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**ARTICLES**

- Risk factors, prevention and control strategies for surgical site infections in veterinary practice in Nigeria - A review** 72  
Oghenemega David Eyarefe
- Risk factors of haemoparasites and some haematological parameters of slaughtered trade cattle in Maiduguri, Nigeria** 83  
Paul B. T., Bello A. M., Ngari O., Mana H. P., Gadzama M. A., Abba A., Malgwi K. D., Balami S. Y., Dauda J. and Abdullahi A. M.
- Seroprevalence and risk factors for reproductive diseases in dairy cattle in Mexico** 89  
Feliciano Milián-Suazo, Ruben Hernández-Ortíz, Laura Hernández-Andrade, Arcelia Alvarado-Islas, Efren Díaz-Aparicio, Felix Mejía-Estrada, Erika G. Palomares-Reséndiz, Isabel Bárcenas Reyes and Horacio Zendejas-Martínez
- Newcastle disease: Seroprevalence and associated risk factors in backyard and small scale chicken producer farms in Agarfa and Sinana Districts of Bale Zone, Ethiopia** 99  
Minda Asfaw Geresu, Kemal Kedir Elemo and Gezahegne Mamo Kassa
- A study on prevalence of paramphistomum in cattle slaughtered in Gondar Elfora Abattoir, Ethiopia** 107  
Getenet Ayalew, Alebachew Tilahun, Alemu Aylate, Ayichew Teshale and Abebaw Getachew

Review

# Risk factors, prevention and control strategies for surgical site infections in veterinary practice in Nigeria - A review

Oghenemega David Eyarefe

Department of Veterinary Surgery and Reproduction, Faculty of Veterinary Medicine, University of Ibadan, Nigeria.

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**Surgical site infections (SSIs) are surgery associated nosocomial infections with multifactorial etiologies. They are adverse events that have placed heavy burden on surgery universally and have bedeviled veterinary surgery practice in Nigeria for decades, with consequent severe morbidity, mortality, financial and psychological burden on animal owners. In this paper, information on current universal trend of SSIs, including risk factors, prevention and control strategies was reviewed with emphasis on principles and practice among small animals and equine surgery practitioners. Principles guiding surgical suite design, surgical team, instruments/equipment, and patient preparation, were emphasized. It was concluded that imbibing the principles and practice of SSIs prevention strategies in Nigeria veterinary hospitals and clinics would impact positively on the veterinary health care system and the society the Veterinarian is committed to serve.**

**Key words:** Surgical site infections, patient, surgery, veterinary.

## INTRODUCTION

Surgical site infections (SSIs) are surgery associated nosocomial infections that account for about 38% of nosocomial infections among human patients (Beldi et al., 2009), 0.8 to 18% among small animal surgery patients (Verwilghen and Singh, 2015; McMillan, 2014; Birgand et al., 2014; Weese and Halling, 2006; Eugster et al., 2004; Vasseur et al., 1985) and 0 to 50% among equine surgery patients; depending on surgical procedure and wound classification (Ahern and Richardson, 2012; Verwilghen, 2015). The multifactorial etiology of SSIs has been associated with poor surgery theatre environment,

operating techniques, surgery team attitude, as well as, poor instruments, surgical team, and patient preparation (Verwilghen and Singh, 2015; McMillan, 2014; Humes and Lobo, 2009; Cheadle, 2006). The effects of SSIs is enormous on patients' welfare and animal care-givers/owners, and include: poor surgical site cosmesis, revision of surgery (de Lissovoy et al., 2009), prolonged wound healing (Verwilghen, 2015), risk of drug side effects (AORN, 2010), increased hospital stay (Jin-joeng, 2013.), increased psychological and financial burden on dog owners (Verwilghen and Singh, 2015), emergence of

E-mail: odayarefe@gmail.com. Tel: +2347064468932.

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multi-drug resistant (MDR) pathogenic organisms and patients' death (Weese et al., 2007). Although SSIs cases are prevalent in veterinary facilities (Turk et al., 2015; Nazarali et al., 2014; Mayhew et al., 2012; Eugster et al., 2004; Beal et al., 2000; Whittam et al., 1999), and the need to prevent re-occurrence emphasized, the lack of SSIs surveillance programs in veterinary healthcare centres, as obtained currently in human surgery practice (Astagneau et al., 2009; Anderson et al., 2014) has led to late detection and sometimes, non-capturing of SSIs cases in many veterinary hospital record systems (Turk et al., 2015). This has made intensive control and eradication of SSIs difficult due to lack of empirical data to justify the need for worry. SSIs is prevalent in Nigeria veterinary surgery practice due to unstandardized environment for surgical procedures, poor theatre manners, poor patient and instrument preparation among others (Tsai and Caterson, 2014). Attempt at prevention had, in most cases led to antimicrobial abuse (Akinrinmade et al., 2012), and possible evolution of resistant microbial strains.

A recent growing concern for veterinary clinical practice proficiency improvement has led to the establishment of the Postgraduate College of Veterinary Surgeons, Nigeria (CVSN), as well as, the development of Veterinary Teaching Hospital (VTH) facilities across the nation. These landmark trends in the veterinary profession in Nigeria; ultimately geared toward disease prevention and control, require information complementation on current global trend in SSIs prevention and control measures. The dearth of literature on this subject in Nigerian professional and institutional journals further heightens the need for this review. This paper therefore comprehensively and systematically presents: risk factors in SSIs, as well as, time proven prevention and control measures that could be adopted to minimize or possibly eradicate SSIs occurrence in public and private surgical facilities nationwide.

## DISCUSSION

SSIs remain the third most common hospital acquired infection with costly implications for surgery in human and veterinary practice (Verwilghen, 2015; Bigand et al., 2014; Anderson et al., 2014). The problem is legendary and dates back to the beginning of practice of the surgery specialty (Milard, 2012; Clark, 1907), when the fear and risk of SSIs prevented quick surgical intervention until the patient was brought near death (Verwilghen, 2015). Earlier infection control measures were implemented following Drs. Ignaz Semmelweis and Oliver Wendell Homes' observations that contaminated hands of attending physicians served as vehicle for the spread of infections (Humes and Lobo, 2009; Adriaanse, 2000). The introduction of compulsory hand scrubbing with chlorinated lime solution before physical examination by attending physicians resulted in an impressive reduction

in mortality rate (from 11.4 to 1.3% within two years) in the Vienna maternity ward (Sabbatani et al., 2014; Adriaanse, 2000), and propelled the commencement of compulsory antiseptics hand washing regimen as a means of infection control among surgeons (McMillan, 2014; Humes and Lobo, 2009). This practice became globally accepted following the publication of the Louis Pasteur germ theory in 1860, on the role of germs in infection causation, and the statement "instead of forcing ourselves to kill the microbes in wounds, would it not be more reasonable not to introduce them" (Ahern and Richardson, 2012; Verwilghen et al., 2011). Infection control practice further became entrenched among communities of surgeons with Joseph Lister's publications on anti-septic surgery concept and thesis on aseptic principles for surgeons (Hermani, 2009). The discovery of antibiotics further enhanced the curbing of SSIs. However, the current trend in microbial multi-drug resistance to antimicrobials calls for the need to identify SSIs risk factors, and strengthen the prevention and control strategies.

## Risk factors in SSIs causation

Endogenous and exogenous sources of wound site bacteria contamination, and patient health status at surgery are major risk factors in the causation of SSIs among human and veterinary patient (Turk et al., 2015; Hermani, 2009). Patients' commensal flora at the surgical site, including skin surface and body tracts (gastrointestinal and respiratory) are sources of endogenous wound site contamination and infection, while bacteria contaminants from surgical team, the environment, surgical materials, instruments and wound dressings are exogenous sources (McMillan, 2014; Cogen et al., 2008). Canine endogenous pathogens associated with SSI have been identified and include: *Staphylococcus pseudintermedius*, *Staphylococcus aureus* *Coagulase-negative staphylococci* (CONS), *Pseudomonas species*, *Enterococci* and extended spectrum  $\beta$ -lactamase (ESBL) producing Enterobacteriaceae (*E. coli*, *Enterobacter* and *Klebsiella spp*) (Weese and Duijkeren, 2010). Staphylococci are the most commonly cultured bacteria from SSIs with *S. pseudintermedius* being the leading cause of SSIs in dogs, and *S. aureus* in horses (Weese et al., 2010). *S. pseudintermedius* is also the most isolated *Staphylococci spp* from small animal healthcare workers as against *S. aureus* in human healthcare workers (Thorup, 2014). Besides, there is an increasing concern about the multidrug resistant potentials of *S. pseudintermedius* which is potentially greater than those produced by methicillin resistant *Staphylococcus aureus* (MRSA) (Weese et al., 2010; Thorup, 2014). The bacterium also has a strong biofilm forming ability that further complicate treatment in implant associated SSIs (Thorup, 2014).

Zoonosis caused by *S. pseudintermedius*, though lower

than that caused by *S. aureus* has also been reported (Weese et al., 2010). A thorough surgical patient clinical screening prior to surgery has been recommended (Centre for Disease Control (CDC), 1999). Patient health status is a major risk in SSIs causation because risk of SSI correlates directly with dose and virulence of microbial contamination and patient's immune resistance (Owens and Stroessel, 2008). Obese patients and those with endocrinopathies such as hypothyroidism, diabetes mellitus, hyperadrenocortism, smoking, diabetes, nutritional status and consumption of certain drugs are SSIs risk in human practice (Mangram et al., 1999).

In animal patients, hypothermia, hypotension, surgical wound classifications and implants (Turk et al., 2015) increased body weight and endocrinopathy in intact animals (Fitzpatrick and Solano, 2010; Nicholson et al., 2002) are risk factors in SSIs causation. Other risk factors are; hair clipped at surgical site > 4 h (Mayhew et al., 2012), increased anaesthesia time (Nazarali et al., 2014), duration of surgery (Eugster et al., 2004; Nicholson et al., 2002; Vasseur et al., 1985), longer tissue manipulation, wound exposure time, noise in the theatre (Kurmann et al., 2011) and non-administration of antibacterial prophylaxis in clean contaminated and contaminated wounds (Whitten et al., 1999).

In equine practice, uncontrolled use of antimicrobial prophylaxis is associated with burden of multi-resistant bacteria (Damborg et al., 2012) and antimicrobial-induced colitis, especially in horses undergoing elective arthroscopic surgery (Weese and Cruz, 2009). Surgeon's training and experience is also a risk factor as complications associated with closure of equine celiotomy incision (Wormstrand et al., 2014) and survival after colic surgery has been linked with years of experience and training of the surgeon (Wormstrand et al., 2014).

## Strategies for prevention and control of SSIs

### **Pre-surgical hand preparation and hand hygiene**

Hand hygiene is a key component in prevention of SSIs (Nelson 2011; WHO, 2009). The hands of surgical staff have higher pathogenic microbial load than those of others due to their increased contact with infected wounds (Verwilghen et al., 2011; Coelho et al., 1984). Transient skin microbes acquired by contact with persons, animals and environment are known for inducing SSIs (Verwilghen et al., 2011). The isolation of zoonotic, biofilm producing, multi-drug and methicillin resistant *S. pseudintermedius* from hands of small animal health care workers (Thorup, 2014), further calls for strict pre-surgical hand wash and hygiene protocol. Pre-surgical hand antisepsis is aimed at eliminating or reducing the skin microbial flora to diminish the risk of SSIs (McMillan, 2014). The practice of the correct

method of pre-surgical hand preparation has been reportedly low among human and veterinary surgeons (Verwilghen et al., 2013). The common tradition is the use of antiseptic solution (chlorhexidine or povidone base soap) to scrub hand and arm with 20 to 25 scrubbing brush strokes, and hand/arm rinse over elbow or pedal controlled tap (Tanner et al., 2008). This traditional scrubbing method has been faulted due to much time involved, its inability to adequately remove resident bacteria from hand, arm and beneath finger nails, and for its compromise of the protective water-lipid layer of the superficial skin (Widmer et al., 2010; Kampf and Kramer, 2004), thus increasing the chances of pathogenic bacteria skin colonization due to impaired skin immunity (Larson et al., 1998). Besides, contact dermatitis due to skin reaction to scrubbing solutions has been reported in some individuals (Larson et al., 2006; Krautheim et al., 2004). Emphasis is currently rapidly shifting from the traditional hand scrub to hand wash and alcohol gel rubs among human surgeons (Verwilghen et al., 2013; Kampf and Kramer, 2004).

Alcohol gel hand rub has the advantages of being easier and faster to use, better efficacy against hand resident microbes compared to disinfecting soap solutions and cause less skin damage with repeated use (Loffler and Kampf 2008; Kampf et al., 2003). For these reasons, it has been recommended by the World Health Organization for pre surgical antiseptic purposes (WHO, 2009). However, low compliance attitude has been observed among veterinary surgeons in shifting from the traditional hand scrub with brush and antiseptic soap solution to adjusting to alcohol hand rub despite observed advantages and WHO recommendations (Verwilghen et al., 2013; Verwilghen et al., 2011). Despite the popularity of alcohol gel hand rubs among human surgeons, cases of non-compliance with usage directive have been observed (Umit et al., 2014).

### **Surgical theatre construction and environmental hygiene practice**

Operating theatre location and construction influence the potential for the risk of SSIs (Gastmeier et al., 2012; Hambraeus, 1988). Although veterinary literature has less information on theatre construction as correlate of SSIs, information in human literature (Sapna and Pradeep 2011) could be extrapolated and applied. It was recommended that the operating theatre be located in a blind wing, or at bottom floor, or topmost floor of hospital facility to control traffic to the area and reduce contamination (Sapna and Pradeep 2011; Lynch et al., 2009; Lidwell, 1982). A clear demarcation of the theatre into zones (the outer, restricted, and aseptic) has also been recommended to minimize contamination (Sapna et al., 2011). The use of wall and floor tiles made of polished stone or marble to ease cleaning and disinfection has



also been recommended (Jeong et al., 2013; Sapna and Pradeep, 2011). Also recommended are installation of a laminar air flow system for filtration and expelling of contaminated air (Tsai and Caterson, 2014; Jeong et al., 2013; Lidwell, 1982).

A functional surgical theatre requires constant cleaning and disinfection. All equipment within the theatre such as surgical lamps requires constant daily cleaning and instantly when fluid splashes on them during surgeries. Installation of anti-microbial copper alloys has been recommended for their bactericidal actions on touch surfaces of drip stands and chairs arms. There is also the need to treat water to reduce chances of hand contamination by water borne pathogenic microbes in surgical theatres (Tsai and Caterson, 2014; McMillan, 2014; Sapna and Pradeep 2011). Increased human traffic into and out of the operating room has been linked with increase in SSIs rate (Radcliff et al, 2013; Pokrywka and Byers, 2013; Panahi et al., 2012; Lynch et al., 2009). Rate of infection increases with the number of people in the theatre (Panahi et al., 2012; Eugster et al., 2004), as a result, restrictions should be placed on the type and number of people that can be allowed into the surgical theatre.

### ***Surgical team manners***

Surgical team members' attitude grossly influences SSIs (Beldi et al., 2009; Lucet et al., 2012). Low attitude of team members, such as, non-compliance with hand disinfection procedures, gloving, hair and nose covering, maintaining of 50 cm distance from the surgical table by non-sterile team members, as well as, indiscriminate opening of the theatre doors and increased noise (talking) are risk factors for SSIs causation that require constant caution for prevention (Verwilghen, 2015; Kumman, 2011; Lucet et al., 2012). The presence of one or more visitors during surgical procedure constitutes SSIs risk and should be avoided (Birgand et al., 2014; Makary, 2013; Beldi et al., 2009; Boer, 2001).

### ***Surgical site preparation for surgery***

The patient's skin microbial flora and endogenous pathogens of mucous membranes and hollow organs are risk factors in theatre wound infection (Hermani, 2009). Clipping of hair coat, scrubbing, application of antiseptic solutions, and draping prior to surgery are conventional practice to forestall SSIs (Tanner et al., 2008). In dogs removal of hair coat with clipper rather than razor blade after anaesthetic induction and immediately before surgical procedure is recommended (CDC, 1999), as coat clipping before induction is marked with incidence of SSIs (Tanner et al., 2008). The hair coat of veterinary patients harbor myriads of microbial organisms and cause contamination of the surgical field that eventually

leads to surgical site infection (Nelson, 2011; Cooper et al., 2000). The efficiency of hair clipping has been linked with timing of hair coat removal. Clipping of hair coat > than 4 h before surgery is fraught with high risk of SSIs and should be avoided as it gives bacteria enough time to be established on clipped skin before commencement of surgery (Mayhew et al., 2012). Surgical patients' hair coat removal with razor blades cause more skin abrasions and compromises skin immunity compared to clippers (Mangram, 1999; Anderson et al., 2014).

Skin preparation with appropriate antiseptic solution has been recommended as a preventive measure for SSIs (Nelson, 2011). Commonly used antiseptic solutions are either of alcohol or aqueous base and often contain chlorhexidine gluconate or iodophores (Loscovish, 2014; Dumville et al., 2013). A combination of 70% alcohol and 4% chlorhexidine has a faster onset, longer duration of action and broad spectrum antibacterial activities and preferred for patients' skin preparation (Loscovish, 2014; Dumville et al., 2013; Hemani, 2009). Concentric scrubbing fashion, beginning from the proposed incision site outward has been challenged in favour of 'back and forth motion' with the theory that the surgical site is inadequately sterilized with the concentric method (MacDonald et al., 2001).

Draping of the surgical site is done to isolate the sterile surgical zone during surgery (Figure 1). Although draping techniques are seemingly easy, poor draping of patient increases the risk of surgical site infection (Showalter et al., 2014; Cooper, 2000). Also, blood soaked areas on drapes are potential sites for microbial multiplication and contamination of the sterile field as blood serves as good medium for bacterial growth. Disposable drapes are currently preferred to washable and re-useable fabrics as washable fabrics, over time, do not retain the water proof characteristics of an ideal draping material, and make them support infection (Showalter et al., 2014; Hopper and Moss, 2010).

### ***Instrument preparation***

Surgical instruments are vital to surgical procedures and their poor preparation has been associated with theatre wound infections (Hopper and Moss, 2010). Metal instruments are conventionally sterilized with moist heat using autoclave at 121°C for 13 min (Sapna, 2011). Recommended cold sterilization involves soaking of instruments in antimicrobial solutions (chlorhexidine, iodophores, and isopropyl or ethyl glycols) not lesser than 3 h prior to surgery (Cooper et al., 2000). Cold sterilization is not however advisable for instruments intended for invasive surgical procedures (Cooper et al., 2000). Gas sterilization with ethylene oxide and with gamma irradiation are also recommended where applicable (Sapna, 2011). Delay in opening of the surgical pack till time of operation is important, as pre-operative delay for an extended length of time results in



**Figure 1.** A draped patient on the surgical table.

commencement of surgery could help prevent instrument contamination (Figures 2 and 3) (Dalstrom et al., 2008; Radcliff et al., 2013).

### ***Pre-operative delay***

Delay in commencement of surgery increases the chances of sterile field contamination through airborne contamination of opened surgical instruments (Hopper and Moss, 2010). Common causes of pre-operative delay include: an extended time in gaining intravenous access and anaesthetic ready time (Radcliff et al., 2013). To minimize preoperative delays, a proactive approach to anaesthesia termed “independent anaesthetic induction” has been advocated (Radcliff et al., 2013). It emphasizes the need for two anaesthetic teams; one to begin induction of anaesthesia for new patients and the other to maintain anaesthesia in the current patient. This approach

reduces the preoperative time and chances of surgery site infection (Radcliff et al., 2013).

### ***Surgical guise or theater wears***

Surgical guise comprises conventional theatre wears (scrubs, sterile gown, a face mask, the sterile gloves, head cover, and shoe covers or boots) designed to reduce contaminants and surgical wound infections (Figure 4) (Salassa and Swiontkowski, 2014). The surgical scrub consists of the short sleeves shirt and the pant or trousers (Amirfeyz, 2007). The scrubs should not be long sleeved, and should not be worn with additional clothing like cardigans or inanimate objects like stethoscopes. It should not be worn outside the hospital or clinic facility and should not be laundered at home with other fabrics (Braswell-spruce, 2012). The human nares are colonized by the normal body flora that can become a



**Figure 2.** Instrument pack opened with instruments arranged for surgery.

source of infection to surgical wounds demanding the use of face mask (Salassa and Swiontkowski, 2014). Head covers help to control the spread, through aerosol, of normal hair microbes into the surgical field. The same principle holds for covering of side beards with facial mask (Fossum, 2015). Although no clear study currently shows the correlation between the use of the leg covers or booties in surgical wound infection rates, it is however inferred that street shoes could serve as sources of contamination to the theatre air and contribute to theatre wound infection (McHugh et al., 2014; Amirfeyz, 2007). Disposable sterile surgical gowns are preferred to reusable ones as their moisture proof nature prevents seep through body fluid that could contaminate wounds. A single use also prevents the discomfort associated with constant washing of the fabrics, which eventually results in wear and tear. A study has shown that the rate of theatre wound infections reduces with disposable gowns

compared with re-usable (Moylan, 1987). Modern surgical practice therefore advocates a single use of disposable surgical gowns, face masks, head covers and surgical gloves (McHugh et al., 2014).

### ***Breaks in surgical asepsis***

A break in asepsis is any event that occurs that alters or compromises the aseptic attribute of the surgeons, surgical instruments or surgical field (AORN, 2010). Common breaks in surgical asepsis could include: a tear on the surgeons' gown sterile package, torn gloves during surgery, dropped face masks; an error in an attempt at putting on the surgical guise and scrubbing by the surgeon. It also includes faulty instruments and surgical field sterilization process, errors in the patients' positioning, prepping and draping, as well as, dropping a



**Figure 3.** Covered surgical instruments due to preoperative delay.

contaminated instrument on a sterile instruments table, and when a sneeze or cough occurs across a sterile field (Sapna, 2011). Surgical glove puncture and contamination of sterile field is common in veterinary and human surgery (Nelson, 2011). Surgical gloves become punctured in 35% of cases after two hours of surgery, but only 20% of glove punctures are noted by the surgeon (Nelson, 2011). Double gloving has been encouraged in orthopaedic procedures where glove punctures often occurs through instruments, on the first finger, with grave SSIs consequences in implant associated procedures (McMillian, 2014). It is recommended that glove be changed after an hour of surgery, and open gloving may be done where assisted intraoperative gloving is not feasible (Duxbury et al., 2003).

Four classes of breaks in surgical asepsis have been identified and preventive measures suggested: Class 1: breaks which are spotted as soon as they occur and contained immediately. Class 2: breaks that are noticed a short while after they occurred and can still be managed;

Class 3: breaks which are almost impossible to contain because they were identified to have occurred far into the operative procedure and Class 4: breaks that are never identified (AORN, 2010). It is important that all non-scrubbed personnel keep a distance of 12 inches from scrubbed personnel (Hopper and Moss, 2010). It is essential that a skilled perioperative nurse who has been well trained to detect lapses in aseptic techniques always be in the operating room. It is important to document common breaks in aseptic techniques that are seen to occur in surgery on a daily basis, so that control measures could be taken to prevent a re-occurrence.

#### ***Prolonged surgical time, techniques and tissue trauma***

Increased stay of patient in operating room more than one hour prior to surgery increases the chances of theatre wound infection (Radcliff et al., 2013). Prolonged



**Figure 4.** A surgeon on gown, face mask, cap and gloves.

instruments contamination prior to surgery. In event of delay, covering of instruments with a sterile towel until surgical time is a major contributor to theatre wound infections (Radcliff et al, 2013; Jeong et al., 2013; Tsai and Caterson, 2014). The explanation given is that traumatized internal organs are exposed in the open for long and tissue perfusion delayed (Radcliff et al, 2013; Jeong et al., 2013; Tsai and Caterson, 2014; Cooper, 2000). A skillful surgeon is aware of the importance of adequate tissue oxygenation to the outcome of the wound healing. Supplementing oxygen intake with a ventilator is recommended in addition to maintaining the optimum body temperature (Anderson et al., 2014).

**Temperature regulation:** Maintaining normothermia is important to the outcome of theatre wounds as studies have shown a positive correlation between adverse body temperature and surgical wound infection (Hopper et al., 2009; Cheadle, 2006). Hypothermia hinders peripheral circulation and minimizes oxygen perfusion in the wound

area. Besides, an optimal temperature is needed by the body to trigger body immune responses to infection (Tsai and Caterson, 2014). Hypothermia reduces neutrophil functions and increase blood loss which would trigger the need for blood support, a high risk factor for theatre wound infections (Anderson et al., 2014). Hypothermia could be managed by using warm fluid for intra-operative tissue flushing, warm water blanket, heating lamp and heating pads (Abelha et al., 2005). Well aerated environment using cool mists and fans, cold intravenous fluids, cooling blankets, as well as, oxygen administration are common means of managing hyperthermia (Sessler, 2009).

**Drain placement:** Postsurgical drains placement has been described as a high risk factor for theatre wound infection especially in veterinary patients (Tsai and Caterson, 2014; Jin-joeng, 2013). Drains and catheters are foreign to the body and their placement compromise the skin immune status in areas along which they pass

through and make them prone to infection (Nakamura et al., 2012). Animal patients are more likely to distort the placed drains and catheters and may require restraint collars to prevent their removal and owners cooperation for monitoring (Tsai and Caterson, 2014).

**Antibiotic use and mis-use:** Antimicrobial prophylaxis (AMP) is indicated in human and veterinary surgical procedures depending on surgical wound classification (clean contaminated or contaminated), those with higher infection rates (implanting of prosthetic devices) and where infection could cause grave consequences (Akinrinmade, 2012) without compromising appropriate aseptic protocols (Eugster et al., 2004). The need for empirical laboratory evidence of microbial type and sensitivity could influence the choice of a narrower spectrum of antimicrobial agent to enhance preservation of patients' normal microbial flora and reduce chances of antimicrobials resistance (Anderson et al., 2014; Carlet et al., 2014). The timing of AMP administration is important to ensure peak serum threshold at the time of incision to complement body immune system against infection. The CDC guidelines for human surgery patients (Bratzler and Houck, 2004) requires administration of selected AMP within 60 min of surgical incision, repeated every 2 half-lives to maintain therapeutic concentration, and discontinuation within 24 h post-surgery (Bratzler et al., 2005; Bratzler and Houck, 2004). This protocol has been confirmed effective in reducing SSIs in human surgery patients (Bratzler et al., 2005) and adopted in veterinary medicine (Verwilghen and Singh, 2015) with some modification and prolongation of antibiotics beyond 24 h in canine tibia plateau leveling osteotomy (TPLO) procedures (Nazarali et al., 2014; Frizpatrick and Solano, 2010). The choice of antibiotics AMP depend on the anticipated microbial challenge (Verwilghen and Singh, 2015). Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major culprit in human and animal patients wound infection (Harper et al., 2013) except in canine species where *S. pseudintermedius* is a leading cause of SSIs (Weese et al., 2010). These organisms have been isolated commonly from the hair coat, nasal mucosa and palms of patients and hospital staff (Thorup, 2014) demanding the need for screening of patients and staff, and decontaminating of carriers with appropriate antibiotics prior to commencement of surgery (Tammelin et al, 2001).

**Staff training and re-orientation:** The need to train, re-train and engage all surgeons and peri-operative staff on various aseptic guidelines and practices in the hospital and surgical environment has been emphasized in the reduction of incidence of SSIs (Anderson et al., 2014; NICE, 2008). A routine training exercise will provide the forum to acquaint newly employed staff with theatre aseptic practices, refresh the memory of old staff and allowing for sustainability (Loscovich, 2014). Learning aids like brochures, videos and mock exercises to test

staff skills have also been recommended (Puntis et al., 1990). Feed-back measure to evaluate the impact of educational programs could help in commendation of staff for areas of success and also in spotting areas of lapses that can be improved upon (Loscovish, 2014).

## CONCLUSION

There is a universal effort at preventing or minimizing theatre wound infections. Efforts are made from design of the surgical suite to establishing principles, guidelines and work ethics within surgical facilities to prevent the occurrence of wound infection, enhance restoration to health, and reduce hospital stay and cost. Imbibing and practicing these established guidelines in veterinary hospitals and clinics in Nigeria should move veterinary patient health care efforts positively towards impacting on the society and the primary patient for which the veterinarian swore an oath of absolute commitment to total care.

## Conflict of interest

The author has not declared any conflict of interest.

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*Full Length Research Paper*

## Risk factors of haemoparasites and some haematological parameters of slaughtered trade cattle in Maiduguri, Nigeria

Paul B. T.<sup>1</sup>, Bello A. M.<sup>2</sup>, Ngari O.<sup>2</sup>, Mana H. P.<sup>1</sup>, Gadzama M. A.<sup>1</sup>, Abba A.<sup>1</sup>, Malgwi K. D.<sup>1</sup>, Balami S. Y.<sup>1</sup>, Dauda J.<sup>3</sup> and Abdullahi A. M.<sup>1</sup>

<sup>1</sup>Veterinary Teaching Hospital, University of Maiduguri, Nigeria.

<sup>2</sup>Department of Veterinary Medicine, Faculty of Veterinary Medicine, University of Maiduguri, Nigeria.

<sup>3</sup>Department of Veterinary Public Health and Preventive Medicine, University of Maiduguri, Nigeria.

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This study was conducted to determine the prevalence of haemoparasites and some haematological parameters of slaughtered trade cattle in Maiduguri abattoir. A total of 120 blood samples were randomly collected from cattle between January and July, 2014. The samples were screened for haemoparasites by examining Giemsa stained thin blood films. Microhaematocrit centrifugation technique was used for determination of packed cell volume (PCV) while total red blood cell (RBC) counts and total white blood cell (WBC) counts were determined by cyanmethaemoglobin and hemocytometer methods. An overall prevalence of 10.8% (CI=0.064, 0.017) was recorded for Anaplasma (5.8%), Babesia (4.2%) and Trypanosoma species (0.8%). Young cattle had significantly ( $p<0.05$ ) higher prevalence of 9.2% (CI= 0.052, 0.157) compared to adults with 1.7% (CI= 0.005, 0.059). Among different sexes, females had a significantly ( $p<0.05$ ) higher prevalence of 7.5% (CI: 0.040-0.136) than males with 3.3% (CI= 0.130, 0.083). Rahaji breed had a significantly higher ( $p<0.05$ ) prevalence of 7.5% (CI= 0.040, 0.136) compared to Ambala with 1.7% (CI= 0.005, 0.059), Kuri and Adamawa gudali each with a prevalence of 0.8% (CI= 0.002, 0.046). Cattle with moderate body condition scores had significantly ( $p<0.05$ ) higher prevalence of 6.7% (CI= 0.034, 0.126) compared with those that have good body condition scores with 4.2% (CI= 0.018, 0.094) while thin and fat cattle were not infected with haemoparasites. Even though all the haematological parameters were within range of normal values, there was a significant difference ( $p<0.05$ ) in mean packed cell volume (PCV) and total white blood cell (WBC) counts between infected and un-infected slaughtered cattle. It was concluded from this study that haemoparasites are endemic in cattle populations in Maiduguri and the prevalence of haemoparasites may be associated with changes in PCV and WBC count.

**Key words:** Haematological parameters, haemoparasites, Maiduguri, Prevalence, risk factors, trade cattle.

### INTRODUCTION

The Nigerian livestock resources was conservatively estimated to the tone of USD 6 billion (Anon, 2006; Akande et al., 2010) and contributes significantly to the

Agricultural component of the Gross Domestic Product (GDP) of which cattle production contributes up to 40% (McIntyre et al., 1992). Cattle production provides

essential source of protein through meat and milk, generates employment, income, farm power and organic manure for arable Agriculture in the Sudano-Sahelian ecological zones of the country (Ikhatua and Asaka, 2000). Among nomadic Fulani and Shuwa-Arab pastoralists, the ownership of cattle also serve as an index of social prestige (Lamorde, 1998). Furthermore, their products such as hide and skins, bones and blood serve as raw materials for industries (Ikhatua and Asaka, 2000).

Among parasitic diseases of cattle, haemoparasitism constitute a disease entity of great economic importance (Jongejan and Uilenberg, 2004; Salih et al., 2015) and has been recognised as a serious threat to food security of Nigeria. The impact of haemoparasites on cattle productivity is also difficult to quantify (Singla et al., 2007; Samdi et al., 2010) but losses in traction power, milk and meat production and costs of control programs have been ascribed to haemoparasites (ILIR, 1997). Haemoparasites and their vectors have a global distribution, and are especially important in Sub-Saharan Africa (Okorafor and Nzeako, 2014). The prevalence of haemoparasites of cattle in Nigeria is generally considered to be very high due to the preponderance of their arthropod vectors (Biu and Kabono, 2005; Kamani et al., 2010; Okorafor and Nzeakor, 2010; Musa et al., 2014). Moreover, 90% of the cattle population in Nigeria are raised under the pastoral husbandry system of Fulani herders (Lorusso et al., 2013). Under this system cattle are extensively grazed on pastures and forests and may be exposed to various arthropod vectors of haemoparasites (Obadiah and Shekaro, 2012). The prevalence of various genera of haemoparasites of cattle (*Trypanosomes*, *Babesia*, *Anaplasma*, and *Theileria*) was previously reported in different parts of the country (Akande et al., 2010; Kamani et al., 2010; Samdi et al., 2010; Enwezor et al., 2012; Ademola and Onyiche, 2013; Okorafor and Nzeako, 2014; Qadeer et al., 2015) and elsewhere in the world (Alim et al., 2012; Velusamy et al., 2014). *Trypanosoma*, *Babesia* and *Anaplasma* species are listed among the most economically important genera of haemoparasites in Nigeria (Biu and Kabono 2005; Akande et al., 2010; Kamani et al., 2010), and their impact on cattle production and productivity accounts for heavy economic losses to livestock producers in the tropics and subtropics (Soulsby, 1982; FAO, 1984). They are responsible for destruction of erythrocytes leading to anaemia, jaundice, anorexia, weight loss and infertility in livestock (Akande et al., 2010).

There is paucity of information on the prevalence and importance of haemoparasites in trade cattle in Maiduguri. Therefore, this study was conducted to

determine the prevalence of haemoparasites of cattle and their associated haematological changes.

## METHODOLOGY

### Study area

This study was conducted in Maiduguri which is the capital and largest city of Borno State, and has an estimated total population of 521,492 (NPC, 2006). Maiduguri has one of the largest cattle markets in north-eastern Nigeria with a high volume of trade in livestock and livestock products through neighbouring Chad, Cameroun and Niger republics. Maiduguri lies between Latitude 11°N and Longitude 13°E and it is characterized by a long period of dry season which lasts between October and May, with a short period of rainfall between June and September (LCRI, 2007).

### Study population and sampling method

A total of 120 trade cattle presented for slaughter at the Maiduguri central abattoir were randomly selected for this study which lasted between January and July, 2014. Characteristics of population such as age, sex, breed and body condition scores were observed and recorded for each sample throughout the study. Sex differentiation was based on the appearance of external genitals while breed identification was based on morphology as described by Yunusa et al. (2013). Ageing was based on rostral dentition as described by Lasisi et al. (2002). Cattle aged less than 3 years old were categorised as young while older ones were considered as adults. The body condition score (BCS) of cattle was evaluated on a 5 point scale based on modification of method described by (DEFRA, 2001).

### Collection and preservation of blood samples

5 ml of blood was collected from the jugular vein at the point of slaughter into EDTA containing bottles which were labelled appropriately and placed on ice packs for onward delivery to the side laboratory, Veterinary Teaching Hospital, University of Maiduguri where they were further processed.

### Determination of haematological parameters

Packed cell volume (PCV) was determined using microhaematocrit centrifugation technique (MHCT) as described by Brar et al. (2011). Blood was introduced into microhaematocrit tubes by capillary action and one end of each capillary tube was sealed with plasticin. The tubes were spun in a microhaematocrit centrifuge (Hawksley, England) at 13000 g for 5 min. PCV was measured with a hematocrit reader (Hawksley, England), and recorded appropriately (Kamani et al., 2010). Total red blood cell counts (RBC) and total white blood cell counts (WBC) were determined by cyanmethaemoglobin and hemocytometer methods described by Coles (1974).

### Preparation of blood film for identification of haemoparasites

A thin blood smear was prepared on a standard microscope glass

\*Corresponding author. E-mail: bpaulgadzama@unimaid.edu.ng.

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**Table 1.** Prevalence of Haemoparasites in Slaughtered trade Cattle based on Sex, Age, Breed and Body Condition Sores.

Risk factors	No. examined	No. (%) Infected	95% CI	
			L	U
<b>Sex</b>				
Male	32	4(3.3)	0.013	0.083
Female	88	9(7.5)	0.040	0.136
<b>Age</b>				
Adult	18	2(1.7)	0.005	0.059
Young	102	11(9.2)	0.052	0.157
<b>Breed</b>				
Adamawa Gudali	2	1(0.8)	0.002	0.046
Ambala	21	2(1.7)	0.005	0.059
Kuri	6	1(0.8)	0.002	0.046
Rahaji	91	9(7.5)	0.040	0.136
<b>BCS</b>				
Thin	9	0(0.0)	0.000	0.031
Moderate	48	8(6.7)	0.034	0.126
Good	60	5(4.2)	0.018	0.094
Fat	3	0(0.0)	0.000	0.031
<b>Total</b>	<b>120</b>	<b>13(10.8)</b>	<b>0.064</b>	<b>0.177</b>

BCS= Body condition score. CI = 95% confidence interval on prevalence.

slide (75 mm by 25 mm), air dried, fixed in methyl alcohol for 3-5 min, stained in 5% Giemsa stain for 30-45 min in a staining jar and rinsed in buffered distilled water (Kamani et al., 2010). Haemoparasites were identified by direct microscopic examination based on morphologic keys as described by Soulsby (1982), using a compound microscope (Olympus, USA)..

#### Data analysis

Data generated were summarized and presented in tables using descriptive statistics. Prevalence of haemoparasites was estimated as  $p = d/n$  (%). Where  $p$  = prevalence,  $d$  = number of individuals having disease at a particular point in time and  $n$  = number of individuals in the population at risk at that point in time (Thrusfield, 2005). The two sided 95% confidence interval (Newcombe, 1998) for various sexes, age groups, breeds and body condition scores of slaughtered cattle were computed on VassarStats.<sup>®</sup> (Website for statistical computation), and  $p < 0.05$  was considered significant.

## RESULTS

The distribution of haemoparasites in slaughtered trade cattle by sex, age, breed and body condition scores is presented in Table 1. Out of 120 blood smears examined, 13 representing 10.8% (95% CI= 0.064, 0.177) were positive for various genera of haemoparasites. Sex-wise, the prevalence of haemoparasites was significantly higher ( $p < 0.05$ ) in females (7.5%: 95% CI= 0.04, 0.136) than males (3.33%: 95% CI= 0.013, 0.083). Age-wise, the

prevalence of haemoparasites was significantly higher ( $p < 0.05$ ) in young representing 9.2% (95% CI= 0.052, 0.157) compared with older cattle which represent 1.7% (95% CI: 0.005-0.059). Among the different breed of cattle examined in this study, the prevalence of haemoparasites was significantly higher ( $p < 0.05$ ) in Rahaji which represents 7.5% (95% CI= 0.040-0.136) while the lowest prevalence was recorded in Adamawa gudali and Kuri breeds which both had a prevalence of 0.83% (95% CI= 0.002, 0.046). Similarly, a significantly higher ( $p < 0.05$ ) prevalence was recorded in cattle with moderate and good body condition scores which represent 6.7% (95% CI= 0.034, 0.126) and 4.2% (95% CI= 0.018, 0.094), respectively. Both thin and fat cattle examined in this study were not infected with haemoparasites.

Some haematological parameters of infected and uninfected slaughtered trade cattle is shown in Table 2. Mean values of packed cell volume (PCV), total white blood cell counts (WBC) and red blood cell count (RBC) of infected and uninfected cattle examined in this study were within normal range. However, there was a significant difference ( $p < 0.05$ ) in mean packed cell volume (PCV) and total white blood cell counts (WBC) between infected and uninfected slaughtered trade cattle. On the other hand, no significant difference ( $p > 0.05$ ) was observed in mean RBC between infected and uninfected cattle.

**Table 2.** Some haematological parameters of infected and uninfected slaughtered trade Cattle in Maiduguri.

Haematological parameter	Mean±SE (Range)	
	Positive (n=13)	Negative (n=107)
PCV (%)	28.5 ± 2.40 (11-40) <sup>a</sup>	32.6 ± 0.49 (20-41) <sup>b</sup>
WBC x 10 <sup>3</sup> /μL	7.2 ± 0.41 (4.0-10) <sup>a</sup>	5.5 ± 0.15 (2.2-11) <sup>b</sup>
RBC x 10 <sup>6</sup> /μL	5.2 ± 0.37 (3.1-8.1) <sup>a</sup>	5.6 ± 0.08 (3.2-8.8) <sup>a</sup>

PCV = packed cell volume, WBC = white blood cell, RBC= red blood cell, All values Mean±SE, Row means with unmatched superscripts (<sup>a,b</sup>) differ significantly at p<0.05.

**Table 3.** Prevalence of various genera of haemoparasites of slaughtered trade cattle in Maiduguri.

Species of Haemoparasites	n =120	
	No. Infected	Prevalence (%)
<i>Anaplasma</i> species	7	5.8
<i>Babesia</i> species	5	4.2
<i>Trypanosoma</i> species	1	0.8
<b>Total</b>	13	10.8

The prevalence of various genera of haemoparasites in slaughtered trade cattle in Maiduguri is presented in Table 3. The three genera identified in this study were *Anaplasma* (5.8%), *Babesia* (4.2%) and *Trypanosoma* (0.8%).

## DISCUSSION

The results obtained in this study indicates that haemoparasites are endemic in cattle populations within Maiduguri and its environs, even though the overall prevalence is not comparable with previous reports. The preponderance of infection with haemoparasites may be attributed to a high prevalence of cattle ticks in Maiduguri (Musa et al., 2014). Biu and Kabono (2005) reported a higher prevalence of haemoparasites in cattle from the same study area. This difference could be attributed to changes in farm management practices, including more aggressive measures of vector control in the last decade. Our result is also at variance with Okorafor and Nzeako (2014) who reported a prevalence of 6.7% for various species of haemoparasites of cattle and Ademola and Onyiche (2013) who reported a prevalence of 5% in Oyo state, Nigeria. Also, our overall prevalence does not agree with Kamani et al. (2010) who reported a higher prevalence of 25.7% for haemoparasites in North-central, Nigeria. These discrepancies could be attributed to local differences in prevalence of haemoparasites due to variations in geographical location (Velusamy et al., 2014) which determines the distribution of the arthropod vectors of the parasites (Shah-Fischer and Say, 1989; Agbede, 2013). However, our result is comparable to

Onoja et al. (2013) who reported an overall prevalence of 9.5% for bovine Babesiosis in slaughtered cattle from Zaria, Nigeria and Kumar et al. (2015) who reported an overall prevalence of 9.3% from cattle in Punjab, India.

The effects of risk factors such as age, sex and breed on prevalence of haemoparasites has been previously reported (Kamani et al., 2010; Alim et al., 2011; Ademola and Onyiche, 2013; Okorafor and Nzeako, 2014). The higher prevalence in females (7.5%) than males (3.3%) agrees with Kamani et al. (2010) who observed a similar trend and attributed their finding to the fact that female animals were generally herded much longer for the purpose of breeding and milk production, thereby prolonging their exposure to challenges of disease. Also, younger cattle less than 3 years old had a higher prevalence compared to their adult counterparts. This finding agrees with Ademola and Onyiche (2013) and could be due to development of immunity in adult cattle with previous infection. The higher prevalence of haemoparasites recorded in Rahaji breed could be attributed to the fact that it is the most numerous breed in Borno State because of their adaptation to arid and semi-arid conditions (Blench, 1999). Moreover, they are usually herded by pastoralists under transhumant conditions which exposes them to the vectors of haemoparasites thereby increasing the risk of infection.

This study has also revealed that haemoparasites were prevalent only in cattle with moderate and good body condition scores but thin and fat cattle were not affected. This finding may be due to the fact that fewer numbers of thin and fat cattle were encountered in the study. Additionally, fat animals usually come from feed lots where they are probably treated against various parasites

and other diseases, thereby minimizing the chances of detecting haemoparasites among them. By contrast, thin cattle may originate from extensively managed herds with poor nutritional background, which increases risk of haemoparasites among them. Moreover, poor nutrition and management are significant risk factors for acquiring other debilitating diseases by extensively managed cattle under transhumant conditions, and could be responsible for loss of body condition.

The mean PCV and total white blood cell counts of infected and uninfected slaughtered trade cattle examined in this study were within normal range (Merck Manual, 2012). However, there was a significant difference ( $p < 0.05$ ) in PCV between infected and uninfected cattle, and this could be attributed to the effects of haemoparasites on blood cells. This finding agrees with Kamani et al. (2010) who reported that infection with *Babesia*, *Anaplasma*, *Theileria* and *Trypanosoma* species, either singly or in combination caused a significant reduction in mean PCV of cattle. It also is known that infection with most haemoparasites leads to destruction of erythrocytes and anemia (Soulsby, 1982; Ademola and Onyiche, 2013). The higher mean total white blood cell counts recorded in infected cattle could be explained on the basis of immune response to presence of haemoparasites. Eosinophilia was previously reported in haemoparasitic infections of ruminants (Ademola and Onyiche, 2013) which may contribute to the observed differences in WBC count between infected and uninfected cattle in the present study.

The three genera of haemoparasites identified in this study (*Anaplasma*, *Babesia* and *Trypanosoma*) were previously reported in domestic animals in Nigeria (Abenga et al., 2004; Bui and Kabono 2005; Kamani et al., 2010; Ademola and Onyiche, 2013; Okorafor and Nzeako, 2014; Qadeer et al., 2015) and elsewhere in the world (Soulsby, 1982; Alonso et al., 1992; Bock et al., 2004; Alim et al., 2011; Sitotaw et al., 2014). The higher prevalence of *Anaplasma* (5.8%) and *Babesia* (4.2%) in this study could be attributed to the availability of suitable environmental conditions which favour multiplication and survival of their tick vectors (Soulsby, 1982; Shah-Fischer and Say, 1989). However, the low prevalence of Trypanosomes in the present study is not unusual because Maiduguri is located in the Northern limit of tsetse distribution (Abenga et al., 2004). Previously, cases of trypanosomosis have been reported in the Sahel around Maiduguri (Maxie et al., 1979). These unusual occurrences have been linked to movement of cattle from tsetse infested to tsetse free zones (Anene et al., 1991). Moreover, mechanical vectors such as biting flies have been incriminated in transmission of trypanosomosis in tsetse free zones (Soulsby, 1982).

A number of antiparasitic drugs have been used for the treatment and control of Bovine haematozoa (Soulsby, 1982) but only diminazene aceturate and imidocarb dipropionate are still in common use (Merck, 2013). These drugs have shown up to 100% efficacy against

*Babesia*, *Anaplasma* and *Trypanosoma* species and could be used to effectively reduce the prevalence of these haemoprotozoa in cattle populations within Maiduguri and environs.

## Conclusion

Even though we recorded a lower prevalence of haemoparasites in this study compared to the previous report in the last decade, it may be concluded that haemoparasites are endemic in cattle populations within Maiduguri, and their occurrence may be associated with changes in some haematological parameters.

## RECOMMENDATION

It is therefore recommended that a stringent measure of controlling haemoparasites in food animals should be instituted in Maiduguri and its environs where these animals are sourced. Such measures should include more aggressive chemotherapy, chemoprophylaxis and control of arthropod vectors through the use of effective insecticides, acaricides and environmental management.

## Conflict of Interests

The authors have not declared any conflict of interests.

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*Full Length Research Paper*

## Seroprevalence and risk factors for reproductive diseases in dairy cattle in Mexico

Feliciano Milián-Suazo<sup>1\*</sup>, Ruben Hernández-Ortíz<sup>2</sup>, Laura Hernández-Andrade<sup>3</sup>, Arcelia Alvarado-Islas<sup>3</sup>, Efren Díaz-Aparicio<sup>3</sup>, Felix Mejía-Estrada<sup>4</sup>, Erika G. Palomares-Reséndiz<sup>3</sup>, Isabel Bárcenas Reyes<sup>1</sup> and Horacio Zendejas-Martínez<sup>1</sup>

<sup>1</sup>Facultad de Ciencias Naturales, Universidad Autónoma de Querétaro. Avenida de las Ciencias S/N Juriquilla, Delegación Santa Rosa Jáuregui, Querétaro, Qro. México.

<sup>2</sup>CENID-PAVET, INIFAP, Carretera Federal Cuernavaca-Cuautla, No. 8534, Col. Progreso, Jiutepec, Mor., C.P. 62550, Mexico.

<sup>3</sup>CENID-Microbiología, Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias, Carretera México-Toluca, Km 15.5, Col. Palo Alto, 05110, D. F, México.

<sup>4</sup>Sitio Experimental Pichucalco, Instituto Nacional de Investigaciones Forestales Agrícolas y Pecuarias, Pichucalco, Chiapas. CP 29520, México.

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The purpose of the study was to estimate seroprevalence of four reproductive diseases: brucellosis, bovine virus diarrhoea (BVD), neosporosis and bovine respiratory rhinotracheitis (IBR) in dairy cattle in Mexico. In a stratified multi-stage design, 4,487 serum samples were collected in 182 herds from different states of Mexico. Epidemiologic and spatial information was also collected to evaluate risk factors and elaborate maps of risk. Overall seroprevalence rates were: Brucellosis 14.7% (with the Rose Bengal test) and 5.1% (with the radial immunodiffusion test), BVD 78.8%, neosporosis 36.8% and IBR 73%. The highest prevalence for neosporosis (46%) and brucellosis (21.8%) was observed in the intensive system. In the familiar and double-purpose systems, the prevalence was 34 and 15.8%, respectively. No big differences were observed for IBR and BVD in the three systems, 69 to 75% for IBR and 63.9 to 87.8% for BVD. The states with the highest prevalence for brucellosis were Hidalgo (77%), Aguascalientes (36%), Guanajuato (30%), and La Laguna (Coahuila and Durango) (17%). Prevalence was low in Veracruz (1%), Chiapas (2%), and Sinaloa (3%); for BVD ranged from 55% (in the state of Sinaloa) to 98% (in the state of Aguascalientes). Prevalence for neosporosis was high in Hidalgo (55%), Guanajuato (53.7%), and Querétaro (47.9%). Risk factors associated to prevalence of brucellosis were: herd size, introduction of animals from different herds, common sheds, production system, and source of replacements. For BVD, herd size, common sheds, intensive production, and large calving intervals were significant factors. Abortion rate, use of fresh colostrum, services per conception, and intensive production were the factors associated with neosporosis. Factors significantly associated to IBR were: use of bull for breeding, and positive serology to parainfluenza virus 3. Areas of risk and probability of disease were related with areas of high density of dairy cattle.

**Key words:** Dairy cattle, reproductive diseases, seroprevalence, epidemiology, Mexico.

## INTRODUCTION

Reproductive infectious diseases are a permanent threat to dairy herds all over the world (Juyal et al., 2011). Diseases like neosporosis, brucellosis, infectious bovine rhinotracheitis (IBR), and bovine viral diarrhea (BVD) alter reproductive performance, reduce productivity, limit access to livestock markets, and, in the case of brucellosis represent a risk to public health (Houe et al., 2006; Anderson, 2007). In addition, they reach other than the reproductive organs causing different clinical manifestations.

Brucellosis, caused by *Brucella abortus* is one of the most important reproductive diseases of cattle. In some cases, co-existence with small ruminants promotes infection with *B. melitensis* (Lopez-Merino, 1989). Since livestock is important and represents an important source of currency for the country (Dirección de Tuberculosis Bovina y Brucelosis, 2000; Rio, 1977), a national campaign for the eradication of animal brucellosis has been implemented in Mexico since 1995. However, brucellosis is still a big problem in cattle, dairy and beef goats, sheep and pigs (Pacheco and Luna-Martínez, 1999), causing losses for about USD 200 million a year (Luna-Martínez, 1999a; Luna-Martínez, 1999b). In humans, an average of 2000 cases a year have been reported for the last 7 years (Pacheco and Luna-Martínez, 1999).

Bovine viral diarrhea (BVD) is found worldwide in cattle causing considerable economic losses due to the impact on health and reproduction (Davies and Carmichael, 1973). Early embryonic death, mummification, congenital defects and abortion are some of the consequences of infection during pregnancy. Fetuses infected during the first 120 days of gestation can develop immunotolerance and become lifelong virus carriers (Fray et al., 2000). In regions with high prevalence over 1 to 2% of the newborn calves are persistently infected (Houe et al., 2006). Reduction in milk production is perhaps the most important feature in lactating cows (Howard, 1990).

Two genotypes of BVDV (BVDV-1 and BVDV-2) have been identified by serology and molecular biology (Cantú and Alvarado, 1998); however, subtypes of the two genotypes have also been described (Río, 1977; Vilcek et al., 2001). BVDV-2 is prevalent in North America (Fulton et al., 2005), in Europe (Jackova et al., 2008; Letellier et al., 1999; Luzzago et al., 2001; Tajima et al., 2001); and in Asia (Nagai et al., 1998). It has been associated with severe clinical disease in adults and with hemorrhagic syndrome in youngsters (Carman et al., 1998). In the past two years, a severe form of BVDV-2

has been reported in Germany and in the Netherlands (Arias et al., 2003; Hurtado et al., 2003; Schirrneier, 2014).

*Neospora caninum* in cattle is recognized as a major cause of abortions and economic losses to farmers worldwide (Dubey, 1999a). Cows aborting in previous pregnancies abort repeatedly or give birth to sick calves or calves with subclinical infection. The life cycle of *N. caninum* is well known, dog is recognized as the final host (McAllister, 1988). Canine-derived oocysts have been found contaminating the environment (Wouda et al., 1999) and are infective for calves (De Marez et al., 1999). Sources of postnatal infection for cows are unknown but vertical transmission is the predominant mode of natural infection. *N. caninum* has been reported World-wide (Dubey, 1999a, b); however, no much information is available for Mexico (Morales et al., 2001a, b). Herd-level prevalence has been estimated in between 10 and 100% (García-Vázquez et al., 2002).

Infectious bovine rhinotracheitis (IBR) is a disease of the upper respiratory tract that causes substantial economic losses to the cattle industry worldwide (Hage et al., 1998). Infection may occur by first exposure to the virus; reactivation of the virus in latency, or by vaccination with live virus during pregnancy (Muylkens et al., 2007; Ormsbee, 1963; Smith, 1997). It causes embryonic death, mummified animals, infertility, stillbirths, or birth of weak calves that die after a few days (Arthur et al., 1991; Blood and Radostis, 1992; Correa, 1986). IBR virus can be transmitted by respiratory, ocular, and reproductive secretions; however, introduction of infected animals to the herd is the most important source of infection (Moles et al., 2002). Cattle of all ages and breeds are susceptible, but the disease typically occurs in animals older than six months (Wentink et al., 1993). Therefore, the purpose of this study was to estimate the seroprevalence and associated risk factors of four reproductive diseases: brucellosis, bovine viral diarrhea, neosporosis and bovine respiratory rhinotracheitis in dairy cattle in Mexico.

## MATERIALS AND METHODS

### Sampling strategy

Data was obtained from a large cross-sectional study in 182 farms conducted between January, 2010 and December, 2012. Farms from different states of Mexico and from three systems of production were included. Systems were intensive [States of

\*Corresponding author. feliciano.milian@uaq.mx. Tel: 52(442) 192-12-00, ext. 5384.



Aguascalientes, Chihuahua, Guanajuato, Hidalgo, Coahuila and Durango (La Laguna), and Queretaro)]; family-type (Jalisco), and double-purpose (Chiapas, Sinaloa and Veracruz). The Intensive system comprises Holstein-Friesian cows kept in closed premises with no access to grazing; herds may hold from 150 to 10,000 head of cattle, with an average of 300. Family-type farms are run mostly by family members, Holstein breeds and cows used have access to grazing for short periods of time during the day; herd size is about 50 cows. Double-purpose mainly utilizes *Bos Taurus indicus* and crosses of this with some *Bos Taurus taurus*, which are primarily used for calving and, as a secondary purpose, milk production.

Samples were collected in a stratified multistage sampling design. Since the population of dairy cattle is located in specific regions, each of these regions was considered as a stratum in the first stage. In the second stage, states were selected within each stratum, and counties within each state. Counties were not randomly selected since not all counties in a state have dairy cattle; they were selected from a counties milk-producing list. Finally, due to the lack of a good sampling frame, convenience sampling was used to select herds and animals within herds. Sampling personnel were advised to select herds from different areas of each county to make a representative sample. To reduce variance of sampling, a sampling fraction by stratum (region) was determined dividing the total number of samples by the total dairy cattle population for the states included ( $3,500/2,000,000 = 0.0018$ ). Subsequently, to determine the number of animals sampled per stratum, the sample fraction was multiplied by the size of the population in each stratum. With a 10% hypothetical prevalence for brucellosis, 1% error and a 95% confidence level, the estimated sample size was 3,500 animals; however, for practical reasons, -due to proportional sampling, in some herds the required number of animals to sample was less than 5, a non-worthy number. The final number of samples collected was 4,487.

### Samples

Ten milliliters of blood were collected from each animal from the middle coccygeal vein with a 20-gauge, 1-inch needle in a 10 ml serum-separator Vacutainer tube (Becton Dickinson and Company, Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ 07417 to 1885, USA). The study was conducted in accordance with the Animal Welfare Legislation of Mexico. Collection of blood samples was performed by a qualified veterinarian following official procedures from the Norma Oficial Mexicana (NOM-041-ZOO-1995) of the National Campaign against Brucellosis in Animals (46). Animals were handled aiming to minimize stress and suffering. Samples were stored at -20°C until analysis.

### Serological tests

Presence of antibodies against brucellosis was determined by the Rose Bengal test and then, some were positively confirmed by the radial immunodiffusion test (RIT). For IBR, the plate seroneutralization in MDK cells with the IBR758 virus was used. Antibodies against BVD were identified by an Enzyme-Linked Immunosorbent Assay using CIVTEST bovis BVD/BDab 80 Hipra, Girona Spain. Antibodies against *Neospora caninum* were determined by an indirect immunofluorescence assay. This method uses two antibodies; the unlabeled first (primary) antibody specifically binds to the target molecule, and the secondary antibody, carrying the fluorophore, recognizes and binds to the primary antibody. This provides signal amplification by increasing the number of fluorophore molecules per antigen. The protocol of this study was approved by the Bioethics Committee of the Faculty

of Natural Sciences in the Autonomous University of Queretaro.

### Epidemiological information

In order to collect epidemiological information, a questionnaire was supplied to all herd owners to identify farm management practices and herd performance. Questionnaire included open items (any answer possible) and closed items (possible answers provided in the questionnaire) related to general characteristics of farms, such as size, breed and production, as well as target questions referring to potential risk factors for disease prevalence.

### Statistical analysis

The statistical analysis was carried out in three steps. First, a univariate descriptive analysis was performed throughout frequencies and descriptive statistics, followed by a bivariate analysis to identify variables potentially associated with disease prevalence. Finally, all variables with a  $p$  value  $\leq 0.20$  were considered for a multivariate logistic regression analysis to obtain adjusted odds ratios. Analysis was performed with Epiinfo tm7.1.0.6 (Centers for Disease Control and Prevention, Atlanta, Georgia, EE.UU) and SPSS (SPSS Inc. 233 South Wacker Drive, 11th Floor, Chicago, IL 60606-6412 EE.UU).

### Spatial information

All farms were spatially located using spatial location devices (GPS). This information was used to estimate risk areas of the disease throughout geostatistical modeling by ordinary kriging. Kriging was used based on the farm prevalence of the disease. These analyses were performed with ArcView from ArcGis 10 (ESRI, Inc Redlands, CA, USA).

### Ecological niche modeling

In order to determine relationship between environmental variables from BIOCLIM (<http://www.worldclim.org>) (Museum of Vertebrate Zoology, University of California, Berkeley, EE.UU) and presence of disease, an ecological niche analysis with Maxent (Princeton University, Center for Biodiversity and Conservation, American Museum of Natural History) was performed. Maps showing predicted relative suitability for the presence of cases were elaborated. Twenty-five percent of the herds were randomly selected to test model accuracy. Environmental data used in the Maxent analyses were: temperature and precipitation, and the 19 environmental variables from BIOCLIM with 2.5 min of resolution converted to a common projection. These variables are coded as follows:

BIO1 = Annual mean temperature  
 BIO2 = Mean diurnal range (mean of monthly (max temp - min temp))  
 BIO3 = Isothermality (BIO2/BIO7) (\* 100)  
 BIO4 = Temperature seasonality (standard deviation \*100)  
 BIO5 = Max temperature of warmest month  
 BIO6 = Min temperature of coldest month  
 BIO7 = Temperature annual range (BIO5-BIO6)  
 BIO8 = Mean temperature of wettest quarter  
 BIO9 = Mean temperature of driest quarter  
 BIO10 = Mean temperature of warmest quarter  
 BIO11 = Mean temperature of coldest quarter

**Table 1.** Average seroprevalence for reproductive diseases by production system in dairy cattle in Mexico.

System of production	Brucellosis	VBD	Neosporosis	IBR	Number of Farms
	Prevalence (%)				
Intensive	21.8	87.8	46	75	74
Double- purpose	2.4	63.9	24	74	82
Familiar	15.8	81.2	34	69	26
Average	14.7	78.8	37	73	

**Table 2.** Average seroprevalence of reproductive diseases by state in dairy cattle in Mexico.

Parameter	Brucellosis	VBD	Neosporosis	IBR	Number of farms
Aguascalientes	36	98	38.7	73	9
Chiapas	2	56	27.9	83	21
Chihuahua	6	95	44.7	81	16
Guanajuato	30	90	53.7	74	5
Hidalgo	77	96	55.0	71	14
Jalisco	16	81	33.9	67	26
Laguna	17	96	39.1	71	13
Queretaro	10	64	47.9	73	17
Sinaloa	3	55	30.6	57	12
Veracruz	1	69	18.6	74	49
Average	22.2	69	36.8	75	182

BIO12 = Annual precipitation  
 BIO13 = Precipitation of wettest month  
 BIO14 = Precipitation of driest month  
 BIO15 = Precipitation seasonality (coefficient of variation)  
 BIO16 = Precipitation of wettest quarter  
 BIO17 = Precipitation of driest quarter  
 BIO18 = Precipitation of warmest quarter  
 BIO19 = Precipitation of coldest quarter

## RESULTS

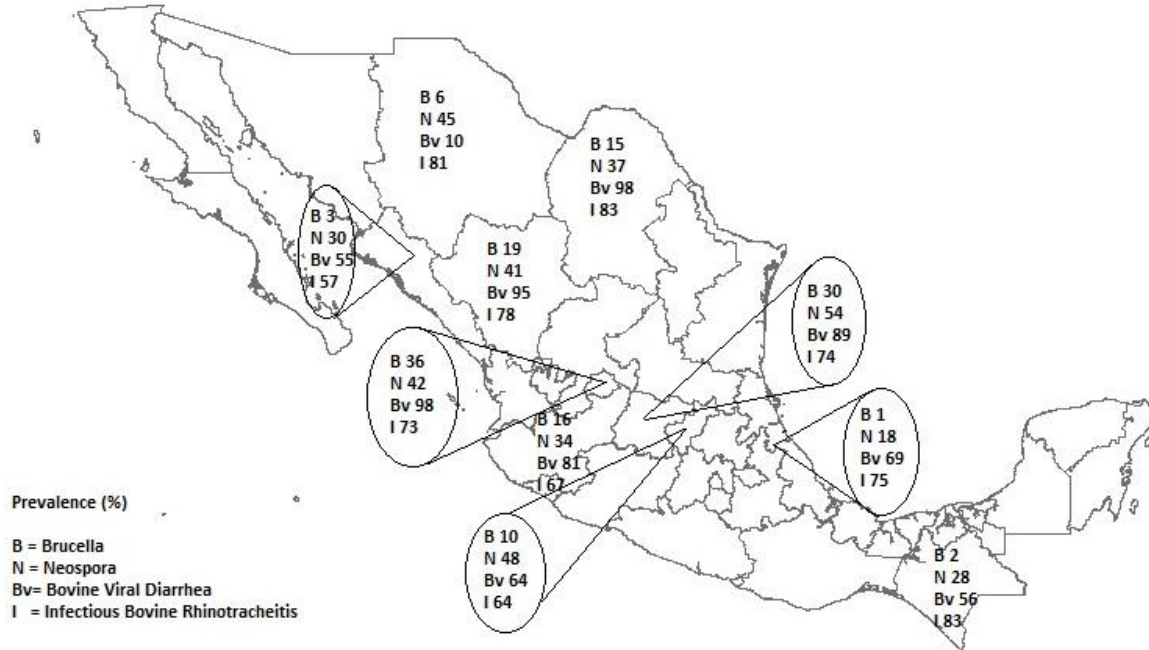
### Seroprevalence

Prevalence for the different reproductive diseases by production system and state are presented in Tables 1 and 2, and Figure 1. The overall seroprevalence for the four reproductive diseases was: brucellosis with the Rose Bengal test 14.7%; brucellosis with the RIT test 5.1%; BVD, 78.8%; neosporosis, 36.8%; and IBR, 73%. By system of production, the highest prevalence for brucellosis (21.8%) and (46%) neosporosis was observed in the intensive system. The lowest prevalence for these two diseases observed in the double-purpose system was 2.4 and 24%, respectively; in the familiar system, prevalence was 15.8 and 34%. No big differences were observed for IBR and BVD in the three systems, ranging from 69 to 75% for IBR and from 63.9 to 87.8% for BVD.

The states with the highest prevalence for brucellosis were Hidalgo (77%), Aguascalientes (36%), Guanajuato (30%), and La Laguna (Coahuila and Durango) (17%). Those with the lowest prevalence were Veracruz (1%), Chiapas (2%) and Sinaloa (3%). With the RIT tests, the states with the highest prevalence were: Hidalgo (25.3%), Aguascalientes (13.5%) and La Laguna (9.5%). The seroprevalence for BVD ranged from 55% in the state of Sinaloa to 98% in the state of Aguascalientes. The states with the highest prevalence for neosporosis were: Hidalgo (Tizayuca) (55%), Guanajuato (53.7%) and Querétaro (47.9%), whereas the state with the lowest prevalence was Veracruz, with 18.6%. The prevalence for IBR was high in all the states included in the study, ranging from 57% in the state of Sinaloa to 83% in the state of Chiapas.

### Risk factors

A multivariate regression analysis to identify factors associated to disease prevalence was performed. Adjusted odd ratios of factors with statistic significance for each disease are in Table 3. Five factors were associated with prevalence of brucellosis: herd size, herds with 200 to 300, and those with more than 300 had more chances of having brucellosis than those with less



**Figure 1.** Seroprevalence of reproductive diseases in dairy cattle in Mexico for states included in the study.

than 200 animals. The OR's were 4.4 (95%CI 1.2 to 7.6), and OR = 5.2 (95%CI 2.4 to 11.2), respectively. Herds introducing more than 30 animals a year had 4.1 (95%CI 2.3 to 7.6) more chances of having brucellosis, compared to herds introducing less than 30 animals. Herds with common sheds, intensive system of production, and origin of replacements (same vs. different herd) were all associated with having brucellosis.

For BVD, herd size ( $\geq 200$  animals), common sheds, production in intensive systems (family-type vs. double purpose), and calving intervals ( $\geq 395$  days) were factors associated to prevalence. Herds with 200 to 300 animals had an OR = 59.3 (95%CI 20.2 to 174), and herds with more than 300 animals had an OR = 7.5 (95%CI 3.6 to 15.8), compared to herds with less than 200 animals.

More than five abortions (OR = 1.12, 95%CI 0.87 to 1.4), the use of fresh colostrum (OR = 1.9, 95%CI 1.5 to 2.6), more than six services per conception (OR = 3.9, 95%CI 2.1 to 7.0), and production in intensive systems (OR = 2.3, 95%CI 2.3 to 3.9) were associated with presence of neosporosis. In the case of IBR, only two factors were significantly associated: type of breeding (insemination vs. bull use) OR = 1.6 (95%CI 1.2 to 2.2), and positive serology to parainfluenza virus 3 (PI3) OR = 2.5 (95%CI 2.1 to 3.1). The respiratory complex bovine infections occurred in conjunction with infections by other viruses associated with respiratory disease, namely, PI-3V and bovine respiratory syncytial virus (BRSV). These other viruses may occur singly or in combination with

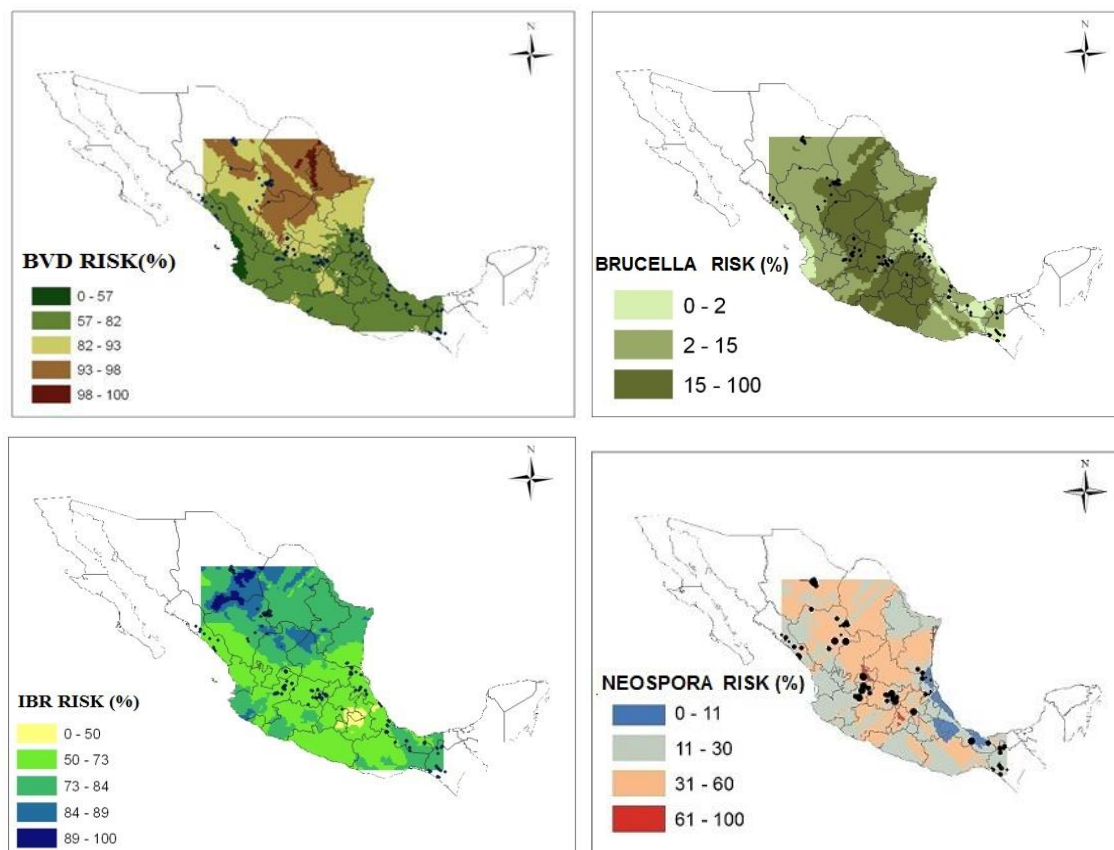
each other.

### Risk maps

Figure 2 shows maps of the continuous surface risk generated by ordinary kriging for brucellosis, BVD, neosporosis and IBR. Colors indicate free, low and high prevalence areas. As expected, high prevalence areas for brucellosis and neosporosis correspond to areas with a high density of dairy cattle in central and central north of Mexico. In the case of BVD and IBR, maps clearly show high risk of these two diseases in practically all the study area. Even when colors indicate differences in risk, most of them indicate high risk prevalence. In the case of BVD, a high risk area is observed in La Laguna, a geographic region including the states of Coahuila and Durango.

### Probability distribution maps

Figure 3 shows maps with the probability distribution of disease provided by Maxent. Color indicates probability, red color means higher probability of occurrence while blue indicates low probability. Black dots indicate prevalence. Conditions for presence of neosporosis is almost all over the study area, especially in central Mexico and the coast of the Gulf of Mexico. Conditions for brucellosis seem to be associated with presence of



**Figure 2.** Maps of risk for reproductive diseases in dairy cattle by ordinary kriging for the study area. Intensity of color represents disease risk in terms of prevalence.

dairy cattle in central and central north of Mexico. IBR and BVD are both more probable of occurring in central Mexico and in the coast of the Gulf of Mexico. It seems that disease presence is more a consequence of the presence of cattle than climatic conditions.

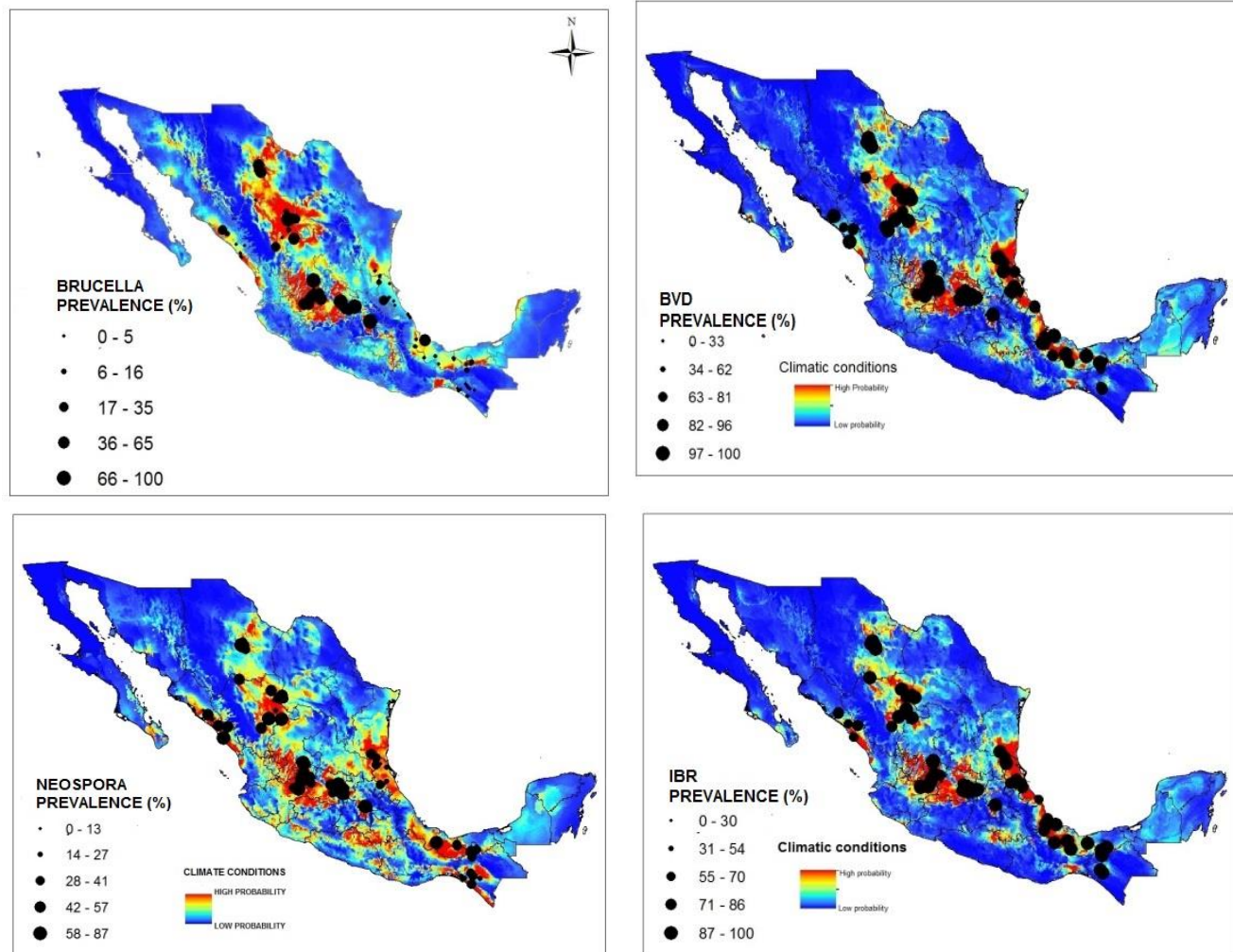
## DISCUSSION

The overall prevalence of brucellosis with the Rose Bengal test was 14.7%, and with RIT test 5.1%. It is possible that results from the Rose Bengal test are influenced by vaccination. Most herds in Mexico use either the *B. abortus* RB51 or the strain19 vaccine, both are allowed. In the intensive and family type systems, calves are vaccinated at 5 months of age, with a boost of 6 months later and a second boost 12 months later. Therefore, the 5.1% from the RIT test is more accurate since this method discriminates the vaccinated from the infected.

Previous studies reported prevalence rates of Brucellosis of 10.3% in La Laguna (states of Coahuila and Durango) (Salgado et al., 1991), and from 42.8 to

75% in Guerrero (Xolalpa et al., 1991). In the present study, the highest prevalence was observed in Tizayuca, Hidalgo (77%). Tizayuca is a dairy complex with about 25,000 cows in an intensive system where contact between animals from different herds is common and entrance of animals from different sources is frequent. Prevalence of brucellosis in double-purpose system was 2.4%. This kind of system occurs in the tropical areas of Mexico, where the average temperature is  $24 \pm 6^\circ\text{C}$  and the number of cattle per hectare is low ( $\approx 2.4$ ) compared to the intensive ( $\approx 9$ ) and family-type systems ( $\approx 3.0$ ). Therefore, conditions for the pathogen are adverse and have less probable transmission than in the intensive system.

Risk factors associated with prevalence of brucellosis were: herd size, introduction of animals to the herd, use of common sheds, intensive production system and replacements coming from different herds. Some of these factors may be modified to reduce the chances of disease transmission, such as introduction of replacements from different herds. As can be modified, other research previously reported no disposal of abortions, presence of dogs in production premises,



**Figure 3.** Maps showing regions with prediction of occurrence for brucellosis, BVD, neosporosis and IBR in Mexico. Color indicates predicted probability, red color indicates higher probability and blue color lower probability. Black dots indicate actual disease prevalence.

milking sick and healthy animals at the same time, and no eliminating of reactors (Rosales et al., 2012).

The 79% prevalence rate for BVD found in this study is similar to those reported previously: between 89.2 and 97% for the states of Aguascalientes, Jalisco, Guanajuato and Zacatecas (Solís-Calderón et al., 2003), 60% for Veracruz (Salas et al., 2009), and 67.2 for Tabasco (Rosete et al., 2014), but is much higher than the 21.1% reported in the state of Hidalgo (Sanchez-Castilleja et al., 2012), and the 14% reported for beef cattle in the south of Mexico (Solís-Calderón, 2005). Risk factors such as herd size and introduction of replacements from different herds have been associated with seroprevalence (Solís-Calderón et al., 2005). Type of milking, reproductive disorders and season (winter) has been previously associated with higher prevalence of

BVD (Cantu and Alvarado, 1998; Sánchez-Castilleja et al., 2012).

Prevalence of neosporosis in this study was 36.8%, close to that previously reported for dairy cattle in Coahuila, Tamaulipas and Nuevo Leon, 42 to 72% ((Garcia-Vazquez et al., 2002; Garcia-Vazquez et al., 2005; Morales et al., 2001b), but higher than that reported in beef cattle in the south (8.5 to 15%) (Garcia-Vazquez et al., 2009). In the northeast the prevalence was 16% (Meléndez et al., 2005) lower than that reported in central Mexico 59% (Garcia-Vazquez et al., 2002). Relationship between seroprevalence and abortions in the herd has been documented. Herds with 13 to 30% of abortions had seroprevalence of 72%, while herds with 12% of abortions or less had seroprevalence of 36% (Morales et al., 2001b).

**Table 3.** Risk factors associated to presence of reproductive diseases in dairy cattle in Mexico.

Disease	Risk factor	Categories	P	OR	95%CI lower	Upper	
Brucellosis	Herd size	< 100					
		201 to 300	0.026	4.4	1.2	16.3	
		≥ 300	0.000	5.1	2.4	11.2	
	Introduction of animals	< 30					
		31 to 60	0.000	4.1	2.3	7.6	
	Type of shed	Individual					
		Mixed	0.000	4.1	2.5	6.9	
	Production system	Double Purpose					
		Intensive	0.001	2.4	1.4	4.2	
	Origin of Replacements	Same Ranch					
Diferent ranch		0.000	6.0	3.2	11.2		
BVD	Total of animals	< 100					
		201-300	0.000	59.2	20.2	174.0	
		≥ 300	0.000	7.5	3.6	15.8	
	Type of shed	Individual					
		Mixed	0.000	2.4	1.5	3.8	
	Calving intervals	< 395					
		More than 395	0.000	3.5	2.1	5.7	
	Production system	Double purpose					
		Intensive	0	21.8	10.8	44.1	
		Family-run	0	5.5	3.3	9.0	
Neosporosis	Abortion	< 3					
		3 to 5	0.000	0.6	0.5	0.8	
		≥ 5	0.37	1.1	0.8	1.4	
	Colostrum type	Treated					
		Fresh	0.009	1.9	1.5	2.6	
	Services per conception	< 3					
≥ 6		0.000	3.9	2.1	7.0		
Production system	Double purpose						
	Intensive	0.009	2.9	2.3	3.9		
IBR	Type of breeding	Artificial					
		Direct breeding	0.001	1.6	1.2	2.2	
	Parainfluenza 3 virus	Negative					
Positive		0	2.5	2.1	3.1		

Previous studies about seroprevalence of IBR in Mexico have reported dissimilar results to the 75% found in this study: 90% in dairy cattle in Queretaro (Escamilla

et al., 2007), 3.4% in Michoacan (Segura-Correa et al., 2010) and 69.5 in central Mexico (Morales et al., 2002). In beef cattle, the seroprevalence was also variable, 5% in Yucatán in Holstein-Cebu cross breeds (Calderon et

al., 1997), 13.6% (Cordova-Izquierdo et al., 2009) to 54.4% (Solis-Calderon et al., 2003) also in Yucatán, and 44.2% in the state of Veracruz, in the Gulf of Mexico (Gutierrez, 2009).

Figure 2 shows areas of risk for the four diseases. The areas of high risk are wide and the risk is high. Risk for brucellosis and neosporosis is specially high in central and central north of Mexico, where the dairy cattle population is dense and the system of milk production intense, suggesting relationship between these two factors.

Figure 3 shows the results of Maxent. Red color indicates favorable conditions for the presence of disease. Conditions for brucellosis are more evident in central and central north, confirming that intensive systems of milk production favor the presence of the disease. Conditions for neosporosis are all over the study area, confirming that this disease affects cattle in all production systems. In the case of BVD and IBR, favorable conditions are present in the center of the country and in the coast of the Gulf of Mexico. The high prevalence in the central region is not surprising, where prevalence rates may be influenced by vaccination. In the coast of the Gulf of Mexico, however, the vaccine is not used but the prevalence is high, suggesting that the seroprevalence is due to real infections.

## Conclusion

Brucellosis, BVD, neosporosis and IBR are four reproductive diseases that are widely distributed in dairy cattle in Mexico. The seroprevalence of these diseases is high and is especially associated with intensive systems of production. Common farming practices such as the introduction of replacements from different farms significantly contribute to increase disease prevalence in the herd. Eventhough vaccination may have a role in the high prevalence of these diseases observed in some parts of Mexico, the high prevalence in some areas where vaccination is not common, suggest that the real prevalence is high.

## Conflict of Interests

The authors have not declared any conflict of interests.

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*Full Length Research Paper*

# Newcastle disease: Seroprevalence and associated risk factors in backyard and small scale chicken producer farms in Agarfa and Sinana Districts of Bale Zone, Ethiopia

Minda Asfaw Geresu<sup>1\*</sup>, Kemal Kedir Elemo<sup>1</sup> and Gezahegne Mamo Kassa<sup>2</sup>

<sup>1</sup>School of Agriculture, Animal and Range Sciences Course Team, Madda Walabu University, Bale-Robe, Ethiopia.

<sup>2</sup>College of Veterinary Medicine and Agriculture, Addis Ababa University, Bishoftu, Ethiopia.

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A cross-sectional study on seroprevalence of Newcastle disease virus (NDV) antibodies in backyard and small-scale chicken producer farms in Agarfa and Sinana districts was conducted using hemagglutination inhibition test (HAI) from February, 2015 to May, 2015. A total of 384 chicken sera were randomly collected from ten kebeles of the selected districts. Hemagglutination inhibition (HAI) test was used to analyze 384 chicken sera for NDV antibodies and the overall seroprevalence rate of 27.86% was found. A higher seroprevalence of 33.04% was observed in Sinana district when compared to Agarfa (20.13%) district. The prevalence in each kebele ranges from 15.63% to 40%; the highest prevalence of 40% was found at Horaboka, but insignificantly associated with Newcastle disease (ND) seropositivity. A Chi-square computed statistical analysis indicated that origin ( $\chi^2=7.6526$ ;  $p<0.006$ ), sex ( $\chi^2=6.9134$ ;  $p<0.009$ ) and type of chicken (layers/broilers) ( $\chi^2=11.2443$ ;  $p<0.001$ ) were the major risk factors for ND infection in the studied areas. The difference, however, was not statistically significant ( $p>0.05$ ) for age (adult/young), breed (exotic/cross/indigenous (local)), contact with other flocks, access to feed and water, and seasonal occurrence. Multivariable logistic regression statistical analysis revealed that origin and type (layers/broilers) were significantly associated with ND seropositivity ( $p<0.05$ ). Consequently, origin was statistically identified to be the major risk factor for ND to occur in relation to other factors (Adjusted Odds Ratio (AOR) =2.12). The study showed that majority of the chicken population in the studied area was susceptible to the pathogenic NDV infection. Therefore, more proactive measures should be taken to protect the chicken population from ND infection to reduce its economic impact to the poultry industry.

**Key words:** Agarfa, chicken, Newcastle disease, risk factors, seroprevalence, Sinana.

## INTRODUCTION

Poultry production plays a major role in the economy particularly of developing countries (Mazengia, 2012).

\*Corresponding author. E-mail: [minda.asfaw@gmail.com](mailto:minda.asfaw@gmail.com) Tel. +251910431505.

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The larger proportion of rural poultry in the national flock population of developing countries makes them worth paying attention to improved management and breeding. At national level in Ethiopia, 99% of the total, 56.5 million, estimated chickens are contributed by village poultry production while only 1% is from intensive exotic breed maintained under intensive management system (Tadelle and Ogle, 2001; Dinka et al., 2010).

In village systems, farmers keep poultry for diverse objectives. They are raised for purposes of hatching, sale, home consumption, sacrifices (healing ceremonies) and gifts (Mazengia, 2012). In Ethiopia, village chickens have been reared for a long time for similar purposes. Constraints which restrict the potential of village chickens in Ethiopia include; low inputs of feeding, poor management, the presence of diseases of various natures and lack of appropriate selection and breeding practices (Ashenafi, 2000; Tadelle and Ogle, 2001).

Among the constraints, poultry diseases are considered to be the most important factor responsible for reducing both the number and productivity of chickens (Tadesse et al., 2005). A growing concern reveals that as there is introduction of diseases of various etiologies into several poultry farms concurrent with importation of exotic breeds to backyard chickens. Furthermore, intensification is aggravating the rapid spread of the prevailing infectious diseases between and within poultry farms. And the distribution of these exotic breeds to farmers is creating a great threat to the indigenous backyard chickens (Zelege et al., 2005a). Among these threats, viral diseases like Newcastle disease (ND) is the major health constraints inflicting heavy losses (Tadelle and Ogle, 2001; Zelege et al., 2005a, b).

Newcastle disease (ND) is one of the most important viral diseases (Orsi et al., 2010). It is an acute infectious viral disease of domestic poultry and other species of birds regardless of variation in sex and age (Haque et al., 2010). The disease is characterized by respiratory, nervous system impairment, gastrointestinal and reproductive problems (Tiwari et al., 2004).

Sources of infection for NDV are exhaled air from infected birds and contaminated feed and water and transmission is mostly via aerosol. Feces, eggs lay during clinical diseases, and all parts of the carcass during acute infection and at death can also act as sources of infection. Chickens infected with virulent NDV may die without showing any clinical sign of illness though young chickens are more susceptible and show sign sooner than older ones. Much of the spread of ND in village is probably via human agents (Ashraf and Shah, 2014). An outbreak of ND is unpredictable and discourage villager from paying proper attention to the husbandry and welfare of their chickens (Spradbrow, 2001).

Various studies have been conducted to determine the epidemiology of ND in various countries in Africa. In study conducted in Ethiopia by Tadesse et al. (2005) and Ashenafi (2000), the seroprevalence rates of 28.57,

29.69, 38.33 and 43.68% were found in Debre Berhan, Sebeta, Adama and Central Ethiopia (among local scavenging chickens kept under a traditional management system), respectively. Another study conducted in two districts of Eastern Shewa Zone, Ethiopia by Chaka et al. (2012) to estimate the seroprevalence of ND (and other poultry diseases being not considered in this study) in the wet and dry seasons and they reported the overall seroprevalence of ND was 5.9% during the dry season and 6.0% during the wet season.

In general, the epidemiology of ND in village poultry in Ethiopia is poorly understood and there is no appropriate investigation and control strategy designed against the disease. This is due to lack of disease monitoring capacity in the Veterinary Services Department of the Ministry of Agriculture and Rural Development (Tadelle and Jobre, 2004). Farmers start to consider, therefore, losses due to diseases as normal and natural (Tadelle, 1996; Nasser, 1998) and they fail to report outbreaks to the veterinary authorities.

Though all the above study reveals that as ND seriously devastating poultry industry in Ethiopia, there is no published data (information) about the seroprevalence of this disease in poultry industry threat in Bale Zone in general and in Agarfa and Sinana districts in particular. This paucity of information on the presence and seroprevalence of ND in backyard and small scale poultry producer farms may reflect a lack of resources for disease surveillance and control in poultry production system.

In addition, the diagnostic coverage of poultry diseases in Ethiopia is limited to the extent that, even from commercial farms, only a few cases are brought to National Animal Health Diagnostic and Investigation Center (NAHDIC), Sebeta or the National Veterinary Institute (NVI), Bishoftu. Most poultry disease outbreaks, particularly in more remote parts of the country, remain undiagnosed and dead chickens are simply discarded (Chaka et al., 2012). Therefore, information on the seroprevalence and significance of ND can only readily be obtained through serological studies on apparently healthy and unvaccinated chickens.

Hence, this study was conducted to determine the seroprevalence of ND that potentially affect backyard and small-scale poultry producer farms in Sinana and Agarfa districts of Bale Zone, and to assess the risk factors contributing to ND seropositivity in the districts. Therefore, our study could complement the paucity of information about seroprevalence of ND and associated risk factors in poultry industry sector of the study areas.

## **MATERIALS AND METHODS**

### **Description of the study area**

The study was conducted in Sinana and Agarfa districts of Bale zone, Oromia Regional State, South East of Ethiopia. Sinana district

is located at 430 km southeast of Addis Ababa. The area is situated at 7° 7' N and 40° 10' E and 2400 masl. The mean average rainfall of the area is 353 mm. For the same period, average annual maximum temperature is 21.2°C and minimum temperature is 9.4°C. The dominant soil type is pellic vertisol and slightly acidic (pH=6). Agricultural production system of the study area is mixed farming. There are about 287,825 bovine, 47,121 ovine, 10,600 caprine, 26,020 equine and 60,000 poultry are found in Sinana district (SDAO, 2014).

Agarfa district is located at 464 kms south east of Addis Ababa. The area is situated at 6°11' N and 40°3' E and 2350 masl. The mean average rainfall of the area is 880 mm and bimodal. The average annual maximum temperature is 24.75°C and minimum temperature is 7.1°C. The dominant soil type is clay soil and slightly acidic (pH = 5.8). Agricultural production system of the study area is mixed farming. There are about 229,206 bovine, 63,485 ovine, 15,674 caprine, 33,777 equines and 40,150 poultry in Agarfa district (ADAO, 2014).

### Sampling method and determination of sample size

The sample size was calculated according to Thrusfield (2007) by considering 50% expected prevalence (P) (since there was no reasonable research done in these districts so far), 95% confidence interval (CI) ( $Z=1.96$ ) with 5% desired absolute precision (d), using the formula  $N = \frac{Z^2 P (1-P)}{d^2}$  for simple random sampling. The calculated required sample size (N) was 384.

Accordingly; the total numbers of sample required for this study was 384 chickens from both backyard and small scale poultry producers.

Sinana district contains 20 kebeles while Agarfa district contains 19 kebeles. Five kebeles from each district were selected purposely by their proximity to roads, accessibility of infrastructure and poultry holdings of each kebele. Prior to commencement of the study, list of all households (HHs) of those kebeles (sampling frame) was obtained from both district Agricultural Office.

**Inclusion criteria:** Apparently healthy chickens with history of no vaccination were included.

**Exclusion criteria:** Apparently healthy chickens with history of vaccination were excluded.

### Study population

The study population was all apparently healthy chickens with history of no vaccination in the selected districts. According to districts agricultural office, there were about 60,000 and 40,150 poultry flock in Sinana and Agarfa districts (SDAO, 2014; ADAO, 2014), respectively. The studied animals were consisting of 384 apparently healthy chickens with history of no vaccination. The chickens sampled were selected by simple random sampling method from backyard and small scale poultry producer farms.

### Study design

A cross-sectional type of study supported by questionnaire survey was conducted to determine the seroprevalence of ND and its associated risk factors in backyard and small scale producer farms in the two selected districts. Questionnaire survey was conducted to have a birds-eye-view of poultry diseases in the afore-mentioned districts. In the two selected districts poultry owners were interviewed with semi-structured questionnaire. Emphasis was given on the frequent clinical symptoms manifested whenever outbreaks of poultry diseases occurred in the respective study sites.

Tentative diagnosis was made based on the classical disease manifestation and vaccine was recommended for healthy chickens accordingly. The questionnaires were prepared, pre-tested and adjusted by translating in to local language (Afan Oromo) and administered by the interviewer. The questionnaire was focused on the potential risk factors and was conducted after carefully explaining the purpose of the work to the interviewees.

### Sera collection and testing

#### Sera collection

After plucking few feathers from the ventral surface of the humeral region of the wing and wiping the site with cotton damped with alcohol, approximately 4-5 ml blood samples were collected from the brachial vein, using plain vacutainer and with 18-20 gauge hypodermic needles. The vacutainer tubes were labeled and set tilted on a table overnight at room temperature to allow clotting. Then sera were filled into storage vials (cryovials) with appropriate identification and stored at -20°C until transported to NVI and the HAI was performed.

#### Haemagglutination-inhibition test (HAI)

HAI test was conducted according to the procedures of Beard and Wilkes (1985) and OIE (2002). The test was undertaken at NVI, Bishoftu, Ethiopia, by running two fold dilutions of equal volumes (0.025 ml) of phosphate buffered saline (PBS) and test serum (0.025 ml) in a U bottomed micro titer plates. Four haemagglutinating units (HAU) of virus/antigen were added to each well and the plate was left at room temperature for a minimum of 30 min. Finally 0.025 ml of 1% (v/v) chicken red blood cells (RBCs) was added to each well and, after gentle mixing, the RBCs were allowed to settle for about 40 min at room temperature. The HAI titer was read from the highest dilution of serum causing complete inhibition of 4 HAU of antigen.

The agglutination was assessed by tilting the plates. Only those wells in which RBCs stream at the same rate as the control wells (containing 0.025 ml RBCs and 0.05 ml PBS only) were considered to show inhibition after greater than or equal to 4 (logarithm to base 2) was taken as positive.

### Data storage and analysis

Data generated from questionnaire survey and laboratory investigations were recorded and coded using Microsoft Excel spreadsheet (Microsoft Corporation) and analyzed using STATA version 11.0 for Windows (Stata Corp. College Station, TX, USA). The seroprevalence was calculated as the number of seropositive samples divided by the total number of samples tested. To identify association of seropositivity with the potential risk factors (origin, sex, age, breed (indigenous/cross/exotic), type (layers/broilers), contact with other flock, seasonal occurrence and access to feed and water were computed by Pearson's Chi-square and multivariable logistic regression tests. A  $p$ -value <0.05 was considered statistically significant.

## RESULTS

### Overall seroprevalence of Newcastle disease in the studied districts

In the present study, an overall seroprevalence of

**Table 1.** Overall seroprevalence of HAI test result of ND in backyard and small scale poultry production system of the study districts.

Haemagglutination inhibition test result (HAI)	Selected districts		Total (N)	Overall prevalence (%)
	Agarfa N (%)	Sinana N (%)		
Positive	31(20.13)	76(33.04)	107	27.86
Negative	123(79.87)	154(66.96)	277	72.14
Total	154(100)	230(100)	384	100

Pearson  $\chi^2$  (1) = 7.6526; Pr = 0.006; N: Number of chickens tested.

**Table 2.** Seroprevalence of NDV antibodies in different selected kebeles of the selected districts.

Selected kebeles from the two districts	Positive samples (N)	Negative samples (N)	Total	Prevalence (%)
<b>Agarfa</b>				
Ali	11	35	46	23.91
Amigna	6	16	22	27.27
Anbentu	5	27	32	15.63
Elebidu	4	20	24	16.67
Ilani	5	25	30	16.67
<b>Sinana</b>				
Besaso	13	40	53	24.53
Horaboka	24	36	60	40
NanoRobe	13	23	36	36.11
Shallo	7	25	32	21.88
Shaya	19	30	49	38.78

Pearson  $\chi^2$  (9) = 15.4957; Pr = 0.078; Pr=Precision value.

27.86% was estimated by HAI test. A higher seroprevalence of 33.04% was observed in Sinana when compared to Agarfa (20.13%) as depicted in Table 1.

### Seroprevalence of NDV antibodies in selected kebeles of the study districts

Of 10 kebeles selected, Horaboka was with the highest ND seroprevalence (40%) while Anbentu was the least (15.63%). There was no significant association between the selected kebeles of the studied districts and ND seropositivity (Table 2).

### Chi-square analysis of association of the putative risk factors with ND seropositivity

A Chi-square analysis revealed that origin, sex, and type of chickens were significantly associated ( $p < 0.05$ ) with ND seropositivity among other factors considered during the study (Table 3).

### Multivariable logistic regression analysis of putative risk factors associated with ND seropositivity

The logistic regression analysis of the putative risk

factors indicated that chickens originated from Sinana were more likely to be infected (AOR= 2.12, 95 % CI: 1.30-3.46) with ND than chickens from Agarfa (Table 4).

## DISCUSSION

The present serological study revealed that the presence of circulating antibodies of ND among chickens sampled from backyard and small scale poultry producer farms of Agarfa and Sinana districts of Bale Zone. An overall seroprevalence of 27.86% was obtained using HAI ( $\geq 4 \log_2$ ) from the two districts. This finding is comparable to 31.2% of anti-NDV antibodies observed by Salihi et al. (2012) in Nassarawa State, 23.6% by Abraham et al. (2014) in Delta State of Nigeria and 32.2% by Tadesse et al. (2005) in central Ethiopia, but the result of the present study is considerably higher than previous report by Zeleke et al. (2005b), Regasa et al. (2007) and Chaka et al. (2012), who reported seroprevalences of 19.8% in the southern and Rift Valley districts, 11% in southern Ethiopia and 6% in Eastern Shewa zone, respectively. However, our result is lower than the prevalence of 43.68% reported by Ashenafi (2000) in central Ethiopia among local scavenging chickens kept under a traditional management system, 46% in village chickens in Borno State (EL-Yuguda et al., 2007) and 54.67% in Nasarawa

**Table 3.** Chi-square analysis of association of the putative risk factors with ND seropositivity.

Variable		Number tested	Number positive N (%)	$\chi^2$ (p-value)
Origin	Agarfa	154	31(20.13%)	7.6526 (0.006*)
	Sinana	230	76(33.04%)	
Age	Adult(>6mos)	219	62(28.31%)	0.0504(0.822)
	Young(3-6 mos)	165	45(27.27 %)	
Sex	Female	321	98(30.53%)	6.9134 (0.009*)
	Male	63	9(14.29%)	
Breed	Cross	197	57(28.93 %)	0.7940 (0.672)
	Exotic	45	14 (31.11%)	
	Indigenous	142	36(25.35%)	
Type	Broilers	74	9(12.16 %)	11.2443(0.001**)
	Layers	310	98(31.61 %)	
Contact with other flock	Yes	170	40(23.53%)	1.7423 (0.187)
	No	214	67(31.31%)	
Access to feed and water	Roaming	218	55(25.23%)	1.7423(0.187)
	Confined	166	52(31.33%)	
Seasonal occurrence	At the beginning of rainy season	306	85(27.78%)	0.0056(0.940)
	At the end of rainy season	-	-	
	Throughout the year	78	22(28.21%)	

Mos; months; \*Statistically significant; \*\*Highly statistically significant.

**Table 4.** Multivariable logistic regression analysis of putative risk factors associated with ND seropositivity.

Variable	ND test result		Odds ratio		p-value
	Negative	Positive	COR (95%CI)	AOR (95%CI)	
<b>Origin</b>					
Sinana	154(67.0)	76(33.0)	1.96(1.21, 3.20)	2.12(1.30, 3.46)	0.003
Agarfa	123(79.9)	31(20.1)	1	1	
<b>Sex</b>					
Female	223(69.7)	97(30.3)	2.35(1.15, 4.81)	0.18(0.02, 1.71)	0.136
Male	54(84.4)	10(15.6)	1	1	
<b>Type</b>					
Layers	213(68.5)	98(31.5)	0.31(0.15, 0.64)	0.06(0.01, 0.60)	0.016
Broilers	64(87.7)	9(12.3)	1	1	

AOR, Adjusted Odds Ratio; COR, Crude Odds Ratio; CI, Confidence Interval; 1, Reference.

State (Salihu et al., 2012).

This could be explained by differences in study settings or by exposure to mild virus strains that induced immunity but did not kill many chickens. The presence of lentogenic, or possibly mesogenic, NDV in backyard/

small scale chicken producing farms in an area may result in a constant cycle of infection that periodically boosts the immunity of all exposed chickens, resulting in a higher proportion of chickens with antibodies (Martin, 1992; Chaka et al., 2012). Another reason for variation

between studies could be subjectivity and variation in HAI cutoff values used for the interpretation of the result. For instance, some authors considered an HAI titer  $\geq 1\log_2$  as positive (Bouzari and Mousavi, 2006; Biswas et al., 2009), whereas others used cut-off titers of  $\geq 3\log_2$  (Tadesse et al., 2005; Zeleke et al., 2005b). However, the present study used  $\geq 4\log_2$  which is similar with the cutoff values used by Gutierrez-Ruiz et al. (2000).

None of the chickens sampled had a history of previous vaccination against ND. It is therefore deduced that antibodies detected in the backyard and small scale chicken producing farms in this study was as a result of natural infection by NDV. Therefore, the 27.86% seroprevalence rate of ND antibodies in the two districts could be attributed to factors such as the management system in traditional production which may serve as a stress factor and favour infection.

Also, the continued exposure to array of infectious agents and wild birds, nutritional deficiencies, the absence of disease control through vaccination, contact of birds of one rural area with those of another rural area through gift and sale of rural chickens which in some cases are diseased or carriers of some diseases may facilitate the spread of diseases like ND among flocks (Musa et al., 2009).

The present study revealed that the origin of the chickens was significantly associated with ND seropositivity ( $p < 0.05$ ) and it was also statistically identified that origin was the major risk factor for ND seropositivity to occur in relation to other factors within the same agro-ecology. The results showed higher individual chicken seroprevalence in Sinana (33.04%) when compared to Agarfa (20.13%). According to districts agricultural office, there were about 60,000 and 40,150 chicken flock in Sinana and Agarfa districts (SDAO, 2014; ADAO, 2014), respectively. Therefore, the higher prevalence recorded in Sinana district can be attributed to more chicken had been sampled (230 chicken sampled) compared to Agarfa (154 chicken sampled) district. Zeleke et al. (2005b) and Tadesse et al. (2005) reported low altitudes do have higher seroprevalence than the high altitude in their study and they were investigated as there were few chickens in the highland area and chicken population number is a factor for the transmission of the disease in their study. Contrary to these findings, the present study investigated significant variation within the almost closer agro-ecology (Sinana 2400 and Agarfa 2350 masl) (variation within higher altitude).

The difference in the seroprevalence between adult ( $> 6$  months) and young (3-6 months) of age was statistically insignificant ( $p > 0.05$ ), which disagrees with the finding of Vui et al. (2002) which stated that the young (3-6 months-old groups) had a significantly lower NDV antibody titre than the adult ( $> 6$  month-old age groups). This can be hypothesized to be due to more frequent exposure of older birds to field virus, which might have

survived the disease at an earlier age (Getachew et al., 2014).

This study also revealed a higher seroprevalence rate among the female (30.53%) compared to male chickens (14.29%) with statistically significance difference ( $p < 0.05$ ). Our finding corroborates the findings of Tadesse et al. (2005), who reported a slightly higher prevalence of 32.63% among female chickens when compared with a prevalence of 31.63% among male chickens in Ethiopia.

In contrary to this finding, a study conducted by Zeleke et al. (2005b) in the Southern and Rift Valley districts of Ethiopia, ND shows a higher prevalence rate among males (21.74%) than among females (19.16%).

The highest seroprevalence was observed in exotic breed than in the indigenous (local) and cross-bred chicken in the present study. The difference, however, was not statistically significant. An insignificant difference ( $p > 0.05$ ) in the seroprevalence between the indigenous (local) and cross breeds of chickens (excluding exotic breed) was reported by Vui et al. (2002) which is consistent with the present findings. In contrast to this, the relatively higher overall seroprevalence rate of ND virus antibodies in local chickens reported by Tadesse et al. (2005) attributed to a number of factors. However, the exotic breed sampled in this study was lower than the indigenous (local) and cross-bred which results in difficulty of interpretation of our findings because the question of breed susceptibility to ND is still controversial (Awan et al., 1994). Hence, this area needs an indebt study to unveil the factors responsible for this difference.

There was statistically significant association ( $p < 0.05$ ) between type of the chickens (layers or broilers and seropositivity of ND in the present study. A higher prevalence recorded in layers than in broilers chicken can be attributed to more layers been sampled. Khan et al. (2011) reported that relatively high level of antibodies against ND in unvaccinated birds observed during the study, (33% in egg-laying hens) indicated a high prevalence of NDV infections in village chickens. The birds showing detectable levels of antibodies were considered exposed, while those having undetectable level of antibody titer against ND were considered as non-vaccinated.

The issue of seasonal ND peaks has always been controversial and may vary according to the environmental, nutritional and socio-economic conditions, under which poultry is kept (Vui et al., 2002). There was no observed seasonal variation in seroprevalence in the present study, suggesting that the disease is widespread and occurs throughout the year in the studied area which is consistent with the report of Chaka et al. (2012). However, in contrary to this finding, Awan et al. (1994) reviewed the literature and found reports of ND peaks during (Asadullah, 1992; George, 1991; Mishra, 1992) and at the end of the dry season while Nguyen (1992) reported that in Viet Nam the ND peaks generally occur at the beginning of the rainy season (September-March)

and Martin (1992) in a review concluded that ND outbreaks are often associated with the change of seasons, specifically at the start of the wet season.

## Conclusion

This study established that ND is endemic in Agarfa and Sinana districts of Bale zone. Higher seroprevalence was observed in Sinana when compared to Agarfa with significance difference. Origin was statistically identified as the major risk factor for ND seropositivity to occur in relation to other factors while age, breed (exotic/cross/indigenous), contact with other flock, seasonal occurrence, and access to feed and water were insignificantly associated with ND seropositivity. This finding, apart from being of economic significance, it is also of nutritional importance because of the high mortality of the birds, which calls for adoption of preventive measures to help curb the devastating effects of the NDV. The prevailing ND seropositivity in the chicken production system indicates the importance of ND in poultry industry of the studied areas and therefore, to effectively control ND, more attention should be given to those areas by adopting prophylaxis through the use of heat resistant ND vaccines for the chickens.

## Conflict of Interests

The authors have not declared any conflict of interest.

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*Full Length Research Paper*

## A study on prevalence of paramphistomum in cattle slaughtered in Gondar Elfora Abattoir, Ethiopia

Getenet Ayalew<sup>1</sup>, Alebachew Tilahun<sup>2\*</sup>, Alemu Aylate<sup>2</sup>, Ayichew Teshale<sup>2</sup> and Abebaw Getachew<sup>3</sup>

<sup>1</sup>Debre Markos, Northern Ethiopia, Ethiopia.

<sup>2</sup>School of Veterinary Medicine, Wolaita Sodo University, Southern Ethiopia, Ethiopia.

<sup>3</sup>Agarfa Federal Agricultural College, Ethiopia.

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A cross sectional study was carried out from October 2010 to April 2011 in Gondar Elfora Abattoir to determine the prevalence of paramphistomosis in cattle (local, cross) breeds which were came from highland, mid highland and lowland areas. Three hundred eighty-four (384) cattle were included for routine ante-mortem and postmortem examination for the presence of paramphistomum. The parasite was examined grossly and under microscope to appreciate the morphology of adult paramphistomum. Out of 384 cattle examined, 199 (51.82%) were found to be positive for paramphistomosis. From 199 infected cattle fluke burden at organ level 125(62.81%) was in rumen, 40(20.1%) was in reticulum and 34(17.09%) was found mixed (rumen and reticulum). The existence of paraphistomum in respect to organ and origin, 56(44.80%) was in rumen and 20(50%) was found in reticulum predominantly in high and low land respectively. The highest infection of cattle with paramphistomum species was found during October to November. However, there is no statistical significance variation ( $p > 0.05$ ) between the prevalence of paramphistomum and that of origin, breed, and age groups of the animals. Integrated control approach using selected anthelmintic therapy and snail control to reduce the magnitude of the problem was suggested as a recommendation.

**Key words:** Elfora abattoir, cattle, Gondar, paramphistomosis, prevalence

### INTRODUCTION

Ethiopia has the largest livestock and draft animal population in the continent. There are approximately 44, 318, 877 cattle, 23, 619, 720 sheep, 23, 325, 113 of goats, 6 million equines, 2.3 million camels and 43 million poultry (CSA, 2011). Its livestock productivity, despite its huge population size, remains marginal due to prevalent

diseases, malnutrition and management constraints. Parasitism represents a major obstacle to the development of sub-sector and bovine paramphistomosis is one of the most important parasitic diseases of cattle causing mortality and production losses in various parts of Ethiopia. Paramphistomosis is the priority disease in

\*Corresponding author. Email: alebmen19@gmail.com Tel: +251913992418.

the highland as well as in lowland areas of Amhara regional state (CSA, 2010).

Paramphistomosis is distributed all around the world, but the highest prevalence has been reported in tropical and sub tropical regions, particularly in Africa, Asia, Australia, Eastern Europe and Russia. The epidemiology of *Paramphistomum* is determined by several factors governed by parasite-host-environment interactions. The major epidemiological variable influencing worm burdens of animals is the infection rate from pastures. It is also influenced by the climatic requirement for egg hatching, development and survival of the larvae in pasture (Ozdamar et al., 2010).

The adult paramphistomum is found in the rumen and reticulum but the immature parasite is found in the duodenum. Adult paramphistomum are mainly parasitic in the fore stomachs of ruminants, although a few species occur in the intestine of ruminants, pigs and horses. Their shape is not typical of the trematodes, being conical rather than flat. All require a water snail as an intermediate host (Taylor et al., 2007).

Paramphistomosis causes a great economic loss in terms of decrease in milk and meat production, loss of weight treatment cost of diseased animals and additional labor required for handling such animals. Mortality rate in young animals is very high (Javed, 2008). It is caused by *P. cervi*, *P. epiclitum*, *P. microbothriodes*, (Chowdhry and Tada, 1994; Kassai, 1999; Rinaldi et al., 2005; Shanila and Hafeez, 2005; Sripalwit et al., 2007). This disease is accompanied by fatal diarrhoea, weakness, dehydration and decreased milk yield, submaxillary edema and death thereby causing great economic loss to the livestock industry (Horak, 1971; Georgi et al., 1999; McLaren et al., 2006; Merianos, 2007; Bianchin et al., 2007). Heavy infections with immature flukes in the upper small intestine can cause serious ill health and death (Panda, 1985; Urquhart et al., 2000).

Outbreaks of disease generally occur in the drier months of the year when the receding water uncovers herbage contaminated with encysted metacercariae in these areas. Dispersal of snails by flooding events and changes in farm-management practices may be responsible for the apparent emergence of the parasite (Foster et al., 2008). In spite of the aforementioned prevailing situation and the presence of a number of problems due to gastrointestinal parasites there is scarcity of well-documented information on the occurrence of *Paramphistomum* in ruminants in Ethiopia. The study was designed with the aims of determining the prevalence of *paramphistomum* in cattle slaughtered at Gondar Elfora Abattoir.

## MATERIALS AND METHODS

### Study area description

The study was conducted in North Gondar, Northwestern part of Ethiopia. Gondar is located 727 km Northwestern of Addis Ababa in

Amhara regional state. It is divided into three major agro-climatic zones: highland, mid-highland and lowland. The altitude ranges from 4620 m in the Semen Mountain in the North to 550 m in the West. The rainfall varies from 880 mm to 1772 mm, while the minimum and maximum temperatures are in the order of -10°C in the highland and 44.5°C in the West (low land). The area is also characterized by two seasons, the wet season from June to September, and the dry season from October to May. According to Gondar town agricultural office (2006), the livestock populations of Gondar registered were, 1,936,514 cattle, 524,083 sheep, 682,264 goats, 2,124,000 poultry, 223,124 donkeys, 12,473 mules, 36,828 horses and 606 camels (CSA, 2011).

### Study population and sampling technique

The study animals were cattle (local, cross) breeds of different ages and body conditions brought from highland, mid highland and lowland areas to the abattoir for the purpose of meat production. Bovine breeds were categorized in to adult (3-7 yrs) and old (>7 yrs). Systematic random sampling technique was used to select the study units i.e. the animals were selected in a way that the first was taken randomly and the rest were selected in 5<sup>th</sup> round.

### Study design and sample size determination

A cross sectional study was conducted to determine the prevalence of *Paramphistomum* infection in cattle from October 2010 to April 2011 in Gondar Elfora Abattoir. The desired sample size was determined by the formula given in (Thrusfield, 2007) with 95% of confidence interval and 5% desired precision with expected prevalence of 50%.

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

Where N=number of sample size,  $p_{exp}$ =expected prevalence,  $d^2$ =Absolute precision.

Therefore, based on the aforementioned formula 384 were considered in the study.

## METHODOLOGY

### Ante mortem examination

Ante mortem inspection was carried out on the animals before slaughter to assess their general health status. During ante mortem examination, detail records about the species, breed, sex, age, origin and body condition of the animals was recorded. General physical examinations of animals were conducted.

### Post mortem examination

During postmortem examination rumen and reticulum was systematically inspected for the presence or absence of adult paramphistomum and fluke burden using the routine meat inspection procedures which consist secondary examination, if evidence of paramphistomum were found they are recorded separately. The primary examination (visualization and palpation) is not showing positive or negative of paramphistomum where as the secondary examination involves further incisions of the rumen and reticulum to observe paramphistomum.

### Identification of *paramphistomum*

For identification, the collected flukes were placed on Petri dish and

**Table 11.** Overall prevalence of bovine paramphistomosis.

Number of cattle examined	Number positive	Prevalence (%)	95% CI
384	199	51.82	[46.70 -56.92]

**Table 22.** Prevalence of bovine paramphistomosis based on origin of the animals.

Origin	No. of cattle	Number positive	Prevalence (%)
Highland	160	82	51.25
Mid highland	90	43	47.77
Lowland	134	74	55.22
Total	384	199	51.82

Pearson  $\chi^2 = 1.2317$  P = 0.540.

**Table 33.** Prevalence of bovine paramphistomosis based on age groups.

Age	Sample size	Number positive	Prevalence (%)
Adult (3-7 yrs)	257	136	52.91
Old (>7 yrs)	127	63	49.61
Total	384	199	51.82

Pearson  $\chi^2 = 0.3734$  P = 0.541.

**Table 4.** Prevalence of bovine paramphistomosis on the basis of organs.

Organ infected	No. of organs infected	Prevalence (%)
Rumen	125	62.81
Reticulum	40	20.10
Both (rumen and reticulum)	34	17.09
Total	199	51.82

Pearson  $\chi^2 = 384.0000$  P = 0.000.

observed through stereo microscope to appreciate the morphology. Final identification of *Paramphistomum* was done based on morphology of flukes; shape, posterior sucker (acetabulum), anterior sucker, terminal genitalium and tegumental papillae following the standard guidelines given by Urquhart et al. (1996).

#### Data analysis

All the data collected during the study from the abattoir were recorded in the format developed for these purpose and later on entered into Microsoft excel spreadsheet. Statistical analysis for categorical variables such as sex, age, origin and breed, was expressed in percentages by using Intercooled STATA 11.0 software.

## RESULTS

### Overall prevalence

In this study, a cross-sectional investigation on the

occurrence of bovine paramphistomosis was carried out between November 2010 and April 2011. A total number of 384 samples (cattle) were included to check the presence of paramphistomum in Gondar Elfora Abattoir. Out of 384 slaughtered cattle, 199 (51.82%) were found to harbor paramphistomum parasites (Table 1). Analysis of the result on the basis of origin and age was made. However, there is no statistically significant variation ( $P > 0.05$ ) in prevalence observed between origin and age of animals examined (Tables 2 to 5).

## DISCUSSION

The study found that the overall prevalence of paramphistomosis in bovine was 51.82% (199/384). This finding is higher than the prevalence rate 20% found by Haridy et al. (2006) from Egypt, 16.6% Jithendran (2000) from India, 23.8% by Juyal et al. (2003) and 5.94% by

**Table 5.** Prevalence of fluke with respect to organ and origin.

Organ infected	Highland	Mid highland	Lowland	Total
Rumen	56 (44.80)	30 (24.00)	39 (31.20)	125 (100.00)
Reticulum	18 (45.00)	2 (5.00)	20 (50.00)	40 (100.00)
Mixed	8 (23.53)	11 (32.35)	15 (44.12)	34 (100.00)
Total	82 (41.21)	43 (21.61)	74 (37.18)	199 (100.00)

Pearson  $\chi^2 = 14.6283$   $P = 0.023$ .

Hafeez (2005) from India, 13.6% in Turkey by Sevimli et al. (2005), 17.1% by Phiri et al. (2006) from Zambia, 28% from Thailand by Morakot and Sakchai (2006). The difference may be due to difference in geographical regions and varied environmental conditions. The occurrence of paramphistomosis in an area is influenced by a multifactorial system that is composed of hosts, parasitic agents, transmission process and environmental effects (Radostits et al., 2000).

The current finding approaches that of Keyyu et al. (2006) which is 42.1% from Tanzania; Chingwena et al. (2002) which is 37.6% from Zimbabwe, and Phiri et al. (2006) which was 51.6% in Zambia. However, this finding is lower than that of Rolfe et al. (1991) reportedly 98%, and Lee and Lee (1971) reportedly 70%. The variation in the rate of prevalence may be attributed to environmental conditions, managemental conditions, parasites and use of antiparamphistome drug agents.

The presence of paramphistomosis and difference in their prevalence is influenced by local climatic conditions presence or absence of water reservoirs, lakes, rivers and availability of suitable intermediate hosts. Maqbool et al. (2002, 2003), Narcis et al. (2004) and Diaz et al. (2007) reported that irrigation canals have a role in distribution of paramphistomosis eggs. An increased incidence of paramphistomosis in adult cattle has been reported in the present study. The finding agreed with the reports of Keyyu et al. (2006) who reported 75.2% prevalence in adults and 47.2% prevalence in young animals. These results differ with those of Juyal et al. (2003) 23.8%; and Shanila and Hafeez (2005) 5.94%. The relatively high frequencies could be associated with nutritional and climatic stress, such as altitude, rainfall, and temperature and livestock management system. As different herds of animals come in close contact at available communal watering and grazing sites (contact points) because of the feed scarcity, the establishment and spread of paramphistomosis were favored. Furthermore, adult animals were significantly more frequently affected than young regarding paramphistomosis because the young may not move to the grazing land (they stay around the house).

During the dry periods, breeding of the snails and development of the larval flukes slow down or stop completely and snails undergo a state of aestivation (Armour, 1975; FAO, 1994; Soulsby, 1982; Urquhart

et al., 1986). Although a decreasing trend was observed along with the advancement of the dry season, relatively high prevalence rates were recorded throughout the study period. This may be attributed to infections acquired during previous peak snail activity season. In addition the existence of permanent suitable ecological conditions in areas like lake-borders, slowly flowing rivers and low lying marshy areas may contribute to persistent but low-grade infection during the dry season.

Two species of paramphistomum were identified during the study period; however, *Paramphistomum clavula* was the most prevalent (57.79%) species compared to *Paraphistomum cervi* (30.15 %) and mixed infection (12.06%). The highest prevalence rate was analyzed during October, when the wet-ecological conditions still prevailed. It has been described that the bionomic requirements for breeding of the planobid snails and development of the intermolluscan stages of the flukes often reach the optimum threshold during the wet months of the year. During the dry periods, breeding of the snails and development of the larval flukes slow down or stops completely and snails undergo a state of aestivation (Radostits et al., 2000).

## CONCLUSION AND RECOMMENDATIONS

In general, paramphistomosis is one of the major obstacles for livestock development in Ethiopia by causing remarkable production losses at different parts of the country. This is due to the fact that the area of origin of the animals is suitable for the survival of the snail intermediate host and the parasite. Paramphistomum burdens varied seasonally and were dependent upon the number of infected host snails. Peak fluke burdens and clinical paramphistomosis occurred in late summer and early winter. Based on the aforementioned conclusion, integrated control approach using selected anthelmintic therapy and snail control should be implemented to reduce the magnitude of the problem. In addition, awareness of the producers about the disease should be raised to enable them actively participate in the control programs. Finally, further information on the epidemiology, ecology and biology of the intermediate host snail should be gathered to help in proposing and implementation of disease control programmes.

## Conflict of Interests

The authors have not declared any conflict of interest.

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