whole virus as an antigen and the competing targets are the H protein. These assays work on the principle that sera-containing antibodies to PPRV either block or compete with the binding of the anti-H mAb and result in the reduction of the expected colour when the enzyme-labeled anti-mAb antibody and substrate chromogen are added.

Assays based on H-specific mAbs use purified or semi-purified PPRV as antigens, strains being either the PPRV Sungri (Singh et al., 2004) or Nigeria 75-1 (Anderson and McKay, 1994; Saliki et al., 2013; Visser et al., 1993). Crude antigens from infected cell cultures were harvested when showing cytopathic effects prepared by cell disruption/sonication and clarification. Concentration is obtained by high-speed centrifugation and pelleting on a sucrose gradient or precipitation using PEG. The resulting concentrated virus is used as the ELISA antigen. Optimized validation conditions relied on sera for which the status for PPR and rinderpest was already established

by the VNT. The H-based C-ELISA developed by Anderson and McKay (1994) utilizes a mAb, designated C77. This ELISA has subsequently been used throughout Africa, the Middle East, and Asia on several species of small ruminants and has proved to be extremely reliable and robust and is currently commercialized by BDSL. However, some studies showed that, in contrast to the highly specific rinderpest c-ELISA, the PPR c-ELISA does detect some antibodies against RPV. The specificity of the test drops significantly when used with rinderpest-vaccinated animals (Couacy-Hymann et al., 2007).

Indirect Immunofluorescence Assay (IFA):

IFA may be used as a serology test although not adaptable to large-scale surveys, mainly implemented in laboratories with suitable facilities, the test is based on the use of formalin- or acetone-fixed infected cell mono layers incubated in successive steps with sera and antisepsis fluorescent conjugates. Reactions are viewed under indirect fluorescence microscopy. With IFA, PPRV immune responses generate a characteristic fluorescence pattern in susceptible infected cells. Although this assay warrants practice and skill, it has advantages compared to indirect ELISA to add specificity since characteristic positive reactions can be discriminated from the background by well-delineated intracellular fluorescence, which corresponds to antigen localized in the cell. To improve the specificity of the test, cells may be optimally infected to obtain 20-40 % fluorescence of the monolayer, hence, facilitating the detection of PPRV-specific fluorescent foci (Libeau et al., 1995).

Recombinant Protein- and Peptide-Based ELISAs:

New generation antigens produced by cloning-specific genes in different expression systems were developed. The insect cell-baculovirus and *Escherichia coli* expression systems appear to be a suitable alternative for producing immunogenic antigens for use in diagnostics. The corresponding delivered proteins were used for the introduction into PPR serology tests. The advantages of recombinants over the whole virus issued from infected cells are multiple. Recombinant proteins overcome the cumbersome and costly production of semi-purified viral particles. These antigens need the requirement of homogeneous and standardized antigens for optimal sensitivity and specificity and can be expressed in large quantities, thus constituting good candidate antigens in diagnostic tests. Finally, the recombinant proteins comply with the demand for safe and reliable tests for serological surveys of PPRV whether the country is infected or not. Of the six structural proteins, the N protein is known as the major viral protein. As such this protein that accumulates in a larger amount during viral infection compared to the other viral proteins is also highly immunogenic and thus constitutes a good candidate to be used as an antigen in standardized diagnostic tests.

Indirect ELISA Based on rPPRV N or Sonicated Transgenic Cells:

To detect the immune response against PPR, an indirect ELISA based on rPPRVN may be used (Ismail et al., 1995). Using rPPRVN instead of whole virus circumvents antigen contamination with cell constituents from the latter, which is the main factor responsible for false-positive reactions in indirect ELISA. This ELISA format although based on a recombinant protein is not in principle specific and is recommended to be used as screening tests for the reason that nucleoprotein is highly conserved among the genus. However, now that the rinderpest is eradicated, the indirect ELISA based on rPPRVN may not provide any evidence that other ruminant morbilliviruses could obscure the reliable serodiagnosis of PPRV. In addition, it is worth stressing that the format favours easy implementation in diagnostic laboratories. If the indirect format is to be widely adopted, it is, however, important to draw attention to a phenomenon linked to this Format: the negative population of an endemic country will behave differently than that of a free country, exhibiting a higher background level. Therefore, extensive validation should be needed, especially the cut-off settlement. An indirect ELISA based on the use of recombinant rPPRVN was first described by Ismail et al. (1995). The test was evaluated for routine diagnosis based on goatSera collected during a suspected outbreak of PPR in Cameroun. Full length of the N gene from a Tibet Strain of PPRV was expressed in *E. coli*. Initial validation of the rPPRVN issued from the Tibet strain was based on the comparison in the c-ELISA format with the TPPRV N issued from Nigeria 75-1 strain using the protocol and mAb developed earlier (Libeau et al., 1995). Differences in the percent inhibition values among both tests were comprised between 5 and 15 %. Based on sera from healthy goats raised in an isolated area (n = 198), the cut-off value of the indirect format based on rPPRVN Tibet was settled at a positive/negative optical density value of 2.18, and a preliminary study for assessing the suitability of the assay for sero-surveillance implemented using serum samples with known status (n = 697). All sera were shown to be reassessed in the indirect format as previously defined by the competitive format.

C-ELISA Based on rPPRVN:

C-ELISA tests based on PPRV recombinant antigens were validated through extensive studies comparative to methods previously developed, particularly against the VNT, and proved to have at least the same diagnostic accuracy in small ruminant populations from various geographical regions. In addition, these antigens helped

increase the improvement of standardized results among laboratories. Test conditions were optimized and the method was revealed to be highly accurate and robust. Expression and purification efficiencies of the recombinant protein obtained by the Baculovirus expression system were evaluated. Crude lysate or affinity-purified rPPRV N was further used as the coating antigen for PPR antibody detection in competitive ELISAs. The first c-ELISA assay based on a recombinant protein described for PPRV was validated with experimentally infected or vaccinated animals and then implemented for sero-surveys. The method originally described in the publication of Libeau et al. (1995) was one of the OIE-approved PPR diagnosis techniques. It was subsequently reproduced by Choi et al. (2005a, 2005b). The mAb used by the ELISA of Libeau and colleagues was chosen for its high affinity for Prh strains. The epitope recognized on the native N is also present on the rPPk protein expressed by baculovirus in insect cells, thus allowing the rPPR N protein to behave as the whole virus in the c-ELISA format. The good performances of this test were further evidenced with African dwarf goats and Sahelian goats field sera during an outbreak of PPR in species of, small ruminants in different geographical areas (Diop et al., 2005; Kwiatek et al., 2007). It is worth stressing that the test is now commercially available (ID Screen® PPR Competition kit from IDVet). *E. coli* expression system has been alternatively explored to express partial and Aull-length PPRV N gene. The histidine-tagged proteins were purified using an N-NTA resin chromatography matrix and eluted. Microtiter wells coated with the purified antigen were tested for reactivity with PPRV-immunized rabbits and specific mAbs for further use in c-ELISA serological diagnosis of PPR infection. N expressed with the E. coli system behaves as the whole virus in the c-ELISA system (Singh et al., 2004) as demonstrated by the specificity of the test with sera from goats having no history of either vaccination or natural exposure to PPRV (n=70). A preliminary study for assessing the suitability of the assay for sero-surveillance was implemented using sheep and goat serum samples collected randomly from an area where the disease was present (n = 120). However, compared to the PPRV antigen-based test, 73 samples out of 93 samples were found positive by recombinant protein-based c-ELISA. Comparison with the VNT was not undertaken due to the limited number of samples analyzed in this preliminary study.

Conclusions:

Clearly, there are several forms (mild to severe) of disease seen following PPRV infection. However, the mechanisms of infection and events very early on infection require further analysis to understand the molecular mechanisms behind infection and dissemination of virus within the host. Histochemical and histopathological investigations have demonstrated that the initial site for virus replication is not within the epithelial cells of the respiratory mucosa, as has been previously reported, but is within the tonsillar tissue and lymph nodes draining the site of inoculation. Pope et al. (2013) proposed that the virus is taken up by immune cells within the respiratory mucosa which then serve to transport the virus to lymphoid tissues where primary virus replication occurs and from where the virus enters the Circulation. Although it has been hypothesized that the initial site of PPRV replication is not within the epithelial cells of the respiratory mucosa, further studies are required to address these questions by assessing the sites of viral replication very early on following infection. C-ELISA is considered a highly accurate standardized and robust test able to measure immune response either due to infection or vaccination. This ELISA format has the potential to replace the conventional VNT method. The profound immunosuppression that follows infection clearly also contributes to the outcome of infection with secondary infection often contributing significantly to the high mortality rates associated with infection. Clearly, extensive investigation into the mechanisms of virus-induced pathology is required to fully understand this economically important viral pathogen.

Data availability:

Not applicable

Authors' contribution:

All authors equally participated in drafting, editing the manuscript and approved the final version of the manuscript.

Ethical approval:

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Giardiasis: An Emerging yet Neglected Zoonotic Disease of Public Health Significance

Mahendra Pal [1]*, Kirubel Paulos Gutama [2], Judit Molnar [3], Firehiwot Abera [4], Surajit Baidya [5]

Narayan Consultancy on Veterinary Public Health and Microbiology, Bharuch, Gujarat, India. [1] Managing Director of Narayan Consultancy on Veterinary Public Health and Microbiology, B-103, Sapphire Lifestyle, Maktampur Road, Bharuch, Gujarat, India, [2] School of Veterinary Medicine, Dambi Dollo University, Dambi Dollo, P.O. Box 260, Ethiopia, [3] Department of Water and Environmental Sciences, Faculty of Agricultural and Food Sciences, Szechenyi Istavan University, 9200 Mosonmagyarovar, Hungry, [4] Food Microbiology Laboratory, Ethiopian Public Health Institute, Addis Ababa, Ethiopia, [5] Department of Veterinary Parasitology, West Bengal University Animal and Fishery Sciences, Kolkata, India,

Abstract

Giardiasis is a neglected protozoan disease that has been reported from developing as well as developed nations of the world.The parasite is estimated to cause more than 28.2 million cases of diarrhoea each year due to contamination of food. Oral route is the prime portal of entry of the parasite though food or water. The disease is caused by *Giardia duodenalis*, a zoonotic parasite, which is widely prevalent in environment and infection can occur in sporadic and epidemic form. Transmission of infection is either direct (person to person) or indirect through water or food. People travelling to areas with poor sanitation are more likely to get the infection. Symptoms of infection include nausea, abdominal cramps, gas and diarrhoea. Chronic infections are also associated with arthritis and irritable bowel syndrome (IBS). Microscopical examination of stool for the parasite is the simplest method to diagnose *Giardia* infection. Real-time PCR is considered as the most sensitive technique for the diagnosis of disease. The patients can be treated orally with drugs like metronidazole and tinidazole.Sometime, the patient with severe disease may be not respond to therapy. Personal hygiene, sanitation and implementation of HACCP in food establishments are imperative for prevention and control of disease.

Keywords: Giardiasis, Neglected zoonotic disease, Public health, Water

Introduction:

Protozoan foodborne diseases, such as amoebiasis, sarcocystosis, toxoplasmosis, giardiasis, cryptosporidiosis, cyclospridiasis, and balantidiasis are important public health problems in many countries of the world; and affect both sexes, and all age groups (Pal, 2007; Feng et al., 2011; Pal et al., 2017; Pal, 2020; Pal et al., 2021). Among these, giardiasis is an emerging protozoan zoonosis of worldwide occurrence (Pal, 2007). It is considered as an under reported foodborne disease of public health significance (Ryan et al., 2019).The disease is commonly observed in warm climates. It is one of the most common causes of waterborne disease in the United States. In USA, *Giardia* infection is responsible to affect over one million people every year (CDC, 2021). Epidemics of giardiasis can occur due to contaminated drinking water or food (Pal, 2007). The children and immunocompromised persons are at higher risk of getting the infection (CDC, 2021). The disease is caused by *Giardia duodenalis*, a protozoan parasite that is highly prevalent in developing and developed nations of the world (Cai et al., 2021). The lifecycle of *Giardia* has two distinct phases: one is a vegetative trophozoite and another is an infective cyst that is resistant to harsh environmental conditions (Pal, 2007).The pathogenic trophozoites infect the intestine, and the hardy cysts are shed in the faeces. The cysts of *Giardia* are detected in the water collected from lakes, rivers, and swimming pools (Carmena et al., 2012). It is mentioned that in several outbreaks of disease, the role of animals in the contamination of water has been suggested (Carmena et al., 2012). *Giardia* infections are acquired by foodborne transmission. The public health impact of foodborne giardiasis is not well studied. The present communication is an attempt to describe the importance of giardiasis as a foodborne and waterborne disease of public health concern.

Etiology:

Currently, there are nine valid species of *Giardia* namely, *G. agilis, G. ardeae, G. cricetidarum, G. duodenalis, G. microti, G. muris, G. peramelis, G. psittaci,* and *G. varani* (Ryan et al., 2021). Among these, *G. duodenalis* (Synonymous *G. intestinalis, G. lamblia*) is identified in humans as well as in wide range of animals (Einarsson et al., 2016; Cai et al., 2021).

Host and Transmission:

Natural infection due to *G. duodenalis* has been reported in humans. The parasite has also been detected in several species of vertebrate animals, such as alpaca, beaver, budgerigar, buffalo, cat, cattle, deer, dog, goat, monkey,

otter, pig, porcupine, sheep, and others (Appelbee et al., 2005; Pal, 2007; Dixon, 2021; Ryan et al., 2021).

Transmission of infection in humans occurs through ingestion of cyst contaminated food. Drinking of contaminated water with cyst of *Giardia* spp. can cause infection. Hand to mouth transfer of cysts from the faeces of an infected person can also result in *Giardia* infection (Pal, 2007).

Clinical symptoms:

Humans: The infection may be asymptomatic in some patients. Clinical manifestations in affected persons show chronic diarrhoea, frequent loose and pale stool, which contain large amount of mucous and fat; epigastric pain, dullness, flatulence, abdominal cramps and distension, fatigue, dehydration, malabsorption, weight loss; nervous manifestations like headache, nausea, vomiting, mental depression, disturbed sleep, irritability, anorexia, and anaemia (Pal, 2007; Einarsson et al., 2016). Dehydration due to diarrhoea in infants may be life-threatening (CDC, 2021).

Animals: The infection is usually subclinical in animals; however, diarrhoea, abdominal pain, bloat and loose faeces are noticed in some cases (Pal, 2007). Young animals are more susceptible to infection than adults (Carmena et al., 2012).

Diagnosis:

Clinical signs are not very characteristic to warrant the diagnosis of disease. Identification of trophozoites and cysts in faecal specimen helps in the diagnosis of infection. ELISA test is used to detect antigen in the faeces of patient (Pal, 2007). If the symptoms persist after the treatment, retesting of the patient for *Giardia* is suggested (CDC, 2021).

Treatment:

Several drugs, such as furazolidone (100 mg qid PO for 7-10 days), metronidazole (250 mg tid PO for 5 days), parmomycin (25-30 mg/kg/d in 3 doses PO for 7 days), quinacrine hydrochloride (100 mg tid PO for 5 days), and tinidazole (2 g once PO) have been advised for the treatment of *Giardia* infection (Pal, 2007).

Prevention and Control:

Currently, no vaccine is commercially available for immunization. Therefore, certain measures, such as protection of food and water contamination from faeces, drinking of safe potable water, proper washing of hands before eating, avoiding swallowing of recreational water, thorough disposal of infective faecal matter, avoidance of eating uncleaned raw vegetables, care when dealing with infected animals, and disinfection of infected site with 2-5% lysol or phenol may help to minimize the incidence of

Giardia infection. In addition, health education of people about the source of infection, significance of sanitary disposal of faeces and personal cleanliness should be imparted (Pal, 2007).

Conclusion:

Giardiasis caused by flagellated protozoan parasite, is a public health problem in many countries of the world including India, and the infection can be spread through ingestion of food and water, and also from person-to-person contact. Disease occurs in humans and also in a wide variety of domestic and wild mammals. The parasite is endemic in areas that have poor sanitation. The disease is an important cause of morbidity in developing nations. Food-borne and waterborne outbreaks of giardiasis are commonly encountered. Laboratory help is imperative for confirming the diagnosis of disease. It is emphasized to undertake epidemiological investigation of cases to establish the source of infection.Additional studies to know the true prevalence and public health impact of foodborne giardiasis will be rewarding.

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Conflict of interest:

There was no conflict of interest among the authors.

Contribution of authors:

All the authors contributed equally in the preparation of the manuscript.

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Rapid Molecular Detection of Fowl Typhoid and Avian Paratyphoid in Poultry: A Review

Samir Dey[*], Kunal Batabyal, Indranil Samanta, Sidhartha Narayan Joardar

Department of Veterinary Microbiology, West Bengal University of Animal and Fishery Sciences, Belgachia, Kolkata-700037, India

Abstract

Fowl typhoid (FT) and Avian paratyphoid (AP) are diseases caused by non-motile bacterium *Salmonella enterica* subsp. *enterica* biovar Gallinarum and motile non-typhoidal serovars (NTS) of *Salmonella* other thanGallinarum and Pullorum. The NTS serovars are non-host specific, associated with subclinical infection in poultry and foodborne diseases in humans. Eradication of FT in commercial poultry in some parts of the world was achieved through improved surveillance and culling. However, FT is an endemic disease of poultry in India with occasional outbreaks. Avian paratyphoid (AP) is an important bacterial disease of chickens worldwide. It is one of several types of diseases caused by infection with *Salmonella* spp. Chicks from hatcheries are most at risk. Infection may occur in birds of all ages. It can also cause huge morbidity and mortality loss. Accurate precision diagnosis of the pathogen is a prerequisite for formulating effective control measures for these infections. The conventional methods of detection of causative pathogens are laborious, less sensitive and time-consuming. Control measures by treatment and vaccination can reduce morbidity and mortality from salmonellosis in birds but do not eradicate infection. Improvement in detection methodology and adoption of rapid DNA-based detection techniques of the major *Salmonella* serovars is of utmost necessity. Rapid Salmonella detection methods of important *Salmonella* serovars were reviewed.

Keywords: *Salmonella*, Fowl typhoid, Paratyphoid,Non-typhoidal serovar

Introduction:

Salmonellosis in poultry can present three disease conditions-fowl typhoid, pullorum disease and avian paratyphoid. Fowl typhoid (FT) caused by Salmonella *enteric* subsp. *enteric* biovar Gallinarum is of major economic significance in many countries of Asia, Africa, Central and South America (Barrow and Freitas Neto, 2011). Pullorum disease by Salmonella *enterica* biovar Pullorum was last detected in India during 2007-08 (Kumar et al., 2012). In contrast,Salmonella*enterica* biovarGallinarum remains a major pathogen in many developing poultry industries including Asia and South America (Shivaprasad, 2000).Non-typhoidal serovars (NTS) of *Salmonella* other thanGallinarum and Pullorum are usually established in subclinical infection. However, eradication of serovar Gallinarum from domestic fowl in the United States and England during the mid-20th century opened up the ecological niche for serovar Enteritidis. Since serovar Enteritidis is usually asymptomatic in chickens, contaminated eggs have entered the human food supply and cause outbreaks of Enteritidis-associated salmonellosis(Matthews et al., 2015).*S.* Gallinarum (43.7%), being the most frequent, followed by *S.* Enteritidis (30.6%) and *S.* Typhimurium (21.9%) were the most prevalent serovars in poultry samples from January 2011 to October 2016 received at National Salmonella and Escherichia Centre (NSEC), Central Research Institute, Kasauli, India (Kumar et al.,

2019).The most common serotypes associated with human illness are *Salmonella* Typhimurium and *S.* Enteritidis in the United States and European countries (Lee et al., 2015). This paper is to review the progress in rapid methods for efficient and reliable Salmonella detection methods usingemerging technologies-conventional culture methods, immunology-based assays, nucleic acid-based assays, and biosensors.

Fowl typhoid and avian paratyphoid in India:

Seropositivity (14.69%) was observed in commercial breeder flocks by rapid serum agglutination test with crystal violet stained antigen in seven states of India (Baksi et al., 2017). However, serological cross-reaction with Salmonella serovar Pullorum and Enteritidis (9, 12:gm) limits its application in clinical diagnosis (OIE, 2018). Rajagopal and Mini (2013)reported an outbreak of FT inthree different poultry farms in Kerala, India.A similar outbreak was reported in West Bengal in backyard poultry (Dey et al., 2016). The prevalence of *Salmonella* inchicken broilers in the Tarai region of Uttarakhand in India was documented in correspondence byKumar et al.(2014).This work involved the isolation of *Salmonella*from a total of 343 faecal samples of poultry and pigs, and from 100 tissue samples of broilers collected between January 2011 and July 2012. Thetotal prevalence of *Salmonella* in poultry was 12.28%(8.4% of cloacal samples and 22.0% of tissuesamples). The detected poultry serovars, in decreasingorder of

frequency, were *S.* Typhimurium, *S.*Enteritidis, and *S.* Gallinarum. In a study by Kumariet al.(2013), 23 *Salmonella* isolateswere reported of which 19 samples were identified as *S.* Gallinarum (9, 12) and 4 samples as *Salmonella* Enteritidis(9, 12: gm) from 134 dead poultry birds collected from23 different farms of Haryana.Kumar et al.(2012) identified *Salmonella* Gallinarum (53), *Salmonella* Pullorum (16), *Salmonella* Enteritidis (13) and *Salmonella* Typhimurium (06) in Hisar and adjoiningdistricts, viz. Jind, Bhiwani, Sirsa, Fatehabad and Rohtak regions of Haryana state of India in the year 2007–08 in from dead broiler birds.

PiruthivirajKumar et al. (2015) confirmed seven isolates as *Salmonella* Gallinarumand three isolates as *Salmonella*Pullorumfrom samples of poultry originating in several Indian states Andhra Pradesh, Telangana, Tamil Nadu,Karnataka, Maharashtra, Haryana,Uttar Pradesh.In a study byArora et al. (2015), 253 *Salmonella*isolates were recovered from disease outbreaks in broiler chickens from January 2011 to December 2013 indifferent parts of Haryanaand these isolateswere grouped into 3 groups namely *Salmonella* Gallinarum (183), *Salmonella* Enteritidis (41) and *Salmonella* Typhimurium (29).Samanta et al.(2014) identified 22 *Salmonella* isolates (6.1%) from cloacal swabs of 6 birds (15%, n = 40), from 4 feed samples (10%, n = 40), 8 drinking water samples (20%, n = 40), and 4 eggs (10%, n = 40) in birds reared in backyard method. The isolates belonged to serovars *Salmonella* Enteritidis (6) and *Salmonella* Typhimurium (2).Besides fowl, the disease FT was also reported in ducks in Thrissur, Kerala (Chacko et al., 2017).

Salmonella detection methods:

a) Conventional Culture method: Traditional detection methods include non-selective and selective enrichment,biochemical characterization, and serological identification (Table 1). Suspected samples of faeces, heart blood, liver,bile etc. are to be collected aseptically, processed withpre-enrichment 2% buffered peptone water or selective enrichment with Rappaport–Vassiliadis (RV) broth, selenite broth or tetrathionate broth at 42°C for 48 hours.Commonly used selective solid media are MacConkey's lactose agar (MLA), Xylose Lysine Deoxycholate agar (XLD),salmonella shigella agar (SSA), and brilliant green agar (BGA).Cultural characteristics on solid media wereused for the initial identification of *Salmonella*.Presumptive *Salmonella* colonies are tested in triple sugar iron agar (TSI) forglucose fermentation and lysine iron agar (LIA) forlysine decarboxylase reactions followed by a urease test for screening *Salmonella* spp.These colonies are further subjected to biochemicaland serologicalconfirmation (Rajagopal and Mini, 2013; Dey et al., 2016).

Chromogenic (BBL CHROM/HiChrome[TM] agar Salmonella) and fluorogenic mediahave improved conventional culture methods with faster detection and identification.Kits for rapid biochemical characterization of *Salmonella* are commercially available, including API 20E (bioMerieux, France), Hi Salmonella identification kit (HiMedia,India).

Table 1: Conventional detection methods of *Salmonella* serovars used by different researchers					
Source	**Selective enrichment**	**Isolation media**	**Serotype**	**Positivity (%)**	**Reference**
Faecal samples	RV Broth	Xylose-Lysine-Tergitol-4 (XLT4) agar	*Salmonella* Arizona (35: z24: z23: -)	6/585 (1.02%)	Kar et al., 2020
Spleen, liver, heart, blood	None	MLA, followed by BGA	SG (1,9,12:--)	Farm outbreak investigation	Dey et al., 2016; Pal et al., 2017
Spleen,liver,gall bladder, heart blood	RV broth	MLA, BGA	ND	Farm outbreak investigation	Rajagopal and Mini, 2013
Cloacal swab	Selenite broth, RV Broth	BGA	*S.* Enteritidis (9,12:g, m,-) *S.* Typhimurium (4,12:i:1,2)	6/360 (6.1%)	Samanta et al., 2014
Heart blood,organs	RV Broth	MLA, BGA, SSA, XLD agar	SG (1,9,12:- -), *S.* Enteritidis (9,12:g, m,-)	23/134 (17.16%)	Kumari et al., 2013
Bile,Heart blood,liver	None	MLA, BGA	SG (9,12:-:-), SP (9,12:-:-),		Kumar et al., 2012

spleen,ova			*S.*Enteritidis (9,12:g,m:-), *S.*Typhimurium (4,12:1:1,2)		
Liver,intestine,spleen,egg	Selenite cystine broth, Tetrathionatebrilliant green broth	MLA, BGA, Hektoen enteric agar	*S.*Heidelberg (1,4,5,12:r:1,2) *S.*Typhimurium (4,12:i:1,2), *S.*Ayinde (1.4,12,27:dz6), *S.*Essen (4.12:gm:-), *S.* Kastrup (6,7:e,n,z15:1,6)	7/260 (2.7%)	Menghistu et al., 2011
Liver, lungs, spleen, heart, intestines and Bursa	Tetrathionate broth (TB)	XLD, (BGA	SG (9,12:-:-), untyped	42/182 (23.08%)	Kashani et al., 2021

* ND = not done

b) Serotyping: Serotyping of the biochemically confirmed isolates is performed from facilities at the National *Escherichia* and *Salmonella* Centre, Kasauli, Himachal Pradesh, India (Pal et al., 2019) or National *Salmonella* Centre at Indian Veterinary Research Institute, Bareilly, Uttar Pradesh, India (Kar et al., 2020) by Kauffmann –White scheme by slide agglutination with O- and H-antigen specific sera.

c) Serological tests: It employ specific mono- or polyclonal antibodies to bind with somatic, lipopolysaccharide (LPS) or flagella antigens for detection of *Salmonella* spp. in a variety of sample matrices. Many assays including enzyme-linked immunosorbent assay (ELISA), latex agglutination tests, immunodiffusion, and immunochromatography are available commercially. Several commercial validatory bodies (like the Association of Official Analytical Chemists, AOAC, USA or similar organizations) certified tests were designed for rapid detection of Salmonella in kit format with ELISA systems (IDEXX SE Ab X2 test for S. Enteritidis), latex agglutination (Oxoid Salmonella test kit), immunodiffusion for motile Salmonellae (Biocontrol/Merck 1-2 Test[®] for *Salmonella*).

Bautista et al. (2002) detected 19 of 22 strains of *Salmonella* spp. in an immune-chromatography-strip based diagnostic kit for *Salmonella* but failed to detect *S. worthington, S.* Choleraesuis var. *kunzendorf,* and *S. johannesburg.* Immunochromatography-based tests may be based on dipstick or lateral flow assay format. Animmunochromatographic assay was developed for the simultaneous detection of *S.* Typhimurium and Enteritidis in a single chip by Moongkarndi et al. (2011). After a 6–24-hr enrichment step, contamination of *S.* Typhimurium and *S.* Enteritidis at 1cfu/ml or greater can be detected.

The commercially available *Salmonella* lateral flow test is simple, rapid and reliable.

d) Nucleic acid-based assays: The conventional methods of biochemical identification are laborious and time-consuming. Therefore, rapid DNA-based detection techniques of the *Salmonella* serovars Gallinarum and Pullorum were used (Pal et al., 2019).

i) Conventional PCR: For confirmation of *Salmonella* serovars, several studies have developed PCR assays to test their ability to detect these *Salmonella* serotypes (Table 2). Allele-specific PCR assay based on *rfbS* (Shah et al., 2005), polymorphic areas of *glg*C and *spe*C genes (Kang et al., 2011; 2012), fimbrial operon gene *bcfD* (Zhuang et al.,2014), flagellar biosynthesis gene *flhB* (Xiong et al.,2016), fimbrial operon gene *sefA* (Gong et al.,2016), flagellar biosynthesis gene *flhB* (Xiong et al., 2017), SPUL 2693 (Xu et al.,2018), *stn*, I137_08605 and *rat*A genes in multiplex PCR (Xiong et al., 2018) were used by various workers.To increase the accuracy and to decrease the time of analysis, some multiplex PCR methods (Batista et al., 2016) were developed allowing the simultaneous identification of multiple pathogens in one sample within a single reaction (Oliveira et al., 2002;Cortez et al., 2006).

ii) Real-time PCR: Real-time PCR or quantitative PCR facilitates direct detection of PCR products in less than half the time of conventional PCR, with no requirement of post-processing steps.Cheng et al. (2008) developeda real-time PCR method with custom-designed primers and a TaqMan probe to detect the presence of a 262-bp fragment of the *Salmonella*-specific *invA* gene.Thequantitative PCR developed by Silva et al. (2011) includes the detection of *Salmonella* spp. and *S.*

Enteritidis, but it was not able to detect *S*. Typhimurium. Rubio et al. (2017) developed the multiplex qPCR for detecting *S*. Gallinarum and *S*. Pullorum. Heymans (2018) developed and evaluated multiplex qPCR targeting the *invA*, the STM4200, and the SEN1392 genes for the simultaneous detection of *Salmonella* spp., *S. enterica* serovar Typhimurium and *S. enterica* serovar Enteritidis in various (food) matrices. The *invA* gene was expected to be detected in all *Salmonella* strains, whereas the STM4200 and SEN1392 genes were expected to

detect *S*. Typhimurium and *S*. Enteritidis strains, respectively. However, one limitation of this qPCR was serovars S. Derby (n = 2), *S*. Goldcoast (n = 1) and *S*. Rissen (n = 5) were also amplified by the STM4200 primer set. The latter two serovars are occasionally identified in poultry meat.Commercial kits based on conventional PCR and real-time PCR are successfully used for routine Salmonella screening in poultry feed, eggs, raw meat etc.

Table 2: Conventional PCR assays for molecular detection of *Salmonella* serovars

Gene	Primers	Oligonucleotides (5'-3')	Amplification product (bp)	Positivity in Salmonella serovar	Reference
invA	forward S139 reverse S141	GTGAAATTATCGCCACGTTCGG GCAA TCATCGCACCGTCAAAGGAACC	284	All serovars	Rahn et al., 1992
glgC	SG-L SG-R	GATCTGCTGCCAGCT CAA GCGCCCTTTTCAAAACATA	174	SG	Kang et al., 2011
speC	SGP-L SGP-R	CGGTGTACTGCCCGCTAT CTGGGCATTGACGCA AA	252	SP,SG	Kang et al., 2011
9R22C9	9R-L 9R-R	CTTTACGGGCAAACCACAGT TGCTGCTCTTTTTCCATCTCA	119	SG strain 9R	Kangetal., 2012
SPUL 2693	Forward Reverse	CGGGGTACCATGGATAAGCGTC ATGAGA CCGGAATTCTCATTTCTGTCCCT CCTCAATGGCT	2160	SG	Xu et al., 2018
fliC	Fli15 Typ04	CGGTGTTGCCCAGGTTGGTAAT ACTGGTAAAGATGGCT	620	Typhimurium	Oliveira et al., 2002
sefA	A058 A01	GATACTGCTGAACGTAGAAGG GCGTAAATCAGCATCTGCAGTA GC	488	SP/SG,*S.*Enteritidis, *S.*Berta	Oliveira et al., 2002

SG – *Salmonella* Gallinarum, SP – *Salmonella* Pullorum

Table 3: Comparative analysis of *Salmonella* detection methods

Method	Reaction time	Sample	Commercial kits	Remarks
Culture	3 step methods-Pre-enrichment, selective enrichment,plating on selective media,then bio-typing, total of 5-6 days	Faeces,organs	Enrichment broth,selective agar	Confirmatory,low-sensitivity
ELISA	8 hrs	Serum, Enrichment broth culture	IDEXX SE Ab X2 test for SE(gm-flagellin based),*Salmonella* Antigen ELISA Kit,Creative Diagnostics	Screening test, specificity issues
Latex agglutination tests	Pre-enrichment, selective enrichment step	Enrichment broth culture	Oxoid Salmonella test kit	Presumptive Screening test, non-motile strains not

				detected
Immunodiffusion	Pre-enrichment, selective enrichment step, 2 days	Enrichment broth culture	Merck 1-2 Test® for Salmonella	Motility based test
Lateral flow assay	Pre-enrichment, selective enrichment step, 20 mins test time	Boiled enrichment broth culture	Merck Singlepath®	Presumptive,Point-of-care tests
Conventional PCR	Pre-enrichment, selective enrichment step, DNA extraction,2 days	DNA from Broth culture	Hi-PCR® Salmonella Semi-Q PCR Kit,HiMedia	Highly sensitive,qualitative
Realtime PCR	Pre-enrichment step of a few hours, selective enrichment in RV broth(optional), followed by DNA extraction(30 mins) and PCR amplification(70 mins)-total 24-48 hrs.	DNA from Broth culture	IDEXX Real PCR™ Salmonella spp.; MicroSEQ® Salmonella spp. Detection Kit,Applied Biosystem, TaqMan® Salmonella enterica Detection Kit, Applied Biosystem; iQ-Check Salmonella, Bio-Rad	Highlysensitive, detect 1−3 colony forming units (cfu) per25 grams of sample

iii) Loop-mediated isothermal amplification (LAMP) assay: Several assays have been successfully established to detect *Salmonella*,however, most of these assays are unable to determine *Salmonella* serovars such as LAMP assay based on *InvA* (Yang et al.,2013), LAMP assay based on *bcfD* (Zhuang et al.,2014).

e) Biosensors:A number of biosensors that use antibodies as a recognition element for *Salmonella* sp. were developed, but more and more devices are now also reported based on nucleic acids-based DNA aptamers by several researchers as reviewed by Paniel and Noguer (2019).

Conclusion:

The traditional methods for Salmonella detection are based on cultural, serological, and biochemical properties using selectivemedia.However, rapid methods for *Salmonella* detection have become increasingly important; many are approved bycountries with advanced rearing systemsand are considered desirable as a future approach.Sample processing techniques in the two steps ofpre-enrichment and enrichment also appeared to affect the sensitivity of *Salmonella* detection in low-concentration, viable but not culturable (VBNC) samples,particularly with the presence of disinfectants. More reliable and efficient new assays with precision are likely to replace the existing conventional methods.

Conflict of interest:

The authors declare that no conflict of interest exists.

Author's contribution:

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Diagnosis of Various Bacterial Zoonotic Diseases in Human and Bovine populations in Haryana State under the umbrella of One Health

Punit Jhandai [(1)], **Shanmugasundaram K** [(1)], **Dolly Gambhir** [(2)], **Tushar Nale** [(3)], **Ajit Shewale** [(3)], **Simmi Tiwari** [(3)], **Harisankar Singha** [(1)*]

[(1)] ICAR-National Research Centre on Equines, Hisar-125001, India., [(2)] State Integrated Disease Surveillance Programme (IDSP), Panchkula, Haryana, India, [(3)] National Centre for Disease Control, New Delhi, India

Abstract

Zoonotic diseases, also known as zoonoses, are infectious diseases that can be transmitted from animals to humans or vice versa. These diseases are of significant concern to public health as they can cause severe illness, and in some cases, death, so early detection and diagnosis are crucial for effective treatment and prevention. The study was conducted from July 2022 to July 2023 to test suspected samples of both human and animal populations for various common zoonotic diseases at district surveillance laboratories and district veterinary surveillance laboratories, respectively, in Haryana state. Zoonotic diseases such as tuberculosis, paratuberculosis, salmonellosis (typhoid), brucellosis, leptospirosis, and scrub typhus were included in the present study. Out of 25,911 animal samples, 216 were positive for brucellosis as detected through ELISA. None of the samples were found positive for bovine tuberculosis and bovine paratuberculosis. Out of 13,693 suspected human samples, 2,048 were positive for human tuberculosis, and among 22,750 samples, 3884 were positive for salmonellosis. For scrub typhus, 15 out of 99 samples were positive, while 4 out of 70 were positive for leptospirosis. The diagnosis of zoonotic diseases is a complex process that requires a multidisciplinary approach. Early diagnosis and prompt treatment of these diseases are essential for ensuring optimal health outcomes and preventing the spread of these zoonotic diseases to humans and animals.

Keywords: Zoonotic disease, Humans, Bovines, Detection, One Health

Introduction:

Zoonotic infections known for their capacity to transfer between animals and humans (or from humans to animals) pose a substantial worldwide public health issue (Jones et al., 2008). These diseases, originating from various pathogens like bacteria, viruses, parasites, and fungi, hold the potential to induce severe illnesses and fatalities among both human and animal communities (Taylor et al., 2001). The inter linkage between humans, animals, and environmental elements amplifies the possibility of transmitting zoonotic diseases (Hassell et al., 2017). Thus, diverse ways of transmission, infection routes, and clinical manifestations of zoonotic infections pose challenges to healthcare systems and veterinary practices worldwide (Behera et al., 2021). Therefore, increased attention, monitoring, and inter-sectoral efforts are of utmost importance to implement successful measures for the prevention and control of zoonotic diseases.

Among the many zoonotic diseases, tuberculosis (TB) caused by *Mycobacterium tuberculosis*, remains a significant public health concern worldwide, affecting both humans and animals (Anaelom et al., 2010). Paratuberculosis, caused by *Mycobacterium avium* subspecies paratuberculosis, is another zoonotic disease primarily affecting cattle, sheep, and goats, with potential transmission to humans (Whittington et al., 2005). Salmonellosis, predominantly known for causing typhoid fever and paratyphoid, represent another significant zoonotic infection with various serovars capable of infecting both humans and animals (Majowicz et al., 2010). These pathogens pose risks of infections ranging from gastroenteritis to severe systemic diseases in humans. Brucellosis, caused by *Brucella* species, is a major zoonotic infection posing significant health and economic burdens globally (Seleem et al., 2010). Leptospirosis, caused by spirochetes of the genus *Leptospira*, is another notable zoonosis with a worldwide distribution and potential transmission between animals and humans (Costa et al., 2015). Moreover, scrub typhus, caused by *Orientia tsutsugamushi*, represents a critical zoonosis transmitted by chiggers, posing significant health risks. The complexity and interdependency of zoonotic diseases necessitate comprehensive understanding, effective surveillance, and control measures involving collaboration among public health agencies, veterinarians, and healthcare providers (Taylor et al., 2001; Morse et al., 2012).

To strengthen the surveillance system of zoonotic diseases in Haryana state, ICAR-National Research

Centre on Equines, Hisar was designated as regional coordinator for National One Health Programme for Prevention and Control of Zoonoses (NOHP-PCZ) scheme under NCDC, New Delhi. In our previous attempt for prioritization of zoonotic diseases in Haryana (India) using one health approach, it was observed that bovine tuberculosis and brucellosis ranked 2nd and 6th in priority list, respectively (Thukral et al., 2023). The objective of the present study was to gather data from both medical and veterinary fields to provide a holistic view of prevalence of bacterial zoonotic diseases in humans and animals and so as to improve surveillance capabilities, enabling early detection and response to disease outbreaks.

Materials and Methods:

Study area and period: Haryana is a state located in the north-westerns parts of India with 22 districts, about 6848 villages and 154 towns. The state of Haryana is situated between 27.39^oN and 30.35^oN latitude and 74.27^oE and 77.36^oE longitude (Shaloo and Kaur, 2016).The present study was conducted from July 2022 to July 2023.

Targeted zoonotic diseases: The study was focused on targeted zoonotic diseases such as brucellosis, bovine tuberculosis, paratuberculosis, salmonellosis (typhoid), leptospirosis, and scrub typhus.

Testing of livestock species for important zoonotic diseases: The samples were collected from suspected animals at the government veterinary hospitals and were sent to disease diagnostic labs for testing. Samples like faeces, blood, urine, and swabs were collected from suspected animals (irrespective of sex and age) and were transported to the laboratory for further testing. The samples were also collected by the Indian Council of Agricultural Research-National Research Centre on Equines (ICAR-NRCE), Hisar as routine surveillance and tested at NRCE. Tuberculin test, RBPT and ELISA were used for the diagnosis of tuberculosis and brucella, respectively. The test kits like Anigen Rapid Bovine TB Ab Test Kit (Maxanim, USA), RBPT antigen (Innovative Diagnostics, France), Bovine Tuberculin PPD 3000/Avian Tuberculin PPD 2500 kit (Applied Biosystems/ BOVIGAM), Brucella Antibody ELISA Kit (Ringbio) were used in this study. All positive controls were supplied with the kits.

Testing of Humans for important zoonotic diseases: Human samples were collected from suspected cases showing symptoms of diseases at government civil hospitals and were sent to district disease diagnostic labs for testing. Widal test and sputum microscopy/CB-NAAT (Widal Test kit by Anamol Lab Pvt. Ltd, Maharashtra) were employed to diagnose salmonellosis and tuberculosis respectively, while IgM ELISA was used to diagnose both scrub typhus [Human IgM (Sandwich ELISA) ELISA Kit - LS-F10546, LS Bio]and leptospirosis [Human IgM (Competitive EIA) ELISA Kit - LS-F27712, LS Bio].

Compilation of reports and analysis: Data regarding abovementioned zoonotic diseases was collected monthly from district surveillance laboratories and district veterinary surveillance laboratories. Further, this data was compiled in Microsoft Excel and analyzed at ICAR-NRCE. Maps were prepared using QGIS version 3.34.1.

Results and Discussion:

Among 25,911 animal samples, 216 were positive for brucellosis (Table 1). The highest positivity rate (7.25%) was observed in Karnal district followed by Panchkula (2.44%), Kurukshetra (1.89%), Sonipat (1.30%), and Kaithal (1.09%). Other districts like Hisar, Yamuna Nagar, Panipat, and Fatehabad showed less than a 1% positivity rate (Table 1, Figure 1). Previous serological studies showed 12- 29% prevalence rate of bovine brucellosis in Haryana (Chand and Sharma, 2004; Kumar and Chand, 2011; Khurana et al., 2012; Chand and Chhabra, 2013) as Haryana is known as dairy state and promote the dairy farming venture. Surprisingly, none of the 115 faecal samples/animals tested for bovine tuberculosis, and 154 faecal samples/animals for bovine paratuberculosis were found positive. The actual extent of the illness remains unclear as routine surveillance data is absent in many developing nations including India. Despite India's progress in achieving the End TB goal, bovine tuberculosis (bTB) remains mostly concealed (Refaya et al., 2020).

Among the human samples, 2,048 out of 13,693 suspected samples were positive for human tuberculosis (Table 1). For human tuberculosis positivity rate in various districts were 59.90% in Nuh-Mewat, 25.9% in Palwal, 18.44% in Kurukshetra, 14.92% in Sirsa, 10.09% in Rohtak, 13.83% in Mahendergarh, 9.87% in Kaithal and 7.34% in Rohtak (Table 1, Figure 2). Many studies were conducted in Haryana and other part of India to diagnosed its positivity rate among household contacts of newly diagnosed tuberculosis patients as an efficient tool for early diagnosis and treatment of active TB, thus minimizing the severity and decreasing transmission (Chawla et al., 2020; Gupta et al., 2016; Nair et al., 2016; Khaparde et al., 2015). Identifying active TB cases allows for targeted public health interventions such as contact tracing, where individuals who have been in close contact with an infected person can be screened and treated, if necessary, for preventing the spread of the disease.

For salmonellosis in human population, 3,884 out of 22,750 were positive for salmonellosis (Table 1). The highest positivity rate (21.88%) were observed in Hisar,

followed by Kurukshetra (17.94%), Kaithal (17.31%), Palwal (15.63%), Rohtak (14.05%), Rewari (12.71%), Mewat (10.43%), Faridabad (8.62%) and Mahendergarh (5.96%) (Table 1, Figure 2). The Widal slide test, an agglutination-based assay, serves as a valuable tool for quickly diagnosing typhoid or paratyphoid fever during the early stages of the illness (Sherwal et al., 2004). Widal test has been used for over a century in developing countries for diagnosis but it has been reported to havelow sensitivity, specificity and positive predictive value (Sherwal et al., 2004; Begum et al., 2009). In different district of Haryana, 9-12% incidence rate were observed for salmonellosis by many researchers (Banerjee et al. 2014; Sharma et al., 2015). The rise in seropositivity might be linked to the population growth and poor hygienic standards. As this disease is mainly preventable, it's crucial to educate individuals about adopting safer hygiene practices and the accessible vaccination methods.

For scrub typhus, 15 out of 99 humans were positive, and 4 out of 70 were positive for leptospirosis. However, 100% positivity rate was observed in Karnal, Kurukshetra and Yamuna Nagar for scrub typhus and similar trend was observed in Karnal and Yamuna Nagar for leptospirosis (Table 1, Figure 1). In rural areas, farmers and agricultural workers are frequently affected, particularly during sowing and harvesting seasons, as well as meteorological events like monsoons. The transmission of leptospiral infection is closely linked to factors such as the presence of farm animals and rodents, known carriers of the bacteria, in agricultural land. The survival of *Leptospira* causing bacteria in wet and humid environments, coupled with regular human engagement in agricultural and animal-rearing activities, serves as the primary contributors to its transmission (Sethi et al., 2010). Northernmost parts in Karnal and Yamuna Nagar districts in the state received high rainfall during monsoon than other part of Haryana (Singh, 2018) and Karnal is fully irrigated districts, having high cultivated land for rice-wheat crops (Kalra et al., 2001). This might be the reason of high positivity rate in these districts for leptospirosis and scrub typhus.

Targeted surveillance would be helpful for better understanding the prevalence of zoonotic diseases in humans and animals. Such as, testing of humans who regularly come in-contact with animals infected with zoonotic diseases and vice versa will be welcome approach for early detection of potential zoonotic disease transmission. It will also help in understanding the root cause of zoonotic diseases, development of comprehensive control strategies, including the implementation of biosecurity measures and health education programs for both animal handlers and the general public.

Table 1: Details of zoonotic diseases diagnosed in animals and humans in Haryana				
Diseases	**Diagnosis test performed**	**Suspected case**	**Positive case**	**District from which positive cases reported**
Bovine brucellosis	RBPT	25,911	216	Karnal, Panchkula,Kurukshetra, Sonipat, Kaithal, Hisar, Yamuna Nagar, Panipat and Fatehabad
Bovine tuberculosis	PPD intradermal/ZN staining	115	0	-
Bovine paratuberculosis	PPD intradermal/ZN staining	154	0	-
Human tuberculosis	Sputum microscopy/ CB-NAAT	13,693	2,048	Mewat, Palwal, Kurukshetra, Sirsa, Rohtak, Mahendergarh, Kaithal and Rohtak
Human salmonellosis	Widal test	22,750	3,884	Hisar, Kurukshetra, Kaithal, Palwal, Rohtak, Rewari, Mewat, Faridabad and Mahendergarh
Human scrub typhus	IgM ELISA	99	15	Karnal, Kurukshetra and Yamuna Nagar
Human leptospirosis	IgM ELISA	70	4	Karnal and Yamuna Nagar
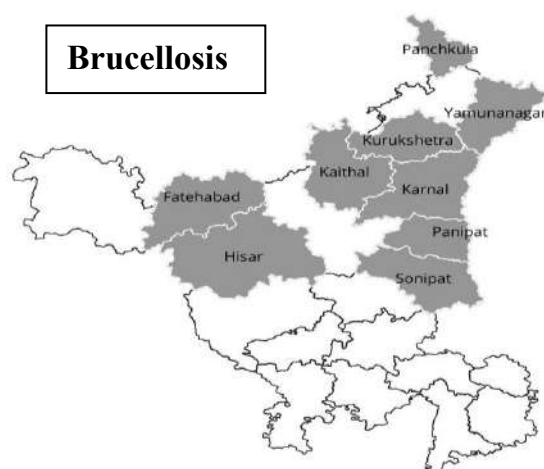
Brucellosis

Figure 1: Map depicting the district of Haryana having positive cases of brucellosis in the animal population

Tuberculosis

Salmonellosis

Leptospirosi

Scrub Typhu
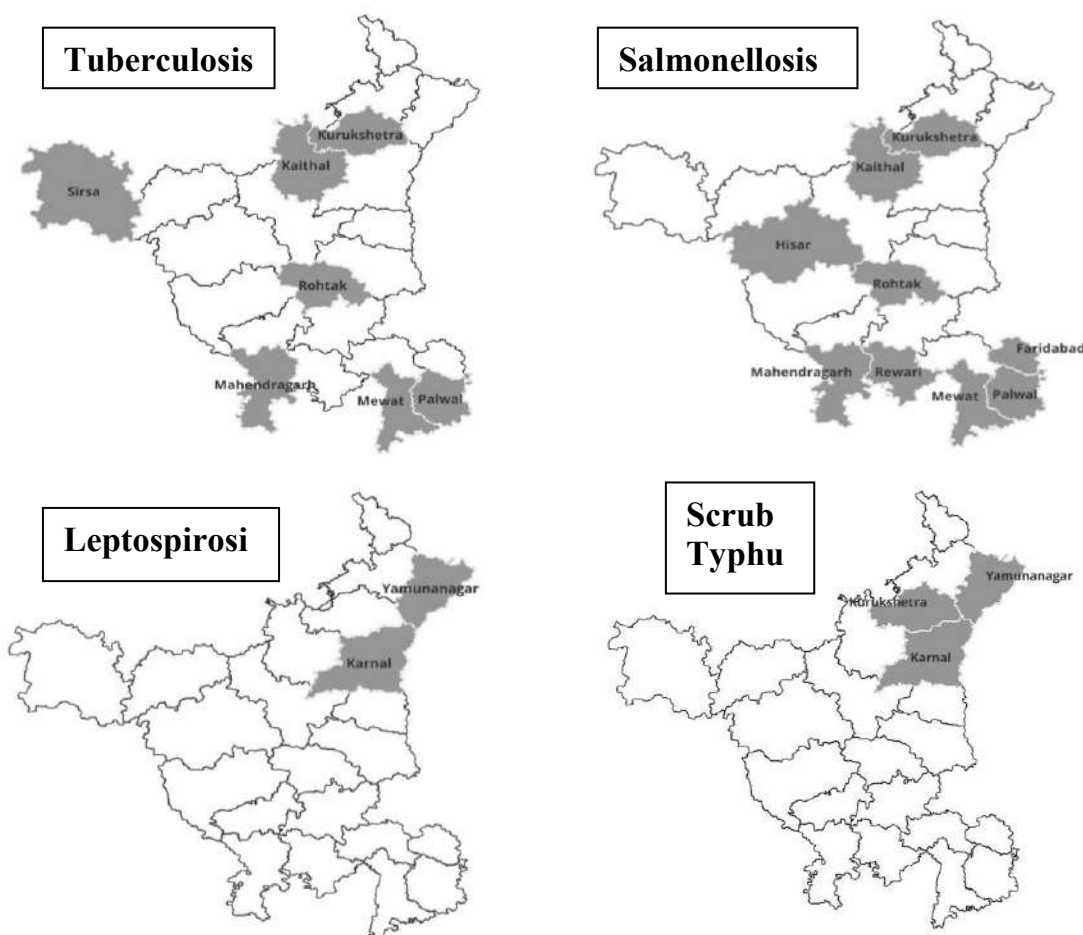
Figure 2: Map depicting the district of Haryana having positive cases for various zoonotic diseases in humans

Conclusion:

To sum up, the identification of zoonotic diseases demands a multidisciplinary approach among public health agencies, veterinarians, and healthcare practitioners. Early diagnosis and prompt treatment of these diseases is necessary to ensure optimal health

outcomes and prevent the spread of these diseases to animals and humans. The findings underscore the need for enhanced surveillance, targeted preventive strategies, and public health interventions, especially in areas prone to zoonotic diseases. This study lays the groundwork for further research, emphasizing the importance of interdisciplinary collaboration to combat zoonotic diseases in efficient manner.

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Conflict of Interest:

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Author's contribution:

Punit Jhandai: Investigation; Conceptualization; Writing – original draft; Formal analysis; Data curation. Shanmugasundaram K: Writing – review and editing; Validation. Dolly Gambhir: Supervision, Monthly reporting. Tushar Nale: Conceptualization, Monitoring and assessment. Ajit Shewale:Conceptualization, Monitoring and assessment. Simmi Tiwari: Supervision; Conceptualization. Harisankar Singha: Supervision; Conceptualization; Writing– review and editing; Validation.

Ethical statement:

Biological samples from humans or animals were collected at the Out Door Patient Department (OPD) of respective hospitals upon consent of human or animal keeper for disease diagnosis.

Acknowledgments:

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A Handy Method to Estimate Titer of Classical Swine Fever Virus in PK-15 Cells

Chayna Singha Mahapatra, Aruna Kuniyal, Shyma K. Latheef, Pronab Dhar*

Division of Biological Standardization, ICAR-Indian Veterinary Research Institute, Bareilly, Uttar Pradesh, India

Abstract

Determination of virus titer is the key factor for development and quality control of classical swine fever (CSF) cell culture vaccines. Like any other non-cytopathic effect producing viruses, classical swine fever virus titration is done by immunological methods *e.g.* Fluorescent Antibody Test (FAT). These classical assays for the determination of Tissue Culture Infective Dose 50 ($TCID_{50}$) are time-consuming and require huge reagents. Hence, they are not very suitable for handling a large number of samples. An accurate and reliable technique for $TCID_{50}$ determination by FAT in cover-slip culture was optimized for titration of classical swine fever. Optimization of FAT in LAB TEK slide chamber could do away the tedious manipulation of cover-slip culture without affecting its final outcome. This newly optimized method of titration has shown 100% similar result to that of one full proof titration method of CSF virus in PK-15 cells in terms of fluorescent positive cells in wells and their fluorescent intensity.

Key words: Classical swine fever; Non-cytopathic; Virus titration; Titer; Immunofluorescence

Introduction:

Classical swine fever (CSF) is a re-emerging disease in many countries. The causative agent of CSF is classical swine fever virus which belongs to the genus *Pestivirus* of family *Flaviviridae* (Edwards et al., 2000; Postel et al., 2019). CSF has been eradicated from a number of countries (Sandvik et al., 2005; Ji et al., 2015) but its endemicity still prevails in parts of Asia and some other countries from South and Central America, and in parts of the Caribbean islands (Postel et al., 2013; Blome et al., 2017).Thus, endemic and re-emerging nature of CSF virus continues to threaten worldwide pork production and food security in developing countries (Saatkamp et al., 2000).

Despite the devastating nature, the disease is well controllable by vaccination in the endemic countries. For that, availability of efficacious and potent vaccine in the field settings is of farthermost important. Currently, as per requirement of any Pharmacopoea, each dose of CSF vaccine must contain at least 100 PD_{50} to pass the potency test (Indian Pharmacopoeia). To avoid challenge experiment of animals for vaccine potency evaluation, an alternate potency test based on the Fluorescent Antibody Virus Neutralization (FAVN) titer of vaccinated animal sera has been evaluated (Manu et al., 2023). To entirely exclude live animal use for the CSF cell culture vaccine evaluation, determination of virus titer in the finished vaccine product (virus titration) would help in quality control. Though most of the live virus vaccines are titrated by observing the cytopathic changes in cell culture produced by the virus, however with a few exceptions, the vast majority of CSF viruses are non cytopathogenic and do not produce visible cytopathic changes (Gallei et al., 2008; Ganges et al., 2020). Being a non-cytopathic effect (CPE) producing virus, virus titrations are done by immunological methods such as Fluorescent Antibody Test (FAT) or Immunoperoxidase Test (IPT) using specific hyper immune sera or monoclonal antibodies (Bouma et al., 2001). The assays were set up to quantify the infectious particle using antibody labeling of viral protein. The FAT is proven to be more sensitive and specific than IPT (Jafari et al., 2015; Zhang et al., 2017).This technique is based on the detection of infected cell at the single cell level using immune-detection of viral proteins by fluorescent microscope (Wang et al., 2020). To avoid pitfalls, virus infected cells must be clearly distinguished from the uninfected ones by emitting a fluorescent signal above the auto-fluorescent background. However, there are still further scopes to improve the detection method by either simplifying the existing FAT steps or by other measures which existed but not used for CSFV titration so far. So, the study is aimed at developing a suitable substitution of existing method/s which will be much faster, economical and easier to perform.

Materials and Methods:

Cells:

Porcine kidney-15 (PK-15) cells (ATCC, USA), free from mycoplasma and pestivirus contamination, maintained at Division of Biological Standardization, ICAR-IVRI, Izatnagar were used for the study. The cells were cultured in Eagle's Minimal Essential Medium (EMEM, HiMedia, India) supplemented with 10% pestivirus free foetal bovine serum (Invitrogen, USA) and 100X antibiotic and antimycotic solution (HiMedia,

India) and kept at 37°C under 5 % CO_2 tension and 100% relative humidity.

Virus:

Porcine kidney (PK)-15 cell culture adapted virus developed by adapting indigenous virulent isolate was used. This very high yielding vaccine strain, named as IVRI-CSF-BS and maintained in Division of Biological Standardization, ICAR-IVRI, Izatnagar was used in the study.

Previous method of titration:

Virus titration was performed in 24 well cell culture plate following protocol by Dhar et al., 2022. Cells were allowed to grow on sterile surface of cover-slips to be used in titration. Briefly, tenfold serial dilutions of vaccine virus were prepared and 100 µL of each virus dilution was transferred to the respective well keeping cell control and plate was incubated. After completion of incubation, cells were fixed and quenched to wane off auto fluorescence due to used fixative. After that addition of detergent acted as porogen helping Mab-FITC (diluted in blocking buffer) to reach viral protein inside cells. Finally stained cells were mounted on slides after washing to remove unbound conjugate.

New method of titration:

Lab-Tek^{TM} chambers were marked properly keeping cell control. PK-15 cells were subcultured and two hundred microliter of PK-15 cell suspension (1×10^5) cells were distributed per chamber (Figure 1). Fifty microliter of 10 fold serially diluted virus was added in respective wells keeping one cell control and mixed gently. Infected cells incubated for 72 h at 37°C, 5% CO_2 in a humidified chamber. Up on incubation spent media was discarded and washed and then developed based on modified FAT protocol. Cells were fixed with 100 µL of 3% para-

formaldehyde. Then fixed cells were washed with PBS and treated with 100 µL of 50 mM NH_4Cl. After that all wells were treated with 100 µL of 0.01% Triton X-100 and washed three times with PBS. Then wells were incubated with 40 µL of 1:40 diluted MAb-FITC (MAb against CSFV-E2 protein,) for 30 minutes keeping in a moist chamber and again washed thrice. Finally sides of the chamber was removed and cover slip was mounted on respective wells.Cells were observed under fluorescent microscope and titer was calculated following Spearman, 1908 and Karber, 1931 method and results were recorded.

Results and Discussion:

FAT in Lab-Tek ^{TM}8-chamber slides was able to reproduce the inoculum titer exactly to that of the FAT titer in 24 well plate cover-slip culture. Using the old method obtained titer was $10^{7.5}$ $TCID_{50}$/mL. Newly optimized method of titration was showing 100% similar result in terms of fluorescent positive cells in wells and their fluorescent intensity. FAT in Lab-Tek ^{TM} chamber showed positive signal up to 10^{-6} virus dilution but no fluorescence was observed in 10^{-7} dilution of virus. Hence calculated titer following the optimized method in Lab-Tek ^{TM} chamber has also produced $10^{7.5}$ $TCID_{50}$ mL^{-1} (Figure 2).

Figure 1: Lab-Tek^{TM} Chamber Slide System for growing cells on a standard microscopic slide for on-spot visualization after staining
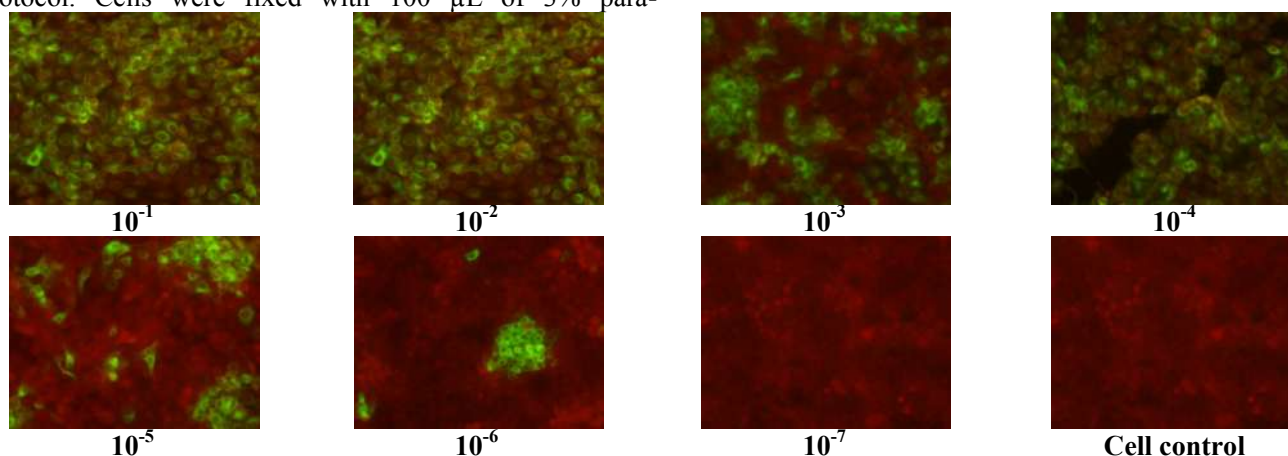
Figure 2: PK15 cells infected with CSF virus showing green fluorescence in cell cytoplasm. The infected and non infected cells in a Titration of the CSF virus in Lab-Tek ^{TM} slide chamber where fluorescence was found up to 10^{-6} dilution of virus. Socalculated titer was $10^{7.5}$ $TCID_{50}$/mL. 10^{-7} dilution of virus and cell control did not show any green fluorescence.

Producibility of CSF virus is measured by its demonstration of viral bodies inside cells and non-cytopathic nature of the virus added extra hardship in doing that. Growth of CSF virus in the cells is usually visualized by using immunological technologies with fluorescent or conjugated antibodies and virus titration is done by specific method like FAT or IPT (Bouma et al., 2001; Chander et al., 2014; Mahapatra and Dhar, 2021). There is always a need of alternatives which can ensure consumption of less sample and reagents, easy to perform and requires less effort and time and incurs less cost. A full proof method of titration of CSF cell culture vaccines employing FAT protocol in 24 well plate cover slip culture is in current use (Dhar et al., 2022). With the use of modified protocol, reagents and buffer consumption can be reduced to $1/4^{th}$. Development of FAT in Lab-Tek ^{TM} slide chamber could also do away the tedious manipulation of cover slip culture without affecting its final outcome. Though use of Lab-Tek ^{TM} slide chamber is higher cost demanding, it can be applied when easy handling is required by unskilled personnel.

Conclusion:

Any non-CPE viruses are difficult to work with and CSF virus was not an exception. Although FAT is gold standard for its detection and titration, the test is not without many disadvantages such as labour intensive, requiring serious cell culture interventions etc. Modified FAT protocol has successfully reduced the buffer consumption and FAT in Lab-Tek ^{TM} slide chamber could have successfully reduced the tedious manipulation of cover slips.

Conflict of Interests

The authors declare that they have no known competing interest.

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Assessment of Gastro intestinal Parasite Prevalence in Dairy Cattle in Three Agro-Climatic Zones of West Bengal

Probhakar Biswas [(1)*], Triveni Dutt [(1)], B H M Patel [(2)], Shyamal Kumar Mondal [(3)], Mrityunjay Mandal [(4)], Sanjoy Datta[(5)], Debasish Saha[(5)], Amitava Roy [(5)]

[(1)] ICAR-Indian Veterinary Research Institute, Bareilly, Uttar Pradesh, Present address: WBUAFS, Kolkata, [(2)] ICAR-Indian Veterinary Research Institute, Hebbal, Bengaluru, Karnataka, [(3)] ICAR-Agricultural Technology Application Research Institute, Kolkata, West Bengal, [(4)] Animal Resources Development Department, West Bengal, [(5)] West Bengal University of Animal and Fishery Sciences, Kolkata

Abstract

Gastrointestinal parasites pose constant threat to dairy animals, greatly affecting their health, well-being, and production. The study was conducted to determine the prevalence of gastrointestinal parasites in dairy cattle in three agro-climatic zones of West Bengal, namely Zone-I (Hilly zone), Zone-II (New alluvial zone) and Zone-III (Coastal saline zone). A total of 1419 faecal samples were collected and analyzed through standard sedimentation and salt flotation technique. In total, 270 animal owners were interviewed in regards to deworming practices. Out of the total 465 samples analyzed, 176 (37.85%) samples were positive for mono or mixed infection in zone-I. The most prevalent were Strongyle (26.02%) followed by Amphistome (22.15%), *Moniezia* sp. (19.14%), *oocyst* of *Eimeria* spp. (12.04%), *Fasciola* sp. (4.09%), *Strongyloides* sp. (4.08%) and *Toxocara* sp. (1.72%). In zone-II, out of 481 samples, 157 (32.64%) were positive and the prevalence of parasites were as Strongyle (28.90%), Amphistome (26.20%), *Fasciola* sp. (14.14%), *Strongyloides* sp. (7.28%)*, Moniezia* sp. (6.86%), Coccidian oocyst (4.99%), and *Trichuris* sp. (3.12%). Out of 473 samples in zone-III, 203 (42.92%) were positive. The highest percentage of samples were positive for Amphistome (31.92%) followed by Strongyle (18.82%), *Fasciola* sp. (10.99%), *Strongyloides* sp. (7.82%)*, Coccidian oocyst (5.92%), *Moniezia* sp. (4.02%) and *Trichuris* sp. (4.86%). The Chi square value indicates a highly significant difference between zones in the healthcare practice of administering dewormer to dairy animals. Overall, 44.81% farmers were deworming adult animals sometimes in all the zones and only 26.67% dewormed their calves frequently. The Chi square value indicates highly significant (p<0.01) difference in deworming of calves in different zones of West Bengal. The findings of the present study may help to develop a strategic control approach for gastrointestinal parasite infections in cattle.

Keywords: Gastrointestinal parasites, Dairy cattle, West Bengal, Deworming

Introduction:

Dairy farming is an essential part of rural life in India, and the livestock business is a major contributor to agricultural economies in India and globally. However, gastrointestinal parasites pose a constant threat to dairy animals, greatly affecting their general health, well-being, and production (Panigrahi et al., 2014; Jas and Pandit, 2017). West Bengal is one of the states where rural resource poor farmers have been involved in dairy cattle farming from ages; however, very scanty data is available about the incidence, kind, and effects of gastrointestinal parasites in this particular region. The prevalent climatic conditions and local management practices have an impact on the epidemiology of gastrointestinal parasites in livestock and when precisely mapped out in the various agro-climatic zones of the region, clarifies basic information from which additional control strategies can be developed. The presence of gastrointestinal helminthiases in animals causes frequently crippling ailments including diarrhoea, decreased feed intake, weight loss, and weakened immune systems, which have a negative impact on the quality of animal products and cause livestock owners to suffer significant financial losses (Murthy and Souza, 2016).

The intricate dynamics of parasite prevalence are influenced by a number of factors, including the local climate, grazing practices, sanitary regulations, and the use of anthelmintics. Evaluation of gastrointestinal parasites in West Bengal's dairy cattle is needed (Shit et al., 2017). By thoroughly examining the prevalence and species variety in three agro-climatic zones of West Bengal, this research seeks to close this gap. This study aims to provide important insights into the complexities of gastrointestinal parasite infestation in this area, which can then be used to inform targeted strategies for parasite control and improve the welfare, productivity, and general health of cattle and buffalo in West Bengal, India.

Materials and Methods:

The study was undertaken to know the prevalence of gastrointestinal parasites in dairy cattle in three agro-climatic zones of West Bengal namely, Zone-I (Hilly Zone), Zone-II (New Alluvial Zone) and Zone-III (Coastal Saline Zone) and also to find out the deworming practice followed by dairy farmers. The data and results were collected from two hundred and seventy (270) dairy farmers in accordance with the research methodology laid down.

Selection of blocks and villages/towns:

A multistage stratified random sampling procedure was used for the selection of three blocks under each district keeping in view the objective of the study. Two Gram Panchayats were randomly selected from each selected block. From each selected Gram Panchayat, a cluster of village or town was selected. The name of the selected blocks is given in Table 1.

Investigation of common gastrointestinal parasites in the study area:

The investigation was carried out in 2014 in three agro-climatic zones of West Bengal. A total of 465, 481 and 473 faecal samples from zone I, II and III, respectively, were collected from both the surveyed households and surrounding areas. Subsequently, the samples were analyzed as per standard sedimentation and salt flotation

techniques. A faecal sample of 10 g (approx) was collected directly from the rectum of the cow with the help of a sterilized spoon into a sterile airtight faecal collection vial. As far as possible, the faecal sample was analyzed on the same day and in few cases the sample fixed in 10% formalin or refrigerated in order to avoid development and hatching. All such samples were analyzed later by qualitative examination.

A part of individual faecal samples was examined qualitatively for the presence of nematode, cestode eggs and coccidian oocysts by standard salt flotation technique and for trematode eggs; the standard sediment technique was performed.

Standard sedimentation technique:

Qualitative faecal examination was done by sedimentation method. Approximately 3g of faecal sample was put in mortar and 8-10 ml of water were added to it. The faecal material was then evenly triturated with pestle. The faecal mixture was then poured into plastic vials with uniform diameter after straining with a strainer and the suspension was then allowed to settle for 15-20 minutes. Then the supernatant was discarded and the sediment was mixed with 5-6 ml of water and with the help of a pipette, the sediment and fluid was taken and one drop was placed on a glass slide and after being covered with a cover slip was observed under 10X magnification.

Table 1: Selected of blocks and Gram Panchayats

Agro-climatic zone	District	Selected Block	Gram Panchayat	No. of Respondents	Total no. of Respondents
Zone-I (Hilly zone)	Darjeeling	Mirik	Chengapanighata	15	90
			Sourani-II	15	
		Kalimpong-I	Bong Nimbang	15	
				15	
		Kalimpong-II	LolayKhasmahal	15	
			PallaKhasmahal	15	
Zone-II (New alluvial zone)	Murshidabad	Bhagawagola-I	Hanumantanagar	15	90
			Kuthirampur	15	
		Murshidabad-Jiaganj block	Bahadurpur	15	
			Dangapara	15	
		Lalgola	Lalgola	15	
			Nashipur	15	
Zone-III (Coastal saline zone)	South 24 Parganas	Gosaba	Chotomollakhali	15	90
			Rangabelia	15	
		Kakdwip	Madhusudanpur	15	
			Rabindra	15	
		Namkhana	Moushuni	15	
			Namkhana	15	
Total	3	9		270	270

Standard Salt flotation technique:

Centrifugal flotation method: Being lighter, worm eggs usually float when suspended in liquid of higher specific gravity than that of eggs. A sample of 5 g faeces was well mixed with 50 ml water and strained through a sieve (1mm mesh) to remove coarse faecal material. The mixture was allowed to sediment for 10-15 minutes or

centrifuged lightly until a clear supernatant appeared. The sediment was then mixed with a saturated solution of salt in a centrifuge tube (50 ml) and centrifuged at 500 g for 2-3 minutes. Eggs being lighter floated to the surface and was removed by touching the surface with the end of a cover slip and transferred to a slide. The eggs of worms were then identified by their morphology (Soulsby, 1982).

Research design in regards to survey:

Based on the nature of the research problem, Ex-post facto research design was followed in the present study as per Kerlinger (1983).

Selection of respondents/animal owners:

Fifteen respondents from each village/town (or cluster) having at least one milch cattle were selected from frame of reference to make a sample size of 270. Ninety respondents (cattle owners) were selected from each district (30 from each block) totaling 270 respondents in whole study.

Tools used for data collection:

A semi-structured interview schedule was developed in consultation with available literature, experts, local veterinary officers, and resource persons. Keeping in mind the objective of the present study, appropriate tools, techniques, scales etc., were incorporated in interview schedule for measurement of different variables.

Methods of data collection:

As far as data collection is concerned, each dairy owner or respondent was interviewed using the developed structured interview schedule during the survey period in regards to deworming or parasite management. The data were based on the memory/ recall of the respondents. Data from secondary sources and observation were also collected.

Results and Discussions:

Incidence of gastrointestinal parasite by faecal sample examination:

Out of the total 465 samples analyzed, 176 (37.85%) samples were found positive for either mono or mixed infection in zone-I as depicted in Table 2. The most commonly occurring gastrointestinal parasites confirmed by faecal sample examination were Strongyle (26.02%) followed by Amphistome (22.15%), *Moniezia* sp. (19.14%), oocyst of *Eimeria* spp. (12.04%), *Fasciola* sp. (4.09%), *Strongyloides* sp. (4.08%) and *Toxocara* sp. (1.72%) in zone-I of West Bengal (Figure 1).

In zone-II, out of 481 samples examined, 157 (32.64%) were found to be positive for either single or mixed infections. The commonly prevalent ova in the faeces of cattle were Strongyle (28.90%), Amphistome (26.20%), *Fasciola* sp. (14.14%), *Strongyloides* sp. (7.28%), *Moniezia* sp. (6.86%), Coccidian oocyst (4.99%), and *Trichuris* sp. (3.12%) (Figure 1).

Feacal sample examinations in zone-III revealed that out of 473 samples, 203 (42.92%) were positive for mono or mixed parasitic infestation. Highest percentage of samples were positive for Amphistome (31.92%) followed by Strongyle (18.82%), *Fasciola* sp. (10.99%), *Strongyloides* sp. (7.82%), Coccidian oocyst (5.92%), *Moniezia* sp. (4.02%) and *Trichuris* sp. (4.86%) (Figure 1).

The findings of zone-I *i.e.*, hilly area is in line with the findings of Rahman et al. (2012) who reported similar findings in different agro-climatic zones of Sikkim. Laha et al. (2013) also reported highest occurrence of Strongyle in organized farms in Meghalaya in consonance with the present findings. The findings of the present study indicated a higher incidence of Amphistome and *Fasciola* sp. than reported by Rahman et al. (2012), which might be due to differences in local conditions and differences in agro-climatic conditions of the areas studied. The probability of the presence of more intermediate hosts in the current study area may be another reason for such findings. In calf faeces, eggs of *Toxocara* sp. and Coccidian oocysts were detected, which can lead to severe health issues. The findings of the present study are similar to those of Rahman et al. (2012) who also reported the presence of *Toxocara* sp.egg and Coccidian oocyst in feaces of cattle in different agro-climatic zones of Sikkim. Shit et al. (2017) also reported prevalence of similar parasites in Bankura district of West Bengal.

Findings of zone-II *i.e.*, new alluvial soil area of Murshidabad district are similar to the findings of Samanta and Santra (2009), who also reported the highest incidence of Strongyle followed by Amphistome. However, the result of the present findings differed from the above-mentioned researcher in terms of the total animals infected and the higher occurrence of *Fasciola* sp. and *Moniezia* sp. The difference in the present findings might be due to differences in the geographical locations of the study, variable management practices etc. Among trematodes, occurrence of *Fasciola* spp. was the lowest and among other nematodes, incidence of *Trichuris* spp. was low. Jas and Pandit (2017) reported

Ova of *Toxocara vitulorum*	**Ova of *Trichuris* sp.**	**Ova of Amphistome**
Ova of *Moniezia expansa*	**Ova of *Moniezia benedini***	**Ova of *Fasciola* sp.**
Ova of Strongyle	**Ova of *Strongyloides* sp.**	**Oocyst of *Eimeria* sp.**
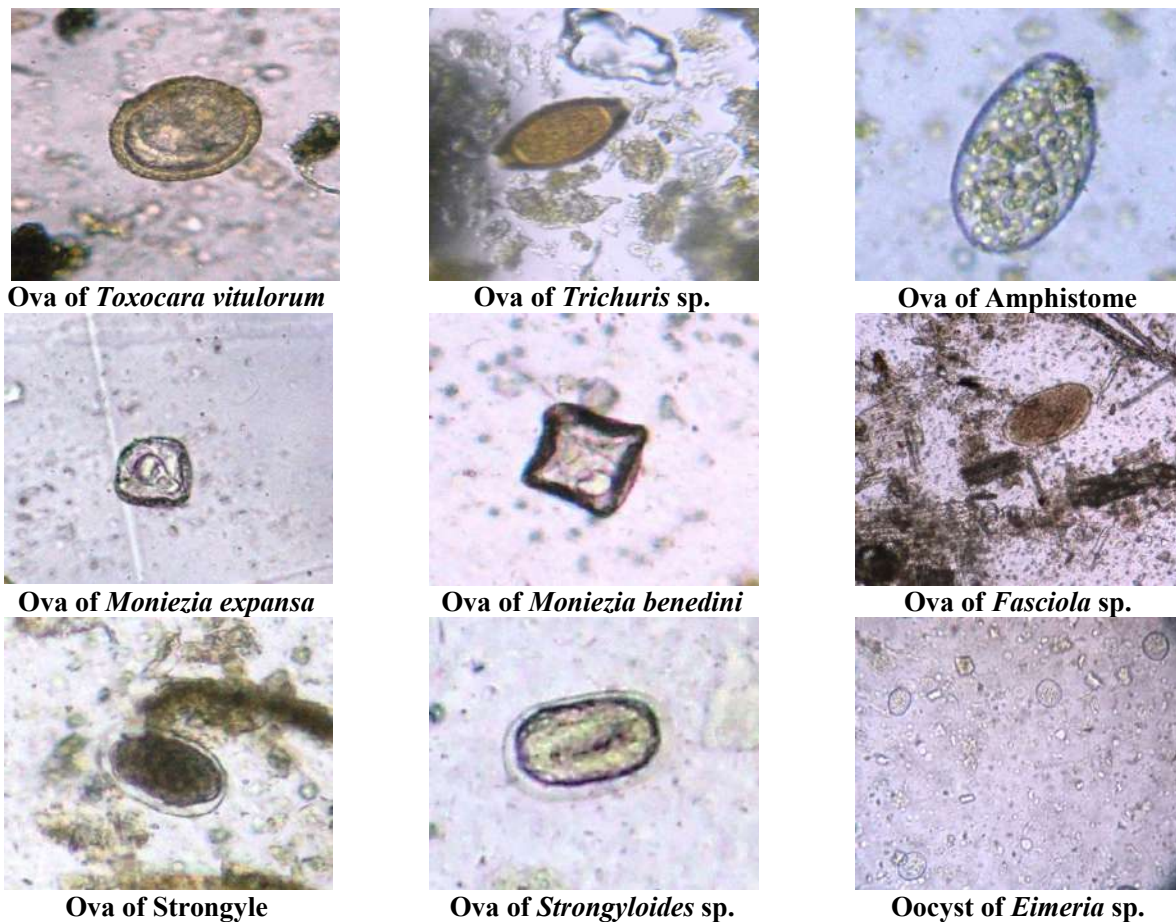
Figure 1: Parasitic ova and oocysts of *Eimeria* spp.

higher incidence of parasites in new alluvial soil zone but the difference may be due to difference in study districts.

The findings of coastal saline zone (zone-III) indicate highest incidence of Amphistome followed by Strongyle, *Fasciola* sp. and others. The results are in disagreement with Rahman et al. (2012) who reported lower incidence of Amphistome in different areas of Sikkim. The probable reason might be due to completely different climatic and geographical features of the study areas. Samanta and Santra (2009) reported a lower incidence or percentage of cattle affected with *Fasciola* sp. in hot and humid regions of Howrah, West Bengal that is dissimilar from the findings of the current study.

Highest percentages of cattle in zone-III were positive for different gastrointestinal parasites followed by zone I and II. There was difference in the occurrence of gastrointestinal parasites in zone-I, zone-II and zone-III not only in number of positive samples but also in species wise variation of parasites, which might be due to difference in agro-climatic condition, cattle management and practice of administering antiparasitic drugs to dairy cattle. Gastrointestinal (GI) parasites cause gastroenteritis, reduction and retardation of weight and growth, low production and reduced immunity, leading to huge economic losses (Jithendran and Bhat, 1999).

Deworming of dairy animals:

Information regarding deworming practices followed by dairy owners in the present study has been summarized in Table 3. In the hill zone of Darjeeling, the majority (45.56%) dewormed their adult cattle sometimes followed by 32.22% deworming never and only 22.22% deworming regularly. In Murshidabad district's, new alluvial soil zone, 47.78% farmers were deworming their animals sometimes, followed by 45.56% deworming regularly and 6.67% farmers who never dewormed their animals. In zone-III, 32.22% farmers were deworming regularly, 41.11% dewormed sometimes, and 26.67% dewormed never. The Chi square value indicates a highly significant difference between zones in the healthcare practice of administering dewormers to dairy animals. The present findings are more encouraging than reported by Rathore et al. (2010) and Singh et al. (2007). Overall, major (44.81%) share of farmers was deworming sometimes in all the zones and this might be due to inappropriate knowledge and awareness regarding the same. Overall, only 26.67% dewormed their calves

frequently. The Chi square value indicates a highly significant (p<0.01) difference in the deworming of calves in different zones of West Bengal.

The findings of the present study are more encouraging than that of Rathore et al. (2010) who reported the practice of deworming by only 4.25% of the cattle owners in Churu district. The findings of the present study indicate that the majority administer dewormers sometimes. Lack of awareness may be a major reason for not following proper deworming along with reasons like unavailability of dewormers, taboos etc. The majority of the farmers administered dewormer sometimes and did not follow the proper schedule, which might be due to lack of awareness about the proper schedule. However, most of the farmers seek local/ herbal measures after detecting problems of worm load/ potbelly, or diarrhea. Deworming is considered one of the most important practices in calf management and a lack of proper deworming can lead to huge losses. Some farmers had the view that administering dewormer to calves at early age may lead to ill health. Most farmers in zone-II practicing deworming also administered liver tonic for a few days after administering the drug with the view that such drugs are harmful for liver and administering liver tonic would protect the calf from the same.

Table 2: Species wise incidence of gastro intestinal parasites in cattle by faecal sample examination						
Sl. No.	Species	Zone-I (n=465)		Zone-II (n=481)		Zone-III (n=473)	
		Positive (%)	Negative (%)	Positive (%)	Negative (%)	Positive (%)	Negative (%)
1.	*Fasciola* sp.	19 (4.09)	446(95.91)	68(14.14)	413(85.86)	52(10.99)	421(89.01)
2.	Amphistome	103(22.15)	362(77.85)	126(26.20)	355(73.80)	151(31.92)	322(68.08)
3.	*Moniezia* sp.	89(19.14)	376(80.86)	33(6.86)	448(93.14)	19(4.02)	454(95.98)
4.	Strongyle	121(26.02)	344(73.98)	139(28.90)	342(71.10)	89(18.82)	384(81.18)
5.	*Strongyloides* sp.	19(4.09)	446(95.91)	35(7.28)	446(92.72)	37(7.82)	436(92.18)
6.	*Toxocara* sp.	8(1.72)	457(98.28)	0(0.00)	481(100.0)	5(1.06)	468(98.94)
7.	*Trichuris* sp.	6(1.29)	459(98.71)	15(3.12)	466(96.88)	23(4.86)	450(95.14)
8.	Oocyst of *Eimeria* spp.	56(12.04)	409(87.96)	24(4.99)	457(95.01)	28(5.92)	445(94.08)

Table 3: Deworming management practices followed by dairy farmers in three agro-climatic zones of West Bengal						
Sl. No.	Calf rearing practices	Zone-I (n=90)	Zone-II (n=90)	Zone-III (n=90)	Overall (N=270)	Significant (χ2)
1.	**Deworming of adult animals**					22.742**
	Regularly (every 6 months)	20(22.22)	41(45.56)	29(32.22)	90(33.33)	
	Sometimes (at least once/year)	41(45.56)	43(47.78)	37(41.11)	121(44.81)	
	Never	29(32.22)	6(6.67)	24(26.67)	59(21.85)	
2.	**Deworming of calf**					36.662**
	Frequent	20(22.22)	36(40.00)	16(17.78)	72(26.67)	
	Sometimes	25(27.78)	44(48.89)	36(40.00)	105(38.89)	
	Never	45(50.00)	10(11.11)	38(42.22)	93(34.44)	

Figures in parenthesis in the text indicate percentage
Significant **p<0.01

Conclusion:

One of the main issues influencing the production and general health of dairy cattle is intestinal parasitism. Gastrointestinal parasites are common in the studied agro-climatic zones of West Bengal. Though, a substantial percentage of animal owners perceive the importance of deworming, but probably lack proper scientific knowledge. So, raising awareness and involving all the stakeholders involved in dairy farming must comprehend the epidemiology of gastrointestinal helminth parasites and have a clear plan for managing and controlling animal resources in order to maximize their potential for output. Control strategies for gastrointestinal parasites include the strategic use of anthelmintics, grazing management, the use of resistant breeds, etc. It is better to use a specific drug according to

the infection after screening of faecal sample. In mixed infection or in the absence of faecal sample examination, treatment of gastrointestinal parasites by broad-spectrum anthelmintics at a proper dose. Deworming in calves is also very important, and it is advised to deworm cattle both pre- and post-monsoon.

Conflict of interest:

The authors declare that there is no conflict of interest.

Data availability:

All the data in relation to the present study are available.

Authors' Contributions:

All authors listed have made a substantial, direct, and intellectual contribution to the work.

Ethical Statement:

According to the guidelines for the care and use of agricultural animals in research (Curtis and Nimz 1988), non-invasive faecal collection was carried out, so handling was kept to a minimum.

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Antibiogram Assay of *Listeria monocytogenes* Isolates from Milk Samples in and Around Kolkata

Monmon Saha [1], Saurabh Majumder [2]*, Chanchal Debnath [3], Amiya Kumar Pramanik [3], Debasish Murmu [3]

[1]Department of Veterinary Public Health & Epidemiology, C.V.Sc. & A.H., R.K. Nagar, Tripura (W), [2] Department of Veterinary Microbiology, C.V.Sc.& A.H., R.K. Nagar, Tripura (W), [3]Department of Veterinary Public Health & Epidemiology, WBUAFS, 37, K.B. Sarani, Kolkata, WB

Abstract

Listeria monocytogenes is well-known globally as one of the most significant foodborne bacterial pathogens. *Listeriosis* may trigger life-threatening illnesses such as severe sepsis and meningitis, sometimes resulting in lifelong harm and even death. This study aimed to determine the occurrence and antibiotic resistance pattern of *L. monocytogenes* in a milk sample collected from in and around Kolkata. For this, a total of 104 milk samples [from individual cow udders (n = 36) and pooled can milk collected from farms (n = 20) as well as from the market (n = 48)] were examined for a period of 6 months, starting from January 2014 to June 2014**.** For the isolation of *L. monocytogenes*, samples were cultured on selective media and tested for their susceptibility to common antibiotics by disk diffusion assay. The results revealed that the overall occurrence of *Listeria species*in unpasteurized raw milk was 14 (13.46%), and *L. monocytogenes* was 5 (4.81%). All five *L. monocytogenes* isolates were subjected to an antibiotic sensitivity test using the Kirby-Bauer disc diffusion method. In this method, three antibiotics tetracycline (100%), gentamycin (100%), and penicillin (100%) exhibited complete sensitivity. However, the isolates showed variable resistance against ampicillin (16.21%), vancomycin (21.62%), and penicillin (43.24%).

Keywords: Occurrence, *Listeria monocytogenes*, Antibiogram, Milk samples, Characterization.

Introduction:

India is the world's largest producer of dairy products by volume and has the world's largest dairy herd. The country accounts for more than 13% of the world's total milk production and is also the world's largest consumer of dairy products, consuming almost all of its milk production (Singh, 2010). There are many organisms secreted through milk; one of them is *Listeria monocytogenes*, which causes significant public health problems. *L. monocytogenes* has been called an "emerging food-borne pathogen" because only recently we have recognized that it can be transmitted through food. *Listeria monocytogenes* is a ubiquitous bacterium. It causes Listeriosis, a serious infectious disease that occurs as a consequence of consumption of food contaminated with this pathogenic bacterium. Listeriosis is a significant public health problem (Rocourt and Catimel, 1985). The first communications/ reports of the presence of *Listeria* in food associated with dairy products, where cow milk was mentioned as a carrier of the fatal Listeriosis (Farber and Peterkin, 1991). According to many communications, consumption of milk and dairy products contaminated with *L. monocytogenes* can lead to individual cases of Listeriosis or a true outbreak of this disease. Of all dairy products, soft cheeses and non-pasteurized milk are the most common causes of Listeriosis. In the process of production of milk and dairy products, it most commonly occurs as a consequence of post-pasteurization contamination. Listeriosis is a serious disease of humans, occurring sporadically or in the form of an epidemic, with a mortality rate of over 25**%** (USDA, 1999).

Materials and Methods:

Isolation and identification of *Listeria monocytogenes* strains: ISO 11290 method was employed to isolate the organisms, whereby pre-enrichment of 10ml sample was done in 20 ml half-strength Fraser broth containing selective supplements (HiMedia) for 24 h at 30°C, which was followed by second enrichment of 0.1 ml of pre-enriched Fraser broth content in 10 ml full strength Fraser broth containing selective supplements (HiMedia) for 48 h at 37°C incubation temperature. After the enrichment procedure, the inoculum was plated on PALCAM agar (HiMedia) and incubated for 48 h at 37°C. The gray-green colonies are surrounded by a diffuse black zone on PALCAM agar. Subsequently, pinpoint colonies of PALCAM were subjected to identification procedures which included Gram's staining followed by a microscopic examination, catalase test, and oxidase test. The characteristic Gram-positive, coccobacillus or short rod-shaped organisms which were catalase positive and oxidase negative, were sub-cultured in Brain heart

infusion (BHI) broth at 25°C for 12-18 h. Subsequently, "presumptive" Listeria isolates were in turn subjected to detailed biochemical tests viz.; methyl red, Voges-Proskauer, nitrate etc. for confirmation of *L. monocytogenes* strains (Farber and Peterkin, 1991).

Antibiotic sensitivity testing of *Listeria monocytogenes* isolates: In the present study, *Listeria monocytogenes* isolates were tested for their susceptibility to antimicrobial agents by the standard Kirby-Bauer disc diffusion method (Bauer et al., 1966) following the National Committee for Clinical Laboratory Standards (NCCLS) guidelines, 1997. All positive *L. monocytogenes* isolates were grown in BHI broth overnight at 37°C. The culture suspension was adjusted to 0.5 McFarland Standard (approximately 1.5 x 108 cells). Within 15 minutes after adjusting the turbidity of the inoculum suspension, a sterile cotton swab was dipped into the adjusted suspension. The swab was rotated several times, pressing firmly on the inner wall of the tube above the fluid level to remove excess inoculum from the swab. Mueller-Hinton Agar (Hi- media®) was used as a medium to study the susceptibility to antibiotics. Then cultured was spread on the entire surface of a dried Muller Hinton agar plate with the sterile culture containing a swab. The culture-inoculated plates were held at room temperature for 10 minutes to allow the evaporation of free surface liquid as adopted by Anon (1997). Commercially available following antibiotics octa disks (Hi-Media®) were used: (D033) Ampicillin (10 mcg), Tetracycline (30 mcg), Cotrimoxazole (25 mcg), Ciprofloxacin (5 mcg), Gentamicin (10 mcg), Erythromycin (15 mcg), Chloramphenicol (30 mcg), Cefalexin (30 mcg). (D034):

Ceftrixone (30 mcg), Ceftazine (30 mcg), Cefotaxime (30 mcg), Lincomycin (2 mcg), Netilmycin (30 mcg), Ofloxacin (2 mcg), Vancomycin (30 mcg), Amikacin (30 mcg). (D0286) Penicillin (10 unit), Erythromycin (15 mcg), Vancomycin (30 mcg), Telecoplanin (30 mcg), Clindamycin (2 mcg), Ofloxacin (5 mcg), Azithromycin (15 mcg), Tetracycline (30 mcg) were placed on the surface of each inoculated plate using a sterile forceps. After incubation for 24 hours at 37°C, the diameter of the zone around each disc was measured, and interpreted by the National Committee for Clinical Laboratory Standards (NCCLS, 1997).

Results and Discussion:

A total of 14 (13.46%) *Listeria* spp. isolates were obtained in this study within which only 5 (4.81%) were found to be *L. monocytogenes* isolates. All isolates were Gram-positive, and coccobacilli, 0.5μm in diameter and 1-5μm in length that do not form spores or capsules, which were catalase positive and oxidase negative. These were MR positive, Nitrate reduction negative, and VP test positive which confirmed these as *L. monocytogenes* (Farber and Peterkin, 1991).

All five *L. monocytogenes* isolates showed different results in the antibiotic sensitivity test. In this study, 3 antibiotics, tetracycline (100%), gentamicin (100%), and penicillin G (100%), exhibited complete sensitivity. However, the isolates showed variable resistance against ampicillin (16.21%), vancomycin (21.62%), and penicillin (43.24%) shownin Table 1 and Table 2 and zone of inhibition of different antibiotics shown in Figures 1, 2 and 3.

Table 1: Antimicrobial drug resistance and sensitivity pattern of *L. monocytogenes* strains							
Antimicrobial agents	No. of isolates tested	*L. monocytogenes* isolates frommilk					
		Resistant		Intermediate		Sensitive	
		No.	%	No.	%	No.	%
Ampicillin	5	1	20	-	-	4	80
Tetracycline	5	0	0	-	-	5	100
Cotrimoxazole	5	4	80	-	-	1	20
Gentamicin	5	0	0	-	-	5	100
Ciprofloxacin	5	5	100	-	-	0	0
Erythromycin	5	3	60	-	-	2	40
Chloramphenicol	5	4	80	1	20	0	0
Cefalexin	5	1	20	1	20	3	60
Ceftriaxone	5	1	20	-	-	4	80
Ceftazidime	5	1	20	-	-	4	80
Cefotaxime	5	1	20	-	40	2	40

Antimicrobial agents	No. of isolatestested	L. monocytogenes isolates frommilk					
		Resistant		**Intermediate**		**Sensitive**	
		No.	%	No.	%	No.	%
Lincomycin	5	2	40	-	-	3	60
Netilmycin	5	1	20	1	20	3	60
Ofloxacin	5	1	20	-	_	4	80
Vancomycin	5	1	20	1	20	3	60
Amikacin	5	2	40	-	-	3	60
Penicillin	5	0	0	-	-	5	100
Erythromycin	5	4	80	-	-	1	20
Clindamycin	5	2	40	-	-	3	60
Azithromycin	5	1	20	1	20	2	60

Table 2: Antimicrobial drug resistance and sensitivity pattern of *L. monocytogenes* isolates
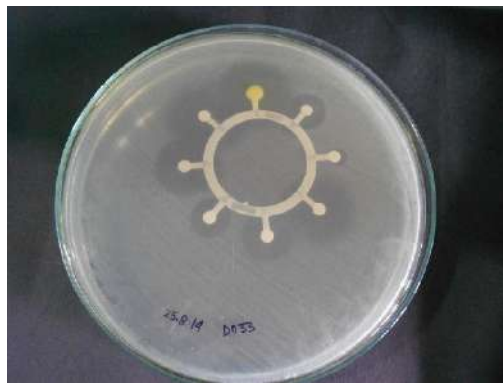
Figure 1: Antibiotic disc sensitivity test of *L. monocytogenes* strains using antibiotics like Ampicillin, Tetracycline, Cotrimoxazole, Ciprofloxacin, Gentamicin, Erythromycin, Chloramphenicol and Cefalexin

Figure 2: Antibiotic discsensitivity test of *L. monocytogenes* strains using antibiotics like Ceftriaxone, Ceftazidime, Cefotaxime, Lincomycin, Netilmycin, Ofloxacin Vancomycin and Amikacin

Figure 3: Antibiotic discsensitivity test of *L. monocytogenes* strains using antibioticslike Penicillin, Erythromycin, Vancomycin, Telecoplanin, Clindamycin Ofloxacin, Azithromycin and Tetracycline

From Table 1and Table 2, it was found that the highest resistance was recorded against Ciprofloxacin (100%), moderate resistance was found against cotrimoxazole, chloramphenicol, and erythromycin and the highest sensitivity was observed against tetracycline, gentamicin, and penicillin (100%). Zone of inhibition of different antibiotics was shown in Figure 1, 2 and 3 against different antibiotics.

The above results were partially correlated with Altunta et al. (2012) who reported a susceptibility pattern of *L. monocytogenes* isolates to antibiotics, such as penicillin G, vancomycin, tetracycline, chloramphenicol, rifampicin, erythromycin, gentamicin, and trimethoprim. However, the percentages of fosfomycin and streptomycin resistances were 92.9% and 7.1%, respectively.

The above result was partially correlated with Sharif et al. (2010) who reported a susceptibility pattern of *L. monocytogenes* isolates to gentamicin, doxycycline, ampicillin, tetracycline, and penicillin G and resistance to ciprofloxacin, cotrimoxazole, nalidixic acid, and erythromycin. Shu Bing et al. (2004) reported the sensitivity of *L. monocytogenes* to 12 antibiotics including gentamicin, vancomycin, kanamycin B, norfloxacin, ofloxacin, erythromycin, chloramphenicol, tetracycline, cephalothin, and cefazolin, were carried out. The study revealed that *L. monocytogenes* was resistant to enrofloxacin and nitrofurantoin. Enurah et al. (2013) reported chloramphenicol was the most effective antibiotic against the *L. monocytogenes* isolates with the least resistance (3.70%) while nalidixic acid proved to be least effective with resistance of 90.74%.

Conclusion:

L. monocytogenes is a psychrophilic bacteria recognized as a pathogen of great importance of food. It is accepted that Listeriosis in humans is a disease that is transmitted mainly through food. The series of outbreaks of the 1980s showed that *L. monocytogenes* causes very serious invasion and often life-threatening disease, constituting an economic burden for both public health services and the food industry. Infection with *L. monocytogenes* is a wide spread zoonosis, affecting mainly cattle, sheep, and goat herds. *Listeria* species are ubiquitous bacteria widely distributed in the natural environment. The ubiquitous character of the bacteria inevitably results in the contamination of numerous food products. All *Listeria* species are small, regular rods, 0.5μm in diameter and 1-5μm in length that do not form spores or capsules. They produce catalase but not oxidase. It is a Gram-positive, facultative anaerobic bacterium with both psychotropic and mesophilic features.

The prevalence of organisms in raw milk, meat, fish, vegetables, and ready-to-eat food is documented in Western as well as Asian countries. The possible causes of the emergence of listeriosis include major changes in food production, processing, and distribution, increased use of refrigeration as a primary means for the preservation of food, and changes, in the habits of the people.

Drug sensitivity test of *L. monocytogenes* with different antimicrobial agents revealed that all the isolates were highest resistant to ciprofloxacin (100%), moderately resistant to cotrimoxazole (80%), chloramphenicol (80%), and erythromycin (60%) and the highest sensitivity was observed against tetracycline, gentamicin, and penicillin (100%). These high resistances to commonly used antimicrobials may be due to indiscriminate use of these drugs.

Conflict of Interest:

No competing interest exists among the authors.

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Prevalence of Zoonotic Parasites of Street Dogs and Its Threats in Madi Valley, Chitwan, Nepal

Rabin Bastakoti [(1)]*, Chet Raj Pathak [(2)], Amit Pandey [(1)], Amazon Acharya [(2)]

[(1)] Madi Veterinay Hospital, Madi Chitwan, Nepal, [(2)] Agriculture and Forestry University, Nepal

Abstract

The enduring companionship between humans and dogs traces back to ancient civilizations. However, the zoonotic diseases carried by dogs pose potential threats to public health. This study aims to examine the prevalence of zoonotic helminths in street dogs from the Madi Valley, a municipality surrounded by a national park. The analysis involves 204 rectally collected samples, and factors like age, sex, infestation patterns, and ward-wise distributions are considered. The formal ether sedimentation technique is employed to detect the presence of helminth ova, and descriptive statistics are utilized for dataset evaluation. Among the 204 samples, 81.86% (n=167) tested positive for helminth ova presence. Out of these, 46.11% (n=77) exhibited multiple parasite infestations in their feces, while the remaining 53.90% (n=90) had single parasite infestations. A significant concern arises from the fact that 77.24% (n=129) of these parasites are zoonotically important. When considering age-wise prevalence, the younger age group of 1-5 years showed a 82% prevalence (n=79), while the adult group of above 5 years exhibited an 83% prevalence (n=88) of parasite ova in their feces. Out of the positive samples, 58.09% (n=97) were from male dogs, whereas 41.91% (n=70) were from female dogs (p-value=0.087). This study underscores the importance of recognizing and managing zoonotic disease risks in the context of human-canine interactions. The presence of helminths among street dogs in the Madi Valley emphasizes the potential health threats and necessitates proactive measures to prevent the transmission of these parasites to humans.

Keywords: Helminth, Street dog, Zoonosis, Madi, Chitwan, Nepal

Introduction:

Dog is the first among the domesticated animals. People kepps dog along with them since ancient era. In that time dog are used to catch prey as a food and protection of them also. Dogs share common habitat with man for food and shelter. Dogs are as a member of society and they share common shelter with human beings like as temple, playground, road, community house etc. Due to this, there is a chance of transmission of disease from dog to the man. Dog ate contaminated food to sustain their life and are more prone to get infection and hence disease transmission. Dogs are important as a vector for transmission of diseases. Many of internal parasites of dog are with zoonotic importance which increases the risk of diseases transmission. Furthermore, children often play with dog and are not aware about personal hygiene hence are likely to get infestation from dog.

Dog are often infected with internal parasite of zoonotic importance, which have public health issue and is equally important for human health. Helminths infestation varied with animal species, meteorological factor of that area and managemental factors. In Nepal, study regarding street dog internal parasite are sparse. In this research we tried to get prevalence of zoonotic internal parasite of street and community dogs and its threat to public of that area.

Materials and Methods:

Descriptions of the study area:

This study was conducted in Madi Municipality, Chitwan Nepal from September to October 2022 AD. Madi municipality is located in southern part of district Chitwan lies between 27.45 N and 84.31 E coordinates, surrounded by national park from three sides and Chure hill from southern part, is buffer zone of Chitwan national park. Madi is valley surrounded by chure hills and forest from all sides and it has 217 km square area and has a population of 38000 (CBS, 2023).

Study design and population:

A cross sectional study carried out to estimate the prevalence of gastrointestinal helminths parasite infestation of street dog in Madi valley, Chitwan, Nepal. The study sample street dog from Madi valley, both sex and all age group of dogs were within the study population. Dentation technique was used to determine age of dog from experienced technician.

Sampling technique:

Dog brought for nurturing camp were taken as sample population and 204 samples were collected. The sample were collected directly from rectum and kept in zip lock plastic bag having formaldehyde soaked cotton pieces

immediately after collection. Samples were examined in parasitological units of Madi veterinary hospital.

Study Methodology:

The samples were processed using Formal ether concentration technique (Arora and Brij, 2010) for detection of ova in feces. 4 ml of thoroughly mixed sample was mixed with 4ml of formaldehyde of 10% concentration and stir for few seconds with mortar and pestle. These suspensions mixture was sieved with double layer of surgical gauze then into a 15 ml centrifuge tube and centrifuged in 2000 RPM for 2 minutes. From the centrifuge suspension, supernatant was discarded and about 2 ml of sediment was taken. 10 ml of formaldehyde and 3 ml of ether was mixed within sediment and this suspension and locked with cap and shaken for 30 seconds and was again processed with centrifugation for 2 minutes in 2000 RPM.

Four layers of different strata were seen in centrifuge tube. Top layer / forth layer of ether seen due to its low density. Third layer of debris was seen which was removed by cotton bud or applicator stick. Second layer of formalin was seen. Last / bottom layer of sediment was used for fecal examination. All top three layers were removed by sudden tilting of centrifuge tube. About 1.5 ml of sediment was used for fecal examination after thoroughly mixing. Cover slip and 1% iodine drops were often used to make laboratory work easy. Microscope with eye piece 10X objectives 40X was used for fecal examination. Ocular microscopy was used to determine the size of ova. The eggs are identified with the base of Soulsby, 1982 and Taylor and Coop, 2016.

Statistical Analysis:

The demographic information of dogs and the result of faecal examination was entered in Microsoft excel and computed descriptive statistics. Chi-square test was used to check association of prevalence and associated factors. In all the analysis 95% confidence interval was considered, and the p value less than 0.05 considered as statistical significant level between the associated factors.

Results and Discussion:

The overall prevalence of gastrointestinal helminthes was found to be 81.86% (n=167) where helminths infection was found to be of various species. This infection is quite high along with zoonotic importance. Out of positive samples 46.10%, (n=77) were mixed infection and remaining 53.89% (n=90) were single infection of parasites. Sex wise distribution revealed that 81% (n=97) male were positive where as 82% (n=73) female were positive, which shows statistically non differences (p=0.878).Among the age wise distribution age group between 1-5 years had 82% (n=79) and more than 5 year

had 83% (n=88) which is statistically non-significant (Table 1). Ward wise distribution of parasites shows all ward are equally likely to be infested varies from 75 to 98**%** (Table 2). Out of positive samples 77% (n=) were major zoonotic infection which include *Ancylostoma* sp., *Dipylidium* sp., *Strongyloides* sp., *Toxocara* sp., *Echinococcus* sp. (Lappin, 2002). Among the infection major parasite found were *Acylostoma* sp., *Toxocara* sp., *Taenia* spp., *Diphyllobothrium* sp., *Spirocera* sp., *Strongyloides* sp., *Spirometra* sp., *Dipylidium* sp. and *Echinococcus* sp. (Table 3).

Table 1: Variables Associated with internal parasites of street dogs of Madi Valley, Chitwan, Nepal				
SN	Associated risk		Positive (n/%)	P-Value	Odd ratio
1	Sex	Male	97 (81%)	0.087	0.994
		Female	70 (82%)		
2	Age	1-5 year	79 (82%)	0.655	0.850
		above 5 year	88 (83%)		

Table 2: Ward wise distribution of internal parasite in street dogs of Madi Valley of Chitwan, Nepal			
SN	Associated risk	Positive (n/%)	P-Value
1	Ward No 1	47 (75%)	
2	Ward No 2	25 (80%)	
3	Ward No 3	51 (83%)	0.3853
4	Ward No 5	26 (87%)	
5	Ward No 6	18 (94)	

Table 3: Major Internal Parasite Species found in street dogs of Madi Valley of Chitwan, Nepal		
S.N	Species of Parasites	No of Positive Samples (n)	% of Positive Samples
1	*Acylostoma* sp.	69	25.7
2	*Toxocara* sp.	50	18.7
3	*Taenia* spp.	37	13.8
4	*Diphyllobothrium* sp.	25	9.3
5	*Spirocera* sp.	23	8.6
6	*Strongyloides* sp.	15	5.6
7	*Spirometra* sp.	15	5.6
8	*Dipylidium*	15	5.6

	sp.		
9	*Echinococcus* sp.	7	2.6
10	others	12	4.5
	Total	**268**	**100**

Overall prevalence of 81.86% is similar with findings from Mexico (Martínez et al., 2007). However, Higher prevalence was recorded as 90% in non-descript dog of UP India (Sudan et al., 2015). Slightly lower prevalence than this study was found in Spain 71% (Eguia et al., 2005) and 46.7% in street dog of Kathmandu valley in Nepal (Satyal et al., 2013). Other studies of Nepal shows Non dewormed dogs had 72.7% prevalence in Kathmandu (Satyal et al., 2013) and 70% at Suryabinayek municipality and 58.75% in Rupendehi district (Sukupayo et al., 2023) and (Yadav and Shrestha, 2017). This shows parasitic infestation is endemic in Madi valley of Nepal which urge need of control program to address this problems. This result also shows higher infestation rate in developing countries this may be due to food availability in garbage, condemned food, human wastages, indiscriminate feeding and gathering of dog near food niche may play role in cross contamination and not well developed mechanism to control street dog as well as mechanism to control parasite. High infestation may be due to high infestation of soil from nematodes larvae, availability of intermediate host to complete life cycle and poor sanitation of surroundings. Among the positive samples 46.10% (n=56) were with multiple infection that shows more ill health of dog and were away from veterinary care and complication in control of parasites. Mixed infection may also indicate the role of dog as a reservoir of zoonotic parasite.

Among the positive sample 77% were zoonotically important species which is alarming in public health point of view. Major zoonotic parasites were *Ancylostoma* sp., *Dipylidium* sp., *Strongyloides* sp., *Toxocara* sp., *Echinococcus* sp. (Lappin, 2002). This much infection may be due to cross contamination from human to dog or vice versa, poor socio economic condition of study area, and lack of awareness about zoonotic importance of dog's internal parasites. Children are more likely to get infestation from parks, temples, playground and roadways where dog's feces were abundant. In context Madi valley this is buffer zone area of Chitwan national park and high risk of infection from sylvatic cycle to urban cycle of infection which is supported by findings of (O'lorcain, 1994) both of them found higher prevalence of infection in stray dogs and wild canids such as foxes.

Sex wise distribution shows 81% (n=970) male and 82% (n=70) female were positive for presence of parasites ova in feces, with no statistically non-significant differences. Previous studies showed no significant difference according to sex from study of Nepal and Ethiopia (Giri, 2010, Degefu et al., 2011) on other hands (Suganya et al., 2019) found higher prevalence in male in UP India. This may be due to the common food sources and habits they share. Age wise distribution shows non-significant differences among below 5 years age and above five years age which indicates all age group are equally vulnerable to get infection. But some studies shows higher prevalence in puppies (Okewole, 2016; Moro et al., 2019). Roaming of street dogs for searching food in condemn offal near meat shop, market may favors same level of infection.

In this study a hook worm *Ancylostoma* spp. was found to be most prevalent parasite across the Madi valley. Many others studies agrees with this findings, (Kutdang et al., 2010). Previous study from Nepal (Satyal et al., 2013) also found higher prevalence of *Ancylostoma* spp. where among the positive sample 52% harbors *Ancylostoma* spp. Some studies shows higher infestations of *Toxocara* spp. (Szabová et al., 2007) while others show *Strongyoides* spp. (Yacob et al., 2007). This indicates variation in infestation level of hygiene, cross contamination, geographical location. *Ancylostoma* is wider spreader in tropic to subtropics and have great threat to public health. Poor levels of hygiene, over-crowing, lack of veterinary services, lack of veterinary care, and lack of knowledge regarding zoonotic importance's of parasite were major factor which may elevate the risk of zoonotic disease transmission.

Conclusion:

In conclusion, gastro-intestinal heminths are endemic in dogs of Madi valley, majority of them possess zoonotic importance. It shows the urgent need of control of infection as well as generating awareness among the peoples regarding zoonosis. Similarly attention should be given to manage street dogs, internal parasite of street dogs to reduce public health risk. This study was done in short duration of years so it is better to conduct detail epidemiological study.

Author's Contribution:

RB: Contribution to generation of research idea, data collection, laboratory examination, interpretation, writing and manuscript preparation. CRP: identification of parasite. AP: Contribute to fecal examination, AA: fecal examination and manuscript preparation

Acknowledgement:

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Prevalence and Risk Factors of Ovine Lung Worm Infection in and Around Ambo, Oromia Regional State, Ethiopia

Firaol Tariku [(1)], Tesfaye Rebuma [(2)], Motuma Regassa [(3)], Mahendra Pal [(4)*]

[(1)] Nono Woreda Agricultural Office, Silk-Amba, West Shewa Zone, Ambo, Oromia, Ethiopia, [(2)] School of Veterinary Medicine, Ambo University Guder Mamo Mezemir Campus Veterinary Teaching Clinic, Ambo, Ethiopia, [(3)] Nono Woreda Agricultural office, Silk-Amba, West Shewa Zone, Ambo, Oromia, Ethiopia, [(4)] Narayan Consultancy on Veterinary Public Health and Microbiology, Bharuch, Gujarat, India

Abstract

A cross-sectional study was conducted from October 2015 to April 2016 to estimate the prevalence of lungworm infection and to assess some of the determinant risk factors associated with ovine lungworm infections in and around Ambo, Central Ethiopia. Fecal samples were collected from randomly selected 349 sheep, kept under extensive management systems, to examine first-stage larvae (L1) using Baermann technique. Additionally, samples were collected from 44 sheep slaughtered in different restaurants and hotels to examine the presence of adult lungworm parasites. The overall prevalence recorded from fecal and postmortem examinations was 23.2% and 31.8%, respectively. A higher prevalence was observed in females (26.2%) than in males (17.7%). Age-wise, a higher prevalence (29.1%) was observed in animals greater than 6 months to 2 years old, while the lowest (13.5%) was observed in animals less than or equal to 6 months. However, the prevalence noted, both in line with age and sex categories, was not statistically significant (P > 0.05). On the other hand, a higher prevalence was observed in sheep with poor body condition (37.6%), followed by moderate (21.9%) and good (12.4%) body-conditioned animals, and the difference was statistically significant (p<0.05). Similarly, the prevalence of lungworm infection in apparently healthy groups was 17.0%, while that of the sick was 52.5% and the difference was significant statistically (p<0.05). The prevalence of lungworm infection during postmortem examination of slaughtered sheep was higher (31.8%) than the result obtained at coprology (23.2%). The present study confirmed that lungworm infection was a common problem among sheep in the study area. Due to its impact on production, emphasis should be given to the control and prevention of lungworm infection in the study area, like the application of repeated deworming and grazing management.

Keywords: Prevalence, Lungworm, Baerman technique, Prevalence, Sheep

Introduction:

Livestock has several benefits for humans, especially in developing nations. In Africa, small ruminant production is a significant portion of the continent's livestock industry (Rege, 1994). Of the 475 million goats and 1,614 million sheep on the globe, 95% and 65 percent, respectively, are found in developing nations. Africa is home to 205 million sheep and 174 million goats, or roughly 17% and 31% of the global population, respectively (FAO, 1993). Africa has a very diverse distribution of small ruminants; they are more prevalent in arid regions than in humid ones (Lebbie et al., 1992).

Due to minimal input requirements such as small initial capital, fewer resources, and maintenance costs such as small ruminant households in African society, these factors account for a larger share of impoverished families' total income. With marginal areas, insufficient pasture, and agricultural leftovers, they can also generate milk and meat in easily consumable quantities. Additionally, because of their rapid production cycle, they can quickly reassemble flocks following calamities and meet demand (Gatenby, 1991).

Ethiopia has a large livestock population in Africa, estimated at 34–40 million TLU. Within the country, 12% of small ruminants and 17% of cattle are found, contributing to the livelihoods of approximately 80% of the rural population (FAO, 1993 and CSA, 2009). Ethiopia is home to diverse indigenous sheep and ranks second in Africa and sixth in the world (CSA, 2009), with an estimated population of nearly 23.62 million sheep (CSA, 2008). 75% of the total sheep population is raised in highlands with altitudes above 1,500 sea levels, while the remaining 25% are reared in the lowlands (Fentahun et al., 2012). In Ethiopia, the livestock sector is the major source of income for rural communities and a significant contributor to foreign currency from exports (Gebreyohannes et al., 2013). Sheep are the dominant livestock in Ethiopia, providing up to 63% of cash income and 23% of the food substance value obtained from livestock production.

Despite this, sheep production and productivity are limited due to parasitic diseases. Lungworms are among the endoparasites frequently found in sheep and affect the production of these animals in Ethiopia and worldwide. Ovine lungworms are widely distributed throughout the world but are particularly common in countries with temperate climates and in the highlands of tropical and sub-tropical countries (Hansen, 1994). In the highland areas, infection with lungworm parasites is the common cause of high mortality and morbidity in the sheep population (FAO, 2002). Lungworm infection in sheep is caused by nematode parasites such as *Dictyocaulus filaria, Muellerius capillaris, and Protostrongylus rufescens* (Radostits et al., 2007). *Dictyocaulus filaria* is acquired by ingestion of infective larvae with herbages, but *Muellerius capillaris and Protostrongylus rufescens* are transmitted when Molluscan intermediate hosts are accidentally ingested by grazing animals (Urquhart, 1996).

The pathogenic effect of lungworms varies depending on where they are located in the respiratory tract, how many infectious larvae they ingest, and the animal's immune system. Lungworms are parasitic nematodes that are known to cause infections of the lower respiratory tract, which are characterized by respiratory distress, trachitis, bronchitis, and pneumonia (FAO, 1994). Lungworm infection manifests clinically as severe, chronic coughing to mild coughing with modestly elevated respiratory rates (Kahn, 2005). Other significant clinical indicators include weight loss, nasal discharge, dyspnea, and nausea (Radostits et al., 2007). The main parasitological method of confirming lungworm disease is by detecting the L1 stage in fecal samples using the Baermann technique, but several factors can affect larval excretion, such as season, lactation, and reproductive effort. On post-mortem examination, the air passage opened to detect adult worms in the lower respiratory tract (lung) (Girisgin et al., 2008).

Control and prevention of these parasites are therefore essential for increasing sheep production and reducing the impact of these parasites on sheep. For proper implementation of control measures, knowledge of parasitic diseases and their prevalence must be studied (Borji et al., 2012). The incidence of respiratory helminthiasis varies from place to place, depending on various risk factors. Several studies were conducted to determine the prevalence and associated risk factors of ovine lungworm infections in different parts of Ethiopia and indicated varied infection prevalence.

Therefore, the objectives of this study were:

- To estimate the prevalence of lungworm infection in sheep in the study area

- To assess potential risk factors of ovine lungworm infection

Materials and Method:

Description of study area

The study was conducted from October 2015 to April 2016 in and around Ambo, West Shewa zone, Oromia regional state. The town is located in the central part of Ethiopia, at a distance of about 110 km from Addis Ababa in the west. The area is situated at latitude of 8^o47 to $9^o20'$N and a longitude of $37^o32°$ to $38^o3°$E. The altitude of the area ranges from 1300-3330 M.A.S.L. The area receives a mean annual rainfall ranging from 800-1000mm with an average of 900 mm, of which 70% (long rain) falls from June to September and 30% (short rain) falls from February to April. The monthly average minimum and maximum temperatures are 15^oC and 29^oC, respectively. The study area comprises 35.3% highland, 14.7% lowland, and 50% midland from the total coverage. The livestock population in the area is estimated to be 133,202 cattle, 52,714 sheep, 43,339 goats, 15,456 donkeys, 9,655 horses, 294 mules, and 138,754 poultries. There are also about 6,202 beehives in both traditional and modern production systems.
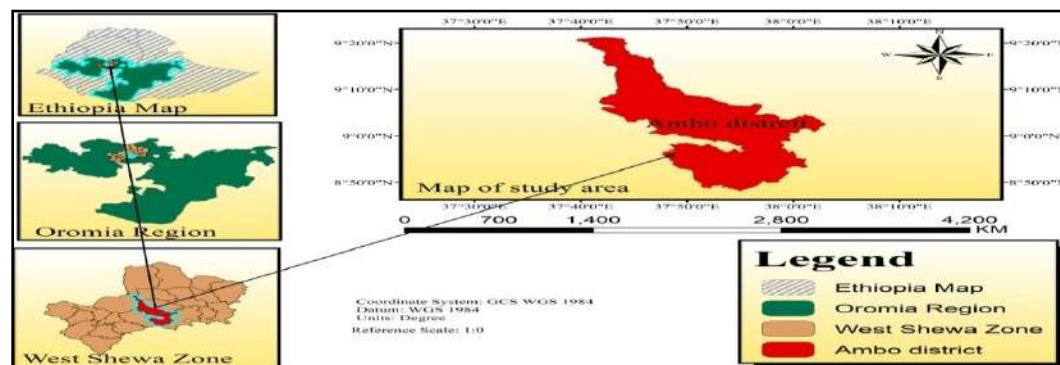

Figure 1: Map of Ethiopia shows study area

Study Population

The study population consisted of 349 sheep randomly selected from the sheep populations in the study area. All the study animals were local breeds and were kept under a traditional management system where animals were allowed to graze freely in the daytime and stay in the pen at night. Of the total sampled animals, 225 were female and 124 were male. The farmers' answers were used to estimate the animals' ages, which were then double-checked by looking at their teeth. The animals were divided into three age groups: younger than six months, between six months and two years, and more than two years, based on the replies. In each age group, there were 37, 127, and 185 animals, in that order. The Ethiopia Sheep and Goat Productivity Improvement Programme (Foreyt, 2013) were followed in the rating of body condition.

The assessment revealed that 105, 151, and 93 animals had good, moderate, and poor BCS, respectively. With regard to clinical respiratory signs, 61 animals had clinical respiratory symptoms, while 288 animals appeared apparently healthy.

Study design

The study was a cross-sectional study conducted in and around Ambo town, both among the field population and sheep brought for slaughter at hotels and restaurants. The Biodata of the individual animals were collected both from clinical assessments and owner information, where appropriate.

Sampling method and Sample size

The sampling method used a simple random sampling technique to select the animals and determine the sample size. The total sample size was calculated using the following parameters: 95% confidence level (CI), 5% desired level of precision, and 34.90% prevalence of lungworm in naturally infected sheep of the Ambo district, as confirmed by (Beyene et al., 2013). The sample size was determined using the formula provided by Thrusfield (2005).

$$n = \frac{1.96^2 Pexp\ (1\text{-}Pexp)}{d^2}$$

Where, n = required sample size

Pexp = expected prevalence

d = desired absolute precision

Therefore, based on the above formula, the total sample size of sheep was calculated to be 349.

Laboratory assay

Coprological examination

Fresh fecal samples were directly collected from the rectum of individual sheep using disposable gloves. The samples were placed in a universal bottle and packed in an icebox. Information on various risk factors, such as date of sampling, sex, body condition, clinical respiratory signs, and age of individual animals, was properly recorded during sample collection. Each bottle was properly labeled to correspond with the animal's identity. The sample was then transported to the Ambo University Veterinary Laboratory Technology. The techniques recommended by (Hendrix, 2006) were used for the identification of lungworm larvae from the collected samples. In the laboratory, the conventional method of the Baermann technique was employed for the detection of lungworm larvae. Fresh feces were weighed and wrapped in gauze, fixed to a string in a beaker filled with water. The Baermann apparatus was left in place for 24 hours. The larvae leave the feces, migrate through the gauze, and settle at the bottom of the glass. After removing the supernatant, the sediment was examined under a lower-power microscope.

Post mortem Examination

For the postmortem examination, the lungs of sheep were collected from sheep slaughtered at different restaurants and/or hotels in Ambo town and transported to Ambo University Veterinary Laboratory Technology after slaughter for examination of adult lungworms. The sex, body condition, and date of sampling of the slaughtered animal were labeled. The air passages were opened, starting from the trachea down to the bronchi, with fine, blunt-pointed scissors to detect the parasites.

Data Management and Analysis

The data was recorded on special formats prepared for this purpose routinely, entered into a Microsoft Excel spreadsheet, and summarized using descriptive statistics. Logistic regression analysis (both univariable and multivariable analysis) was employed using STATA version 11 to analyze the association between individual as well as group risk factors against lungworm infection. The assumed risk factors are considered significant when the p-value is less than 0.05. Age, sex, clinical respiratory signs, and body condition score were considered as risk factors to see their association with the prevalence rate.

Results and Discussion:

Coprological Examination

A total of 349 sheep were examined for lungworm infection using the Baermann technique in Ambo town and its surroundings. The survey showed an overall prevalence of 23.2%. In this study, a number of hypothesized risk factors, including age, sex, body condition, and the presence or absence of respiratory clinical signs, were considered to observe the trend of prevalence. Accordingly, the prevalence in males was 17.7%, while that of females was 26.2%. Similarly, the prevalence in lamb was 13.5%, young adults were 29.1%, and adults were 21.1%, and no statistically significant difference was noted between categories in the respective risk factors (P > 0.05).

Among the hypothesized risk factors, the infection prevalence was observed to be associated with sheep body condition. Accordingly, the prevalence was noted to be 12.4% for good, 21.9% for moderate, and 37.6% for poor body condition. The difference noted was significant statistically (P<0.05). Similarly, the prevalence of lungworm infection in apparently healthy groups was 17.0%, while that of the sick was 52.5%, and the difference was significant statistically (p<0.05).

Table 1: Prevalence of lungworm infection in ovine hosts in relation to different risk factors (variables)

Factors	Category	No. of examined	Proportion (%)	95% CI
Age	≤ 6 month	37	5 (13.5)	2.3 – 24.7
	> 6 month-2 year	127	37 (29.1)	21.2 – 37.1
	>2 year	185	39 (21.1)	15.2 – 27.0
Gender	Female	225	59 (26.2)	20.4 – 32.0
	Male	124	22 (17.7)	11.0 – 24.5
BCS	Poor	93	35 (37.6)	27.7 – 47.6
	Moderate	151	33 (21.9)	15.2 – 28.5
	Good	105	13 (12.4)	6.0 – 18.7
Resp. cl. sign	Showing resp. cl. sign	61	32 (52.5)	39.8 – 65.1
	Apparently healthy	288	49 (17)	12.7 – 21.4
Total		349	81 (23.2)	19.1 – 28.0

The data were further regressed in a univariable logistic regression analysis (Table 3), and those predictors with a p-value less than or equal to 0.25 were further subjected to a multivariable logistic regression analysis. As all four predictors considered in univariable logistic regression fulfilled the benchmark criteria, they were all subjected to multivariable regression analysis; however, body condition score and status of respiratory signs fitted the final model significantly (Table 2).

Table 2: Univariable logistic regression analysis of different risk factors with lung worm infection in sheep

Factors	Category	No. of examined	Proportion (%)	OR	95% CI for OR	P – value
Age	≤ 6 month	37	5 (13.5)	Ref		
	> 6month-2 year	127	37 (29.1)	2.6	0.95 – 7.3	0.062
	>2 year	185	39 (21.1)	1.7	0.6 – 4.7	0.296
Gender	Female	225	59 (26.2)	Ref		
	Male	124	22 (17.7)	0.6	0.4 – 1.45	0.074
BCS	Poor	93	35 (37.6)	Ref		
	Moderate	151	33 (21.9)	0.5	0.26 – 0.81	0.008
	Good	105	13 (12.4)	0.6	0.11 –0.48	0.000
Resp.cl. sign	Apparently healthy	288	49 (17)	Ref		

	Showing resp. cl. Sign	61	32 (52.5)	5.4	2.98 – 9.7	0.000

Table 3: Multivariable logistic regression analysis of different risk factors associated with lung worm infection in sheep						
Factors	Category	No. of examined	Proportion (%)	OR	95% CI for OR	P – value
BCS	Poor	93	35 (37.6)	Ref		
	Moderate	151	33 (21.9)	0.5	0.29 – 0.98	0.045
	Good	105	13 (12.4)	0.29	0.13 – 0.63	0.002
Resp. cl. sign	Apparently healthy	288	49 (17)	Ref		
	Showing resp. cl. Sign	61	32 (52.5)	4.5	2.4 – 8.5	0.000
						47.1

Post mortem examination

A total of 44 sheep were examined through postmortem examination in Ambo town in different restaurants. Out of these, 14 (31.8%) were positive for lungworm infection (Table 4).

Table 4: Postmortem based Lung worm infection prevalence in sheep				
Factors	Category	No. of examined	Proportion (%)	95% CI
Sex				
	Female	18	44.4	6.1 – 40.1
	Male	26	23.1	20.1 – 68.7
BCS				
	Poor	5	8	3.96 – 20.3
	Moderate	22	27.3	7.7 – 46.9
	Good	17	23.5	2.1 – 45
Total		44	31.8	19.4 – 47.1

The comparison of the overall prevalence of lung worm infection was found to be higher in a postmortem examination (31.8%) than in a Coprological examination (23.2%) (Table 5).

Table 5: Coproscopic and post mortem result of lung worm infection in sheep			
Type of examination	No. of examined	Proportion (%)	95% CI
Coproscopic	349	81 (23.2)	19.1 – 28.0
Post mortem	44	14 (31.8)	19.4 –

Lungworm infection (verminous pneumonia) is a chronic parasitic disease that affects the respiratory systems of animals. This results in substantial economic loss due to unthriftiness, loss of body condition, reduction of growth rate, poor skin quality, morbidity, and mortality by predisposing the animal to secondary infection (Radostits et al, 2000). The current study revealed the importance of lungworm parasites in and around Ambo in all indigenous breeds of sheep kept under an extensive traditional management system. Of the total sheep examined, an overall prevalence rate of 23.2% and 31.8% was recorded by fecal and postmortem examinations, respectively. This overall prevalence agrees with the work done by other researchers, who reported 22.7% in and around Bahir-Dar (Dawit, 2009); 21.57% in and around Atsbi (Tegegne et al., 2015); 25.78% in Banja District and 22.1% in and around Wolaita Soddo (Rahmeto et al., 2016).

However, the present finding was lower compared with the findings of (Foreyt, 2013) in three peasant associations from some areas of the country: (Moges et al., 2011) in Wogera District; (Bekele and Aman, 2011) in Tiyo District; and (Tefera and Mekuria, 2016) in Debre Birhan, who reported 34.90%, 67.69%, 57.1%, and 56.3%, respectively. On the other hand, the present report was higher than (Selam et al., 2015) in and around Wukro; (Denbarga et al., 2013) in and around Bahir-Dar; and (Ibrahim and Godefa, 2012) in Mekelle town, reporting a prevalence of 13.1%, 17.5%, and 13.4%, respectively.

The differences in the prevalence of lungworms in sheep in the above studies might be associated with differences in methods employed in the detection of lungworm larvae, the difference in the study areas attributed to climatic factors like humidity and weather and other factors that favor the survival of the larvae of the lungworm and the sample size variation used by researchers.

The reason for the low prevalence of the disease in this study could be attributed to the establishment of an open-air clinic in rural Kebeles, an increase in the number of private veterinary pharmacies, and increased farm awareness to deworm their sheep. The reason for the increase in prevalence in this study could be explained by the fact that all earlier researchers conducted their research in different management systems, but in the present study only extensive management types were examined. A higher prevalence of infection was noted where the husbandry of sheep was extensive type than in the semi-intensive type because sheep with extensive management type have a higher chance to ingest the intermediate host (snail and slugs) for lungworms with indirect life cycles (*Protostrongylus rufescens and Muellerius capillaries*) or are they possibly infested with larvae as well as easily obtained lungworms (D. filarial) from the herbage (Soulsby, 1982).

The study showed a higher level of prevalence was observed in female (26.2%) animals compared to the level of prevalence observed in male animals (17.7%), with an insignificant difference (p > 0.05). This result agrees with the earlier study of (Addis et al., 2011) in Gondar town and (Eyob and Matios, 2013) who reported an insignificant difference in lungworm infection between sexes. However, Ibrahim and Godefa (2012) reported significant variation in the infection rate of lungworms in males and females. The difference may be due to the improper distribution of sample selection between the two sexes, as observed by (Addis et al., 2011), where almost all female sheep were sampled.

Regarding age, a higher prevalence of lungworm infection was observed in the groups of >6 months to 2 years (29.1%) as compared to age groups of less than or equal to 6 months (13.5%) and greater than 2 years (21.1%). The reasons for this lower proportion in the age group of ≤ 6 months could be attributed to the sampling of a small and disproportionate number of animals or might be associated with the infrequent grazing behavior of animals less than 6 months of age and the acquired resistance of adult animals. Accordingly, as the age of animal's increases, their susceptibility to lungworm infection decreases (Urquhart, 1996).

The body condition of animals was found to be significantly associated with the prevalence of lungworm infection in both univariable and multivariable logistic regression analyses. A higher infection rate was observed in animals with poor body condition as compared to other groups. This, in part, may be attributed to the nutritional status of the animal. The odds of animals with medium and good BCS (OR=0.5, 95% CI (0.29, 0.98); OR=0.29, 95% CI (0.13, 0.63), respectively, are less likely to be infected with lungworm than poor body-conditioned

animals. The finding was in agreement with the reports of (Marshet et al., 2014) in and around Wukro, (Tegegne et al., 2015) in Banja District, and (Tefera and Mekuria, 2016) in Debre Birhan.

This study was conducted in the dry season, when feed shortages are a serious problem for sheep kept under an extensive management system in Ethiopia. Therefore, in the dry season, free-ranging animals are not able to meet their maintenance requirements and lose a substantial amount of weight. It is well known that poor nutrition lowers both the resistance (ability to resist the parasites) and resilience (ability to tolerate or ameliorate the effects of the parasite) of the animal, thus enhancing the establishment of worms and increasing the prevalence in poorly conditioned animals (Walkden-Brown and Kahn, 2002; Kahn, 2005). In connection with lungworms, it is reported that poorly nourished animals appear to be less competent at getting rid of lungworm infection, although it is not unusual for well-fed animals to succumb to lungworm infection (López et al., 2013).

The prevalence of lungworm infection by coproscopic examination was significantly higher (52.5%) in animals showing clinical respiratory signs than those that were apparently healthy (17.0%). The odds ratio (OR) of infection in animals that showed clinical respiratory signs was 4.5 times higher than that of sub-clinically infected animals. This report is consistent with that of Eyob and Matios, 2013 and Foreyt, 2013. The occurrence of disease without clinical signs earlier would shed larvae without showing clinical signs and those that show clinical signs but without larvae could be due to failure of procedures and the existence of other disease conditions that is differential to verminous pneumonia. As a result, animals that show clinical respiratory signs have a high chance of being infected with a lungworm infection.

The prevalence of lungworm infection at postmortem examination of slaughtered sheep was higher (31.8%) than the result obtained through coprology (23.2%). This finding is consistent with the observations of Denbarga et al., 2013 and Fentahun et al., 2012 but not in agreement with the reports of (Addis et al., 2011). This difference could be attributed to the difficulty in detecting these nematodes by coproscopic methods (Girisgin et al., 2008) and may be due to a lower number of female animals examined compared to males in the postmortem examination.

Conclusion:

The result of the present study showed that lungworm infection is a problem for sheep in and around the Ambo area. In the present study, the infection prevalence of lungworms was estimated to be 23.2% on Coprological and 31.8% on a postmortem test. There was no significant

difference between the age and gender categories of animals in the study area. The prevalence noted along body condition status was different statistically. A higher prevalence was recorded in those sheep with poor body conditions than in those with medium and good body conditions. Although most sheep infected with lungworms are clinically characterized by respiratory signs, some sheep appeared apparently healthy. A higher prevalence was observed by postmortem examination than by coproscopic examination.

In light of the above findings, the recommendations are forwarded:

✓ Regular Strategic deworming practices need to be adopted

✓ Sick individuals and sheep with poor body condition need to be treated

✓ The efficacy of anthelmintic in use has to be monitored regularly

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Authors' Contributions:

All authors made a substantial, direct, and intellectual contribution to the work.

Conflict of interest:

The authors declare that they have no conflict of interest.

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Molecular Characterization and Antibiotic Resistance Patterns of ESBL-producing *Escherichia coli* Strains from Bovine Faecal Samples in West Bengal

Zahid H. Hazarika, Samir Dey, Siddhartha Narayan Joardar, Indranil Samanta, Sharmila Latchubhukta, Tousif Mondal, Kunal Batabyal*

Department of Veterinary Microbiology, Faculty of Veterinary and Animal Sciences, West Bengal University of Animal and Fishery Sciences, Kolkata – 700037, West Bengal, India

Abstract

Escherichia coli is mainly a commensal and can cause intestinal and urinary tract infections in bovines at times. Mastitis is also one of the *E. coli* complications. *E. coli* is one of the enteric pathogens that remain associated with bovine diarrhoea. These commensals may also possess different antimicrobial resistance genes which can make their treatment quite difficult making the bovine diarrhoea cases more serious. The study was aimed to detect the *E. coli* isolates from bovine faecal samples followed by their characterization, detection oftheir ESBL and ACBL-producing genes, followed by antibiogram. A total of 46 *E. coli* strains (45.09%) were isolated from bovine faecal samples (n=102) from different districts of West Bengal. All the isolates showed typical characteristics and were found to possess a *16S rRNA* gene specific to this genus. During the detection of ESBL and ACBL positivity, 21 (45.6%) and 37 (80.4%) strains showed phenotypical characteristics. A total of 24 (52.17%) and 41 (89.13%) isolates showed the presence of any one of the beta-lactamases (ESBL and ACBL) producing genes in genotypical characterization. All the ESBL-producing *E. coli* isolates showed a high level of resistance to amoxicillin/clavulanic acid, ceftriaxone, ceftazidime, ampicillin/cloxacillin, cefotaxime (all 100%), levofloxacin (95.83%), cefoxitin (91.67%) and tetracycline (87.5%), whereas gentamicin, imipenem, amikacin (all 100%) and doxycycline (91.67%) were found to be sensitive against these pathogens.

Keywords: Antibiogram, ACBL, Bovine Faecal samples, ESBL, *Escherichia coli*

Introduction:

Escherichia coli is a very commonly found commensal in bovines and other mammals. It can cause diseases like calf diarrhoea/bovine diarrhoea along with other pathogens, the host's immunological and nutritional status, the environmental and managemental issues (Izzo et al., 2011). Bacterial diseases like diarrhoea can lead to huge production loss and loss of conditions in bovines. Enterotoxigenic *E. coli* (ETEC) can cause bovine infections leading to diarrhoea with morbidity and mortality loss throughout the world (Izzo et al., 2011; Acha et al., 2004).The possession of antimicrobial resistance genes like the genes encoding ESBL and ACBL in *E. coli* strains is a growing concern around the globe including India. The pathogens carrying the resistance genes pose a major challenge for the treatment of infections as the enzymes encoded make the pathogen resistant to higher generation of cephalosporins (Tenover et al., 1999). Consequently, there is an increase in the use of last-resort antimicrobial drugs (*i.e.* carbapenems) for treatment. Again, *E. coli* strains carrying the resistance genes can easily transfer those genes to other pathogens leading to the spread of the determinants (Hu et al., 2016). In this background, the present study was aimed at the detection and characterization of ESBL and ACBL-producing *E. coli* strains from bovine faecal samples from different districts of West Bengal followed by further characterization and to know their antibiotic resistance patterns *in-vitro*.

Materials and Methods:

Collection and transportation of faecal samples:

A total of 102 faecal samples from bovines irrespective of age and sex, were collected from private owners from Purba Barddhaman, Nadia and Hooghly districts of West Bengal during January - June 2020. The faecal samples were directly collected directly from the rectum of the animals into sterile vials with sterile peptone water (HiMedia, India) using sterile swabs. All the sample vials were transported under ice cover to the laboratory for further processing within 24 hours of collection.

Isolation of *E. coli* from faecal samples:

The collected faecal samples were incubated in the laboratory at 37^0C for overnight. After that, it was streaked onto MacConkey's agar (HiMedia, India) followed by incubation at 37^0C for 16 hours. The rose pink-coloured colonies were randomly picked and transferred to EMB agar (HiMedia, India) for further confirmation. The next day, brown colonies with a

'metallic sheen' were picked up and streaked onto nutrient agar (HiMedia, India) slants for further biochemical confirmation.

Morphological and Biochemical Identification:

All the pure cultures were subjected to different morphological and biochemical tests as per methods described by Quinn et al. (2011).

Detection of *E. coli* by PCR:

Bacterial DNA extraction: For genotypic detection of *E. coli,* DNA was extracted from all the positive isolates as per standard procedure.

Detection of *E. coli* in the faecal sample by PCR: The tentatively confirmed *Escherichia coli* isolates were subjected to PCR for genotype-based detection of the 16S *rRNA* geneas described by Wang et al. (1996) with some modifications. Positive control was supplied by the Department of Veterinary Microbiology for each PCR reaction.

Detection of ESBL Property in *E. coli* isolates:

Phenotypic Confirmatory Tests for ESBL Production: The antibiotic discs containing cefotaxime (30μg, HiMedia) and ceftazidime (30μg, HiMedia) with or without clavulanate (10μg, HiMedia) were used and a difference of ≥5 mm between the zone diameters of either of the cephalosporin disks and their respective cephalosporin/clavulanate disk was considered to be phenotypically positive for ESBL property in the isolates (Bauer et al., 1966; Patel et al., 2015).

Detection of bla_{CTX-M}, bla_{TEM} and bla_{SHV} Genes in *Escherichia coli* isolates by PCR: Detection of the ESBL-producing genes like the bla_{CTX-M}, bla_{TEM} and bla_{SHV} geneswere studied in all positive *Escherichia coli* isolate by PCR assay, as per the protocol of Weill et al. (2004) with some modifications.

Detection of ACBL Production in *E. coli* isolates:

Phenotypic assays for detection of AmpC beta-lactamase in *E. coli* isolates: All the *Escherichia coli* including ESBL-producing isolates were subjected to cefoxitin–cloxacillin double-disc synergy (CC-DDS) test for phenotypic confirmation of ACBL property following the previously described protocol (Tan et al., 2009).

Detection of AmpC Gene in Escherichia coli isolates by PCR: Detection of the bla_{AmpC} gene in all positive *Escherichia coli* isolates by PCR assay was performed as per the protocol of Féria et al. (2002) with some modifications.

Antimicrobial Sensitivity test of the ESBL-producing *E. coli* isolates:

All the ESBL-producing isolates were tested for their sensitivity and resistance to different antimicrobials by the disc diffusion method (Bauer et al. 1966; Patel et al. 2015). The antibiotic discs used were Amikacin (30μg), Amoxicillin/Clavulanic acid (20/10μg), Ceftriaxone (30μg), Doxycycline (10μg), Ceftazidime (30μg), Imipenem (10μg), Gentamicin (10μg), Levofloxacin (5μg), Ampicillin/Cloxacillin (10μg), Cefoxitin (30μg), Cefotaxime (30μg), and Tetracycline (10μg).

Results and Discussion:

E. coli infection in animals is an economically important disease. This study tried to detect the prevalence of *E. coli* in bovine faecal samples along with the detailed characterization of the isolates. A total of 46 (45.09%) bovine faecal samples showed growth of the characteristic pink-coloured colonieson MacConkey's agar, producing 'metallic sheen' on EMB agar plates and showed typical results in morphological (Gram-negative bacilli) and biochemical [Indole-Methyl Red-VogesProskauer-Citrate (+ + - -)] characterizations (Quinn et al., 2011; Samanta, 2013). All these isolates were confirmed to be *E. coli* (45% positivity) [Table 1] by detection of the 16S *rRNA* gene PCR like other researchers like Masud et al. (2012), Dereje (2012), Taghadosi et al. (2018) and Mohammed et al. (2019) who detected 44%, 43.1%, 26.3% and 46.4% *E. coli* isolates, respectively from bovine faecal samples which showed almost similar prevalence of *E. coli* like the present findings.

During the detection of phenotypical beta-lactamases production, 21(45.65%) *E. coli* isolates were found to be positive for ESBL production in double disc diffusion assay whereas 41(89.13%) isolates were ACBL positive (Table 2) (Klimiene et al., 2018). This type of finding was also reported by Vinueza-Burgos et al. (2019) (94.3% for ACBL) and Kar et al. (2015) [50% for ESBL] for bovine *E. coli* isolates. Molecular detection of beta-lactamase-producing genes in all the positive isolates showed bla_{CTX-M} (13, 28.26%) bla_{SHV} (9, 19.6%), bla_{TEM} (2, 4.34%) bla_{AmpC} (41, 89.13%) genes with variable frequencies (Table 2) with CTX-M being the most common gene among ESBL ones. The reports are almost by the reports of Geser et al. (2012) [bla_{CTX-M} - 94%, bla_{SHV} - 6%, bla_{TEM} - 0%], Kar et al.(2015)[bla_{CTX-M} - 72%, bla_{SHV} - 94%, bla_{TEM} - 50%] and Upadhyay et al. (2015) [bla_{CTX-M} - 56.3%, bla_{SHV} - 9.7%, bla_{TEM} - 12.6%] who also reported CTX-M as the dominant ESBL genotype in *E. coli* isolates from different sources. ESBL positivity in *E. coli* isolates was also reported by Casella et al. (2017) from different countries. The detection AmpC gene in *E. coli* isolates almost matches with the

reports of Vinueza-Burgos et al. (2019) [94.3%], Banerjee and Acharyya (2020) [88.9%] but quite higher than the reports of Kar et al. (2015) [11.1%] and Casella et al. (2017) [4.2%] maybe possibly due to geographical variation.

All the ESBL-producing *E.coli* isolates (n=24) showed almost similar high-level resistance to amoxicillin/clavulanic acid, ceftriaxone, ceftazidime, levofloxacin, ampicillin/cloxacillin, cefoxitin, cefotaxime, and tetracycline. However, no resistance was observed against amikacin, doxycycline, imipenem, and gentamicin (Table 3).These findings are almost in accordance with the reports of Banerjee and Acharyya (2020), Ibrahim et al. (2016) and Hinthong et al. (2017). Faruk et al. (2016) reported that ampicillin, cefotaxime, ceftazidime, and cefuroxime (all 100%), tetracycline (93.54%) were highly resistant but imipenem (100%) highly sensitive to the ESBL *E. coli* strains isolated from cattle in their study which almost matches with the

current findings. Ali et al. (2016) also found resistance against drugs like ampicillin (86.11%), amoxicillin-clavulanic acid (63.89%), cefotaxime (100%), ceftazidime (66.67%), tetracycline (72.22%) and gentamicin (61.11%) by ESBL *E. coli* pathogens in their study. The resistance profile of the *E. coli* isolates in the present study reflected the usage pattern of antibiotics in the studied animals.

Table 1: Detection of *E. coli* isolates from bovine faecal samples from different districts of West Bengal

Name of the Districts	No. of samples studied	No. of *E. coli* strains isolated (%)
Purba Barddhaman	41	13 (31.70)
Nadia	37	22 (59.46)
Hooghly	24	11 (45.83)
Total	**102**	**46 (45.09)**

Table 2: Detection of *E. coli* isolates from bovine faecal samples from different districts of West Bengal

Name of the Districts	No. of *E. coli* isolated (%)	Phenotypical ESBL positivity in *E. coli* strains	Frequency of ESBL genes in *E. coli* strains 24 (52.17%)			Phenotypical ACBL positivity in *E. coli* strains	Frequency of AmpC gene in *E. coli* strains
			CTX-M	TEM	SHV		
Purba Barddhaman	13	6	5	1	1	10	11
Nadia	22	10	7	0	5	18	20
Hooghly	11	3	1	1	3	9	10
Total	**46**	**21 (45.65%)**	**13 (28.26)**	**2 (4.34)**	**9 (19.6)**	**37 (80.43%)**	**41 (89.13%)**

Table 3: Antibiogram of ESBL-producing *E. coli* strains (n=24) isolated from bovine faecal samples in West Bengal

Sl. No.	Antimicrobials (Conc. in µg)	Isolates sensitive		Isolates intermediately sensitive		Isolates resistant	
		No.	%	No.	%	No.	%
1.	Amikacin (AK - 30)	24	100	0	0	0	0
2.	Amoxicillin/Clavulanic acid (AMC - 20/10)	0	0	0	0	24	100
3.	Ceftriaxone (CTR - 30)	0	0	0	0	24	100
4	Doxycycline (DO - 10)	22	91.67	2	8.33	0	0
5	Ceftazidime (CAZ - 30)	0	0	0	0	24	100
6	Imipenem (IMP - 10)	24	100	0	0	0	0
7	Gentamicin (GEN - 10)	24	100	0	0	0	0
8	Levofloxacin (LE - 5)	0	0	1	4.17	23	95.83
9	Ampicillin/Cloxacillin (AX-10)	0	0	0	0	24	100
10	Cefoxitin (CX - 30)	0	0	2	8.33	22	91.67
11	Cefotaxime (CTX - 30)	0	0	0	0	24	100
12	Tetracycline (TE - 10)	0	0	3	12.50	21	87.50
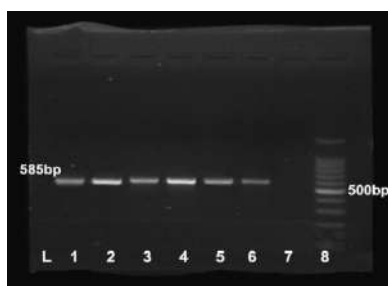
Figure 1: PCR amplification of the 16S *rRNA* gene (585bp) in *E. coli* isolates (Lanes 1-5: Test samples, Lane 6: Positive control, Lane 7: Uninoculated Negative control, Lane 8: 100 bp ladder)

Conclusion:

The study, therefore, can be concluded as approx. 46 bovine faecal samples screened here were found to be infected with *E. coli* strains (45.09%) from different districts of West Bengal. A total of 21(45.6%) and 41(89.1%) strains were found to possess ESBL and ACBL positivity which is quite alarming. All the ESBL-producing *E. coli* isolates showed a high level of resistance to amoxicillin/clavulanic acid, ceftriaxone, ceftazidime, ampicillin/cloxacillin and cefotaxime (all 100%), levofloxacin (95.83%), cefoxitin (91.67%), and tetracycline (87.5%). Antibiotics *viz.* amikacin, imipenem, gentamicin (all 100%) and doxycycline (91.67%) were detected to be quite sensitive against these pathogens. These drug-resistant *E. coli* strains may be of great clinical significance as these can easily create animal health hazards due to unsuccessful treatment with common antimicrobials and may be zoonotic too in a few cases.

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Conflict of Interest:

The authors declare that there is no competing interest among the authors.

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Study on Different Types of Farming Practices Adopted by Poultry Farmers in North 24 Parganas District of West Bengal

Debasish Saha [(1)]*, Prabhakar Biswas [(2)]

[(1)] Department of Veterinary and Animal Husbandry Extension Education, [(2)] Department of Avian Sciences, F/O: VAS, WBUAFS, 37 KB Sarani, Kolkata – 700 037, West Bengal

Abstract

The study was conducted in North 24 Parganas district of West Bengal, India. Two blocks were purposively selected from the district having a high poultry population from the two zones (*i.e.* non-saline and saline zones). From each zone, two clusters with three villages each, based on proximity were selected randomly on a proportionate basis to form a total sample size of 150 respondents. A structured interview schedule was prepared, pretested, and administered personally for data collection. Data were computed and analyzed statistically. It was observed that the poultry rearing practices in the backyard system were done mostly in a small kaccha house under the scavenging system with little supplementary feed. In the semi-intensive and intensive system scientific poultry rearing practices including housing and feeding were followed. The productivity of birds was found to be higher in the intensive system as compared to the semi-intensive and backyard systems. Most respondents received training about poultry farming in intensive broiler farming, whereas most of the respondents used scientific housing arrangements in their farm in intensive and semi-intensive poultry rearing.

Keywords: Fowls, broilers, Poultry housing, Hens, Poultry farming, Animal production

Introduction:

The increase in world population projected over the next 50 years will affect food production and delivery system. Currently, about 800 million people are undernourished to varying degrees, most of them living in underdeveloped countries in Africa and Asia (World Bank, 1996). Protein hunger is a common occurrence in infants and particularly in India child malnutrition (63%) is alarming (Deshai, 1998). Poultry, therefore, in this scenario plays a wonderful role in to fight against nutritional insecurity. The renowned scientist of the Indian Agricultural Economy, Dr. M. S. Swaminathan opined that India has reached its target of cereal security; now what is required is nutritional security, which can be achieved by making available animal protein in adequate quantity. Poultry production in India has reached a distinct and spectacular growth in the last 40 years. This sector plays a vital role in augmenting the income of the weaker sections besides providing nutritious and delicious food through eggs and meat to the people. Today India ranks 4ᵗʰ in egg production and 22ⁿᵈ in broiler production in the World (Singh, 2000). Unfortunately, the growth of the sector is mainly confined to a few pockets of our country. The ICMR recommends 180 eggs and 11 kg of poultry meat per capita per year. But the per capita availability of eggs and poultry meat is 86 eggs and 2.2kg meat per head per year, respectively which is far below the recommended levels. Again, poultry farming is possible in widely different agro-climatic environments as the fowl have remarkable physiological adaptability. The requirement of small space, low capital investment, quick return, and well-distributed turnover throughout the year make poultry farming remunerative in both rural as well as urban areas. Thus, in a nutshell, the rural poultry production system has a vast scope. There is hardly any systematic data on rural poultry production. With the increasing emphasis on small animals, we need first to have empirical data to develop some faithful strategies, particularly for the weaker section of the community. Keeping this in mind the present study was a modest attempt to find out the existing status of rural poultry production in North 24 Parganas district of West Bengal.

Materials and Methods:

The study was conducted in North 24 Parganas district of West Bengal, India. Two blocks were purposively selected from the district having a high poultry population from the two zones (*i.e.* non-saline and saline zones). From each zone, two clusters with three villages each, based on proximity were selected randomly on a proportionate basis to form a total sample size of 150 respondents. A structured interview schedule was prepared, pre-tested, and administered personally for data collection. The data collection was taken up during January to March 2003. Data thus generated were computed and analyzed through different statistical tools viz. frequency distribution, percentage analysis, etc.

Results and Discussion:

i) Duration in poultry farming: As reported in Table 1, the majority of the intensive poultry farmers *i.e.,* 68.1 percent had been rearing poultry for less than 4 years while 31.9 percent had 4 – 6 or more than 6 years of experience. On the other hand, the majority of semi-intensive (57.2%) and backyard (64.6%) poultry farmers had more than 4 years of experience in farming and the rest below 4 years.

ii) Training in Poultry: A majority (78.7%) of the poultry farmers under intensive and 14.3 percent under semi-intensive systems had received training in poultry, whereas, none of the farmers under the backyard system had received any formal training (Table 1).

iii) Flock size: The prevalent breeds in the backyard were non-descript desi birds, on the other hand in the intensive system only white broilers and Rhode Island Reds were reared and kuroilers in the semi-intensive system. Under the intensive system majority (54.4%) of the broiler farmers kept up to 500 birds, followed by farmers having 500-1000 birds (17%), 1000-1500 birds (12.8%), 1500-2000 birds (4.2%) and above 2000 birds [8.5%] (Table2). The layer farmer kept upto 500 birds on average. On the other hand, a majority (85.79%) of the semi-intensive poultry farmers kept 16-20 birds and the rest (14.3%) above 20 birds. Under the backyard system of poultry, the respondents (44.8%) generally kept upto 5 birds, whereas, 35.5%, 13.5%, 4.2% and 2.1% respondents kept 6-10, 11-15, 16-20, and above 20 birds, respectively (Table3). The average flock size, however, in the intensive, semi-intensive, and backyard systems were 1449, 42, and 16.9 birds, respectively.

Table 1: Distribution of respondents according to their duration of poultry rearing (N= 150)

Categories	Intensive		Semi-Intensive	Backyard	Total
	Broiler	**Layer**			
Duration					
< 2 years	16(34.1)	–	2 (28.5)	17 (17.7)	35 (24)
2 – 4 years	15(31.9)	1 (2.1)	1 (14.3)	17 (17.7)	34 (22)
4 – 6 years	9 (19.1)	–	1 (14.3)	23 (24)	33 (22)
> 6 years	6 (12.8)	–	3 (42.9)	39 (40.6)	48 (32)
Total	**47 (100)**		**7 (100)**	**96 (100)**	**150 (100)**
Training received in Poultry					
Yes	36(76.6)	1(2.1)	1(14.3)	–	38(25.4)
No	10(21.3)	–	6(85.7)	96(100)	112(74.6)
Total	**47 (100)**		**7 (100)**	**96(100)**	**150 (100)**

Table 2: Distribution of respondents according to their average flock size under intensive system (N= 47)

Flock size (birds)	Intensive	
	Broiler	**Layer**
Upto 500	26(54.4)	1 (2.1)
500 – 1000	8 (17)	–
1000 – 1500	6 (12.8)	–
1500 – 2000	2 (4.2)	–
> 2000	4 (8.5)	–
Total	**47 (100)**	

Table 3: Distribution of respondents according to their average flock size under semi-intensive and backyard system (N= 103)

Flock size (birds)	Semi-intensive	Backyard
Upto 5	–	43 (44.8)
6 – 10	–	34 (35.4)
11-15	–	13 (13.5)
16-20	6 (85.7)	4 (4.2)
Above 20	1 (14.3)	2 (2.1)
Total	**7 (100)**	**96 (100)**

Housing:

A. Backyard System: In this system respondents generally made small houses with locally available materials *viz.* bamboo, mud, wood, net, jute stalk, tiles, tin, straw, etc. Generally, the adult birds were housed together during the night with little consideration of space available per bird. Chicks were kept separately to avoid huddling and consequent death. Although, birds were left in free range during the daytime, however, some respondents housed the birds in poultry houses or bamboo baskets in the afternoon to avoid disputes with neighbours and attacks from predators.

Metallic, plastic, or earthen feeders and waterers are provided by a few respondents. The poultry houses were constructed at different heights from the ground to prevent the attack from predators.

B. Intensive and semi-intensive type of houses: Mixed farming was prevalent among intensive and semi-intensive poultry farmers. Under the intensive system, nearly 98% of respondents were rearing broilers whereas, only 2% kept layer birds. All the respondents keeping birds under the semi-intensive system were rearing dual-purpose birds. Integrated fish, duck, and chicken farming was also found among the semi-intensive farmers. The details about the housing are studied and discussed below (Table 4).

i) Space: All the respondents under the intensive system provided upto 0.5 sq. ft. space per chick. In the case of broiler birds, the growers were provided 0.5 to 1.0 sq. ft

(42.5%) and 1.1 to 1.5 sq. ft. (55.4%) space, whereas, 2.1 percent layer farmers provided 1.5 to 2 sq. ft space per bird. Under the semi-intensive system, all the respondents provided up to 0.5 sq. fit space for chicks, whereas, 28.6 percent and 71.4 percent provided 1.1 to 1.5 and 1.5 to 2.0 sq. ft. space, respectively (Table 5).

ii) Height from ground level: As reported in Table 4, the majority (85.2%) of the respondents had a poultry house, 1 to 2 ft. and 2 to 3 ft from ground level. It is further seen that an equal number of respondents (44.7%) rearing birds under intensive system of the poultry had the sheds at about 1 to 2 ft and 2 to 3 ft and only 8.5 per cent respondents made the house above 3 ft height from ground level. Similarly, 28.5 percent of each of the semi-intensive poultry farmers had a house at less than 1ft, 1 - 2ft, and 2 - 3 respectively.

iii) Housing Material: As reported in the case of backyard poultry farming the respondents made use of locally available as well as conventional materials under the intensive system. The walls were made of either brick and net or bamboo net in the intensive (74.5% and 25.5%, respectively) as well as in semi-intensive (28.6% and 71.4%, respectively) systems. It is further indicated in Table 4, that the majority of the respondents (83% intensive and 85.7% semi-intensive) used tiles as a roofing material. Straw was made use of by only 12.8 percent intensive and 14.3 percent semi-intensive poultry farmers. A mere 4.2 percent of intensive farmers had concrete roofs. Generally, the flooring was of mud (59.6% intensive and 100% semi-intensive) followed by 40.4% intensive farmers having a flooring of brick filled with cement. In the intensive system, sawdust was popularly used by poultry farmers (93.7%) as a flooring material. However, all the semi-intensive farmers and the rest of the intensive farmers used small pieces of straw as the litter material.

In the intensive and semi-intensive system, the space provided per bird was appropriate. The flooring was generally made of mud, which could lead to high moisture content in the litter and consequently, the breeding of diseases. Roofs were generally made of a tile which aligns with the findings of Mishra et al. (2000).

iv) Material for feeders and waterers: A perusal of Table4 indicates that 70.2 percent of intensive and the

entire semi-intensive poultry farmers used earthen pots with metallic trays and only 29.8 percent of intensive farmers made use of plastic waterers. With regards to feeders majority (61.7% intensive and 100% semi-intensive) of farmers used indigenous metallic feeders, whereas, 36.2 percent and 2.1 percent respondents used plastic and earthen feeders, respectively.

v) Arrangement of a poultry house: All the intensive as well as semi-intensive poultry farmers changed the litter, disinfected the room, and cleaned equipment in the poultry house. The majority (76.6%) of intensive and 71.4 percent semi-intensive poultry farmer had an electric supply in their farms. Sixty-eight percent and 100 percent had their farm well connected with brick roads, 44.6 percent and 57.1 percent planted trees around poultry houses. Eight-one percent and 71.4 percent of the farms were located in less crowded places, under intensive and semi-intensive systems, respectively. Table 4 further shows that the entire intensive poultry farmer had a chick guard and maintained the temperature in the poultry house. Sixty-eight percent of intensive farmers had white-washed their farms. Further, among the respondents who white-washed their sheds were generally found to be doing it, 1, 2, or 3 times a year *i.e.* 8.5 %, 40.4%, and 19.2%, respectively. Under an intensive system, 40.4% of farmers had brooder houses situated away from the adult birds, and 42.5% and 57.5% provided 5-6 hrs. and more than 7 hrs. of artificial light, respectively. The semi-intensive farmer did not make these arrangements at their farm.

Fifty percent of the farmers were generally keeping less than five hundred birds in an intensive system, however, farms with more than 2,000 birds were also found in the area of the study. Under the backyard and semi-intensive system, the average flock size was found to be 42 and 16.9, which is similar to the findings of Dana (1998) and higher than those reported by Panda and Nanda (2000) who concluded that the average flock size was 10.29 birds in Orissa. The higher productivity breeds were kept in intensive and semi-intensive systems, however, in the backyard system non-descript breeds were found. The improved indigenous breeds need to be introduced to reap more benefits.

Table 4: Distribution of respondents according to the height of poultry house from ground level (N= 54)				
Category Height (ft)	**Intensive**		**Semi-intensive**	**Total**
	Broiler	**Layer**		
0 – 1.0	1 (2.1)	–	2 (28.5)	3(5.5)
1.0 – 2.0	20 (42.6)	1 (2.1)	2 (28.5)	23(42.6)
2.0 – 3.0	21 (44.7)	–	2 (28.5)	23(42.6)
Above 3 ft	4 (8.5)	–	1 (14.5)	5(9.3)

	Total	47 (100)	7 (100)	54 (100)
Materials used in Poultry House				
Wall: i. Brick +Net	35(74.5)	–	2 (28.6)	37(68.5)
ii. Bamboo Net	11(23.4)	1 (2.1)	5 (71.4)	17(31.5)
Floor: i. Brick with cement	19(40.4)	–	–	19(35.2)
ii. Mud	27(57.5)	1 (2.1)	7(100)	35(64.8)
Roof: i. Tiles	38(80.9)	1 (2.1)	6 (85.7)	45(83.3)
ii. Concrete	2 (4.2)	–	–	2(3.7)
iii. Straw	6 (12.8)	–	1(14.3)	7(13)
Litter material: i. Saw dust	44(93.7)	–	–	44(81.5)
ii. Small pcs of straw	2 (4.2)	1 (2.1)	7(100)	10(18.5)
Types of material used for feeder and waterer				
Waterer: i. Earthen pots with Metallic tray	32(68.1)	1(2.1)	7 (100)	40(74.1)
ii. Plastic	14(29.8)	–	–	14(25.9)
Feeder: i. Earthen	–	1(2.1)	–	1 (1.8)
ii. Plastic	17(36.2)	–	–	17(31.5)
iii. Metallic	29(61.7)	–	7 (100)	36(66.7)
Types of arrangement made in poultry house				
Chick guard	46(97.9)	1(2.1)	–	47(87.3)
Maintenance of temperature	46(97.9)	1(2.1)	–	47(87.3)
Litter change	46(97.9)	1(2.1)	7 (100)	54(100)
Room disinfected	46(97.9)	1(2.1)	7 (100)	54(100)
Clean equipment	46(97.9)	1(2.1)	7 (100)	54(100)
Electricity supply	35(74.5)	1(2.1)	5 (71.4)	40(74.1)
Trees planted around house	21(44.6)	–	4 (57.1)	25(46.3)
Brooder house situated away from adult birds	18(38.3)	1(2.1)	–	19(35.2)
Farm connected with road	31(66)	1(2.1)	7 (100)	39(72.2)
Farm in less crowed place	37(78.7)	1(2.1)	5 (71.4)	43(79.6)
White wash	32(68.1)	–	–	32(59.3)
Duration. Once a yr	4(8.5)	–	–	4(7.5)
ii. Twice a yr	19(40.4)	–	–	19(35.2)
iii. Thrice a yr	9(19.2)	–	–	9(16.6)
Artificial light: i. 5 – 6 hrs	19(40.4)	1(2.1)	–	20(37.3)
ii. > 7 hrs	27(57.5)	–	–	27(50.0)

Table 5: Distribution of respondents according to space provided per bird (N= 54)						
Space/bird (sq. ft.)	**Intensive**			**Semi-intensive**		**Total**
	Chicks	**Broiler**	**Layer**	**Chick**	**Adult**	
<0.5	47 (100)	–	–	7 (100)	–	54 (100)
0.5 – 1	–	20 (42.5)	–	–	–	20 (37)
1.1 – 1.5	–	26 (55.4)	–	–	2 (28.6)	28 (51.9)
1.5 – 2.0	–	–	1 (2.1)	–	5 (71.4)	6 (11.1)

Feeding:

Seventy percent of the expenditure in poultry rearing accounts for the feed cost.

A. Backyard system: Under the backyard system of poultry farming the practice prevalent in the rural areas is of leaving the birds for scavenging in the morning and giving a handful of grain during the daytime. Some supplementary feed is offered in the night shelter too. The birds generally scavenge on insects, worms, grasses, seeds, flowers, etc. which are locally available. Table 6 shows the distribution of respondents according to the supplementary feed ingredients given. All the respondents offered rice/broken rice and kitchen waste to the birds, 89.6 percent of respondents gave boiled rice as supplementary feed and 39.5 percent gave broken wheat to the birds. 28.1 percent of respondents sometimes purchased readymade ration from the market for feeding the birds kept in the backyard system. The source of drinking water was the open drains around the hand pump and sometimes fresh water was provided in waterers.

B. Intensive and semi-intensive system: As shown in the study layer farmers and semi-intensive respondents gave different types of rations to different age groups of birds *i.e.,* starter, grower, and layer ration to birds. The broiler farmers also gave starter ration between 1 to 5 weeks and thereafter provided with finisher ration.

i. Frequency of feeding: A look at Table 7 reveals that the majority of the intensive (57.4%) and semi-intensive (14.3%) farmers feed their birds 3 times a day the rest feed the birds twice a day. The semi-intensive farmers generally left the birds for scavenging in the morning and evening and gave feed in return so that they could make up for the deficient amount.

ii. Amount of feed: The data in Table8 shows that the amount of feed given varied from 80-90, 90-100 gm, 100-110 gm, and 110-120 gm, for the broiler farmers *i.e.* 4.5, 17, 46.7, and 29.7 percent, respectively, while the layer farmer gave an amount of 120 to 130 gm of feed. The entire respondents under the semi-intensive system gave 80-90 grams. of feed only, since they left the birds to scavenge also. It is further seen that 80.3 percent of intensive and 57.1 percent semi-intensive farmers gave feed supplements to their birds (Table 8).

iii. Source of drinking water: All the intensive and semi-intensive poultry farmers used water from hand pumps. Additionally, 14.8% of respondents under intensive systems also used tap water (Table 9).

In the backyard system, birds were left in free range in the daytime with some supplementary feeding. Singh and Johori (1990), Dipelou et al., (1996), Rangaker and

Rangaker (1996), and Dana (1998) also reported similar findings.

However, in the intensive and semi-intensive system scientific feeding practices were followed, which shows that the farmer were well acquainted with the feeding regime and realized the importance of proper feeding for better productivity of birds.

Table 6: Distribution of respondents according to supplementary feed ingredients given by backyard poultry owners (N=96)

Ingredient	Percentage given
Rice/Broken rice	96 (100)
Boiled rice	86 (89.6)
Broken wheat	38 (39.5)
Kitchen waste	96 (100)
Readymade ration purchased from market (rarely)	27 (28.1)

Table 7: Distribution of respondents according to frequency of feeding of birds (N=54)

Frequency	Intensive		Semi-Intensive	Total
	Broiler	Layer		
2 times	20 (42.6)	–	6 (85.7)	21 (38.8)
3 times	26 (55.3)	1 (2.1)	1 (14.3)	33 (61.2)
Total	47 (100)		7 (100)	54 (100)

Table 8: Distribution of respondents according to amount of feed provided (N=54)

Feed/day	Intensive		Semi-Intensive
	Broiler	Layer	
80 – 90 gm	2 (4.5)	–	7 (100)
90 – 100 gm	8 (17)	–	–
100 – 110 gm	22 (46.7)	–	–
110 – 120 gm	14 (29.7)	–	–
120 – 130 gm	–	1 (2.1)	–
Given feed supplement	36 (78.2)	1 (2.1)	4 (57.1)

Table 9: Distribution of respondents according to source of drinking water (N=54)

Source	Intensive		Semi-Intensive
	Broiler	Layer	
Hand-pump	46 (97.9)	1 (2.1)	7 (100)
Tap	7 (14.8)	–	–

Conclusion:

Regarding the poultry rearing practices the respondent in the backyard system reared poultry in a small kaccha house, under the scavenging system with little

supplementary feed. In the semi-intensive and intensive system scientific poultry rearing practices including housing and feeding were followed. The productivity of birds was found to be higher in the intensive system as compared to the semi-intensive and backyard systems. Most respondents received training about poultry farming in intensive broiler farming, whereas most of the respondents used scientific housing arrangements in their farms in intensive and semi-intensive poultry rearing.

Conflict of interest:

The authors declare that they have no conflict of interest.

Data availability:

All the data in relation to the present study are available.

Author's contribution:

All authors equally participated in designing this article, data analysis and interpreting the results, drafting, editing the manuscript and approved the final version of the manuscript.

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Prevalence of *Cryptosporidium* Species Isolated from Calves from North 24 Parganas, South 24 Parganas and Nadia Districts of West Bengal

Rahul Bhanja [1], **Jaita Biswas** [1], **Sayantani Dule** [1], **Soumitra Pandit** [1], **Ruma Jas, Abhijit Nandi** [1], **Rahul Barua**[2], **Shubhamitra Chaudhuri** [3], **Tapan Dutta** [4], **Sanjoy Datta** [5], **Kunal Batabyal** [6], **Surajit Baidya** [1]*

[1] Department of Veterinary Parasitology, Faculty of Veterinary and Animal Sciences, West Bengal University of Animal and Fishery Sciences, 37, K. B. Sarani, Kolkata, WB, [2] Department of Veterinary Public Health, WBUAFS, Kolkata, [3] Department of Veterinary Clinical Complex, WBUAFS, Kolkata, [4] Department of Veterinary Microbiology, CVSc & AH, CAU, Aizawl, [5] Department of Animal Genetics & Breeding, WBUAFS, Kolkata, [6] Department of Veterinary Microbiology, WBUAFS, Kolkata

Abstract

The present work was carried out to study the morphology of *Cryptosporidium* oocysts recorded in diarrhoeic calves. A total of 148 faecal samples of diarrhoeic calves were screened for *Cryptosporidium* oocysts by modified Ziehl-Neelsen staining technique for morphological studies. Out of 148 samples, 31 (20.9%) samples were found positive for oocyst of *Cryptosporidium* species. The morphometric analysis revealed that the range of longitudinal diameter of the oocysts were 5.05±0.349-5.275±0.248 μm and the transverse diameter of the oocysts were 4.67±0.354-4.775±0.184 μm with a shape index (length/width) of 1.08-1.104.Based on oocyst dimension and shape index, it was assumed that ovoid and sub-spherical oocysts belonged to *Cryptosporidium parvum.*

Keywords: *Cryptosporidium* oocyst, Calves, Modified Ziehl-Neelsen, Morphometry

Cryptosporidium is a zoonotic apicomplexan intracellular obligatory parasite (Rossle and Latif, 2013). The first species of this genus was described by Tyzzer in 1907 and was named *Cryptosporidium muris*; two years later,the author described a similar species in mice and named *Cryptosporidium parvum* (Tyzzer, 1907). *Cryptosporidium parvum* oocysts are smaller than those of *C. muris* and typically infect the small intestine of the host. Cryptosporidiosis is a common protozoan infection in cattle, whereas bovine cryptosporidiosis is also an important source of human *Cryptosporidium* infection. It has enormous zoonotic importance because this parasite is transmitted through contaminated food and water and infects humans and animals (Feng et al., 2007; Helmy and Hafez, 2022). This parasite is ubiquitous in nature and not easily destroyed by ordinary disinfectants. *Cryptosporidium* has the potential for autoinfection, which makes it more fatal. The symptoms like persistent watery diarrhoea, vomition, nausea, abdominal cramps, etc., associated with *Cryptosporidium* spp. are self-limiting, but in immune-compromised individuals, symptoms can become more severe and may persist for months if left untreated. It also causes substantial economic loss in the livestock sector in terms of reduced milk production in dairy cows (Esteban and Anderson, 1995) and decreased weight gain in calves (Anderson, 1992; Innes et al., 2020). Herein, we report the prevalence of *Cryptosporidium* isolates obtained from diarrhoeic calves from three districts of West Bengal,

India. For identification, thorough morphological and morphometric studies were also carried out.

Faecal Samples were collected from three districts, namely, South 24 Parganas, North 24 Parganas and Nadia of West Bengal, India. A total of 148 faecal samples were collected from calves under three months of age from organised and unorganised farms. Samples were collected directly from the rectum and stored in a sterile container without preservatives. After collection, faecal matters was stained immediately at the laboratory with modified Ziehl Neelsen's staining method as described by Henriksen and Pohlenz, 1981 with some modifications, for microscopic evaluation and the rest portion was preserved in 2.5% potassium dichromate solution at 4°C for further studies.

For morphometric study, oocysts in the stained faecal preparations were measured under the light microscope by the method described by Pellardy (1965). The micrometer was calibrated under a light microscope and it was found that 1 unit of ocular micrometer equals to 2.5μm.The transverse and longitudinal diameters of 50 oocysts were measured, and the shape index of each oocyst was calculated as follows.

$$\text{Shape index} = \frac{\text{Longitudinal diameter of the oocyst}}{\text{Transverse diameter of the oocyst}}$$

Faecal specimens were analysed for the presence of *Cryptosporidium* and examined by direct microscopy

with modified ZN staining at 400× magnifications. Identification was made on the basis of the oocystic dimensions of *Cryptosporidium* described by Upton and Current (1985), Lindsay et al. (2000) and Kumar et al. (2004).Based on morphology, the smaller, spherical to ovoid purple colour oocysts were found (Figure 1 A and B). Out of 148 samples, 31 (20.9%) samples were found positive for oocyst of *Cryptosporidium* species.

The morphometric studies (Figure 2) revealed that the range of longitudinal diameter of the oocysts was 5.05±0.349-5.275±0.248 μm and the transverse diameters of the oocysts were 4.67±0.354-4.775±0.184 μm with a

shape index (length/width) of 1.08±0.0302-1.104±0.035 (Table 1).

Table: 1 Morphological analysis of *Cryptosporidium* oocyst based on micrometry:			
Sample no: (n=10)	Longitudinal Diameter (LD): (mean±SD)	Transverse Diameter (TD): (mean±SD)	Shape Index (SI)=LD/TD (mean±SD)
1.	5.05±0.349	4.67±0.354	1.08±0.0302
2.	5.17±0.354	4.7±0.307	1.101±0.031
3.	5.125±0.428	4.77±0.389	1.073±0.027
4.	5.225±0.463	4.8±0.497	1.09±0.042
5.	5.275±0.248	4.775±0.184	1.104±0.035
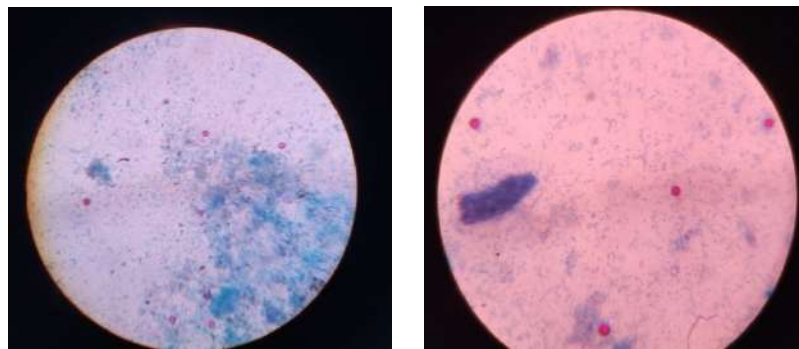
Figure 1: *Cryptosporidium* oocyst in modified Z-N staining under a light microscope. (A) Purple colour spherical oocyst of *Cryptosporidium* under 40X. (B) Purple colour spherical oocyst of *Cryptosporidium* under 100X.
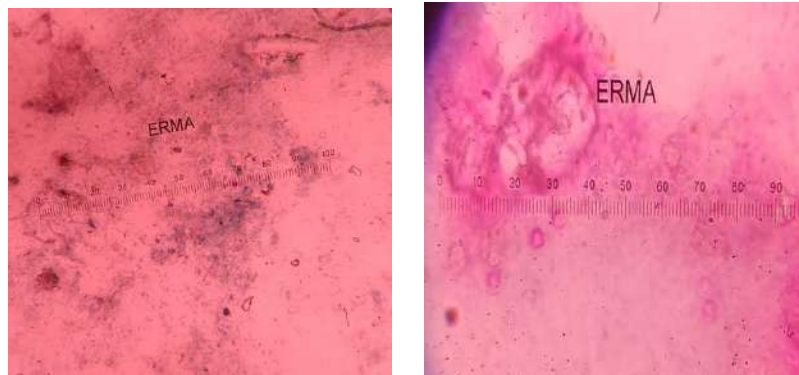
Figure 2: Micrometry of *Cryptosporidium* oocysts

Previously, morphometric studies revealed that the measuring size of *C. parvum* is 5.0 × 4.5 μm [range, (4.5–5.4) × (4.2–5.0) μm] (Tyzzer,1912; Upton and Current, 1985) and the shape indexwas1.16 (1.04- 1.33) (Tilley, 1991).Oocysts of *C. parvum* were also reported to range in size from 4.5 to 5.4 X 4.2 to 5.0 μm, with mean size of 5.0 X 4.5 μm and a shape index of 1.1 (Upton and Current, 1985), from 4.7 to 6.0 X 4.4 to 5.0, with a mean size of 5.0 X 4.7 μm and a shape index of 1.06 (Fayer et al., 2001), and from 5.0 to 5.5μm X 3.7 to 5.0 μm with a mean size of 5.2 X 4.3 μm and a shape index of 1.20 (Fall et al., 2003).

Based on oocyst dimension and shape indices, it was assumed that ovoid and sub-spherical oocysts belonged to *C. parvum*, which was responsible for causing undiagnosed diarrhoea as well as malnutrition, and decreased weight gain in neonatal calves. However, further, confirmation based on molecular characteristics is required to confirm the multi-species nature of *Cryptosporidium*.

Conflict of interest:

Authors declare no conflict of interest for this investigational report.

Data availability:

All raw data and backup photography are preserved at the Department of Veterinary Parasitology, WBUAFS

Ethical statement:

Authors maintained all ethical concern during sample collection and do not require IAEC certificate as it's not experimental.

Author's contribution:

All the authors equally participated in designing, data analysis and interpreting the results, drafting, editing the manuscript and approved the final version of the manuscript.

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Clinical management of pox like lesions in Black Bengal goats

Sanjib Datta [1], Indranil Samanta [2]*

[1] Veterinary Officer, Block Animal Health Centre, Barasat-I, North 24 Parganas, West Bengal , [2] Department of Veterinary Microbiology, West Bengal University of Animal and Fishery Sciences, 37, K.B. Sarani, Belgachia, Kolkata

Abstract

The present case study observed the occurrence of pox like lesions, with fever, anorexia, dyspnoea and infrequent abortion followed by death in Black Bengal goats (n=50) in North 24 Parganas district of West Bengal. None of the animals were vaccinated against goat pox. The treatment started after collecting the case history from the farmers with Injection Chlorpheneramine maleate, Injection Ceftriaxone-Tazobactam, Injection Paracetamol, Injection Meloxicam, Injection Vitamin A D_3 E, Topicure® spray (Natural Remedies, India) on the pock lesions, followed by eye drop containing Ciprofloxacin. The therapy successfully prevented the secondary bacterial infection and recovery of the animals occurred 10-15 days after onset of the treatment.

Keywords: Black Bengal goats, Pox, Treatment

Rearing of goats act as sustainable source of income for the farmers and it also helps in women empowerment as most of the female members of the farmer family take care of the animals. Different infectious or non-infectious health hazards create the major obstacles in the profit making process through goat rearing. The Black Bengal goat is considered as the most compliant, early maturing, prolific, and productive breed of the world which is commonly found in West Bengal and Bangladesh (Hossain, 2021). The meat and skin of the breed belong to the superior quality and are popular among the consumers. The breed is also popular for rearing due to its scavenging nature which helps in collection of the nutrients from uncultivable land, river bank, residence, and even from the hilly area. Although the breed is resistant to the most of the tropical animal diseases, the occurrence of skin infection, PPR and pneumonia are still reported (Nooruddin et al., 1987; Kashem et al., 2011). The present case study reported the occurrence of pox like lesions in Black Bengal goats with clinical signs of systematic involvement and successful therapeutic management.

The Black Bengal goats (n=50) irrespective of sex and age in few Gram Panchayet of Barasat-I block (North 24 Parganas, West Bengal) suffered from fever (104.6°F-107°F), anorexia, dyspnoea, hypersensitivity to touch, typical pock lesions in the eyes, hooves, under the abdomen / tail, udder, vagina, testicular area and infrequent abortion (Figure 1,2,3,4). Few adult goats (n=5) died before the onset of treatment. None of the animals were vaccinated against goat pox.

Figure 1: Typical pock-like lesion in the mouth of a Black Bengal goat

Figure 2: Typical pock-like lesion of a Black Bengal goat

Figure 3: Typical pock-like lesion in the udder and hooves of a Black Bengal goat

Figure 4: Typical pock-like lesion in the eye of a Black Bengal goat

The treatment started after collecting the case history from the farmers at Block Animal Health Center, Barasat-I Block with Injection Chlorpheneramine maleate, Injection Ceftriaxone-Tazobactam, Injection Paracetamol,

Injection Meloxicam, Injection Vitamin A D$_3$ E, Topicure® spray (Natural Remedies, India) on the pock lesions and eye drop containing Ciprofloxacin. The therapy successfully prevented the secondary bacterial infection and recovery of the animals occurred 10-15 days after onset of the treatment (Figure 5).

Figure 5: Recovered goat (early phase)from typical pock-like lesion

The present case study observed the occurrence of pox like lesions, with fever, anorexia, dyspnoea and infrequent abortion followed by death before the onset of treatment in Black Bengal goats. Similar kind of pock lesions with pustules and dry scab was observed in eye, nostril, mouth, vagina and base of tail in Vembur sheep in Tamilnadu (Malmarugan et al., 2015). The respiratory distress and fever was reported from Tellicherry goats suffering with goat pox virus infection (Pothiappan et al., 2015). In the present study, the fever was followed by development of flat and discoloured lesion on unpigmented skin. Later the lesions ulcerate and purulent discharge was observed. The respiratory distress was observed due to development of lung lesions and swelling of retropharyngeal lymph nodes which generates pressure onupper respiratory tract. The open skin lesions attract flies and secondary pneumonia is common due to bacterial co-infection (Mondal et al., 2004). Treatment with the antibiotics and topical spray successfully prevented the secondary bacterial infection and fly attacks.

Conflict of interest:

The authors declare that there is no conflict of interest regarding the present research work.

Ethical statement:

Not applicable

Author's contribution:

SD conducted the study, IS interpreted and wrote the manuscript.

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