

# Journal of Veterinary Medicine and Animal Health

Volume 5 Number 5 May 2013



*Academic  
Journals*

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## Case Report

## Mixed infection of *Trichophyton* species in a Nigerian part Arab horse with dermatophytosis

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Accepted 6 May, 2013

**A five-year-old Nigerian part Arab horse kept in the University of Nigeria, Nsukka demonstration farm was observed to have non-exudative, scaly circumscribed lesions on its body. Two dermatophytic fungi, *Trichophyton mentagrophytes* and *Trichophyton verrucosum*, were isolated from its hair plucking and skin scrapings. This report describes the trend in the diagnosis and differentiation of the two species of dermatophytes in a con-current infection of horse.**

**Key words:** Dermatophytes, horses, *Trichophyton*, mixed infection.

### INTRODUCTION

Dermatophytosis is one of the most common and economically important causes of several dermatoses that have been observed in equine species (Fadok, 1995; Ural et al., 2008). In equines, it is caused by the infection of hair roots and follicles by filamentous fungal organisms belonging to the group called dermatophytes (Shimozawa et al., 1997). These organisms are primary cutaneous pathogens and comprise the largest group of fungi that cause skin disease particularly of the stratum corneum (Quinn and Markey, 2003; Ural et al., 2008). They produce keratinase enzyme which have keratinolytic effect enabling them to utilise keratin as substrate (Grappel and Blank, 1972). This enables them to colonize and invade the cornified epidermis and keratinized adnexal structures such as hair and nail that are derived from it (Weeks et al., 2003; Bernado et al., 2005; Issa and Zangana, 2009), producing classical circumscribed, alopecic, crusty and scaly skin lesions generally called ringworm (Fadok, 1995; Quinn and Markey, 2003).

Dermatophytic agents are classified into three ecological groups as anthropophilic (mostly associated with humans), zoophilic (associated with animals) and geophilic (found in soil) (Weitzman and Summerbell, 1995). These ecological

adaptations have enabled them to have a wide range of host (Quinn and Markey, 2003), and their zoonotic and public health importance have been well recognized (Shams-Ghahfarokhi et al., 2009).

In horses, *Microsporum* and *Trichophyton* species have been reported to be the causative agents of dermatophytosis (Quinn and Markey, 2003; Ural et al., 2008). *Trichophyton equinum* is the most commonly involved agent and has been reported in many countries (Hasegawa and Usui, 1975; Al-Ani et al., 2002). Other *Trichophyton* spp. that have been isolated include *Trichophyton mentagrophytes* (Shimozawa et al., 1997; Quinn and Markey, 2003) and *Trichophyton verrucosum* (Shimozawa et al., 1997; Khosravi and Mahmoudi, 2003). These fungal species however were isolated from single infections in horses.

Diagnosis of dermatophytosis is often based on the clinical presentation and history, microscopic and cultural (which involves the observation of the obverse and reverse sides of the fungal colonies) morphological studies, biochemical tests, use of selective media and hair penetration test (Weitzman and Summerbell, 1995; Quinn and Markey, 2003; Weeks et al., 2003).



## CASE REPORT

A five-year-old horse in the University of Nigeria, Nsukka demonstration farm was found to have non-exudative, circumscribed, alopecic lesions on its body. Anamnesis revealed that prior to the introduction of the horse into the farm, a mare with generalized circumscribed alopecic lesions was present and both were kept together. Few weeks thereafter, the infected mare died. Several weeks later, patches of raised hair were observed on the head, lateral abdominal region and hind limbs of the horse in the present case (Figure 1A). Consequently, the hairs detached leaving alopecic, grey, and shining areas with diameters of approximately 1 to 3 cm. The lesions progressed to the hind limb, inguinal and ventral abdominal regions of the body. The lesions on the head became encrusted with severe desquamation of the epidermal tissue debris. Ticks were also found attached to the axillary, perineal and navicular regions of the horse.

On presentation to the University of Nigeria Veterinary Teaching Hospital (UNVTH), physical and clinical examination showed that the animal was in good general body condition. De-ticking was done by hand-picking and tick bite wounds were dressed after disinfection by topical application of gentian violet. Systemic chemotherapy given included penicillin/streptomycin combination and multivitamin. However, the circumscribed lesions were observed to have increased to about 4 to 5 cm (Figure 1B) with those on the face and hind limbs coalesced to form large alopecic areas (Figure 1C and D). The horse was observed to be emaciated. A mycotic cause was suspected on the basis of the characteristic annular, alopecic, scaly and non-exudative appearance of the lesions.

Sample collection for microbiological examination was done as were described by Weeks et al. (2003). Following disinfection with 70% alcohol, skin scrapings and hair plucks were taken from the advancing border of the lesions using sterile disposable scapel blade. The samples were transported to the laboratory using clean, dry sterile petri dish.

Mycological examination was done by digesting a portion of the sample with 10% potassium hydroxide (KOH) for direct microscopic examination of typical hyphae or arthroconidia (Quinn and Markey, 2003). Isolation of the fungal agents were done by culturing using Sabouraud dextrose agar (SDA) supplemented with cyclohexamide (0.4 mg/L), chloramphenicol (0.05 g/L), and gentamicin (0.16 mg/L) (Nweze, 2011). Cultures were incubated aerobically for 2 weeks at 25 and 37°C, and were observed daily for growth of dermatophytes (Weeks et al., 2003). Identification of dermatophyte species was performed by macroscopic examination of colonial appearance. Culture slide smear stained with lactophenol cotton blue was used for microscopic identification following the methods described by Bernado et al. (2005)

and Issa and Zangana (2009). Biochemical study (urease hydrolysis) and culturing on SDA supplemented with 5% salt (Issa and Zangana, 2009) were done to further confirm the identity of the isolated dermatophyte species (Campbell et al., 1996; Weeks et al., 2003; Issa and Zangana, 2009).

Hair plucked from the margin of the head lesion and scales collected from this site did not reveal fungi by direct microscopy (KOH). At 8 days of incubation, two different fungal isolates were observed macroscopically at the spots where hair strands touched the agar (Figure 2A and B). Both isolates had similar obverse morphology - white, buff initially but later became folded with raised centre (Figure 2C). On the reverse, one of the isolates gave colourless/tan pigmentation, whereas the other gave wine red (reddish-brown) pigmentation (Figure 2D and E).

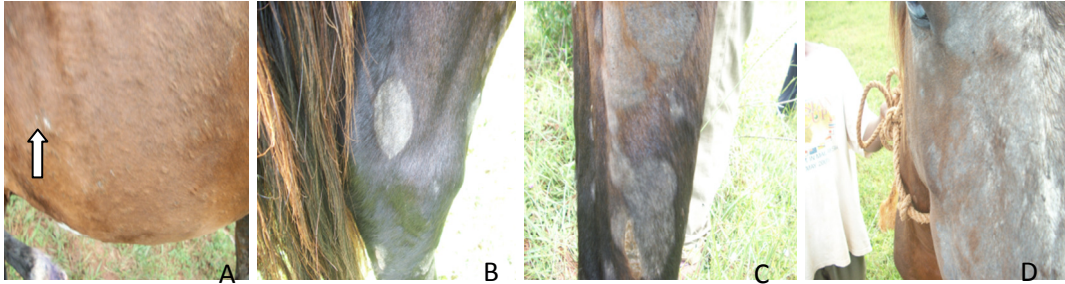
The isolates were purified using SDA supplemented with chloramphenicol (5%) to ensure that further studies were conducted using pure isolates (Figure 2C and D). Of the two isolates, only one (with reddish-brown reverse) yielded growth on SDA supplemented with 5% salt while, only the other (tan coloured) isolate gave characteristic heaped/"button-shaped" growths at 37°C (Figure 3A). Microscopic examination of the stained culture smear of the non-pigmented isolate showed chlamydospores arranged in chain-like appearance, whereas that of the red wine pigmented isolate revealed multi-septated, clavate, smooth and thin-walled macroconidia (Figure 3A and E). The isolates were also observed to be urease positive (turned urea agar from yellow to pink) after 7 and 15 days of incubation, respectively (Figure 3B and C).

## DISCUSSION

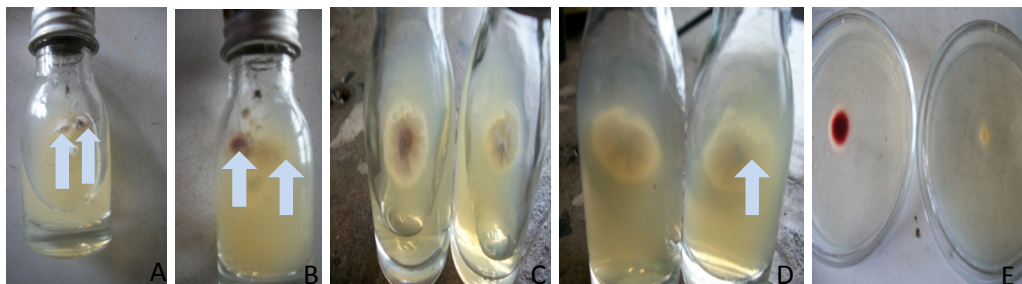
Equine dermatophytosis is caused by dermatophytic fungi which are keratinophilic and keratinolytic (Connole, 1990; Ural et al., 2009). It has been well documented that apart from keratinase, dermatophytes also produce proteolytic and lipolytic enzymes that have major roles in mycotic invasion and pathogenesis (Weitzman and Summerbell, 1995). These factors enable them to destroy skin and associated structures in a centrifugal pattern giving rise to alopecic, scaly or crusty annular "ringworm" lesions as were observed in the present case (Quinn and Markey, 2003; Ural et al., 2009).

Diagnosis of dermatophytosis is based mainly on history and clinical manifestation, direct microscopic examination, cultural and microscopic studies (Quinn and Markey, 2003).

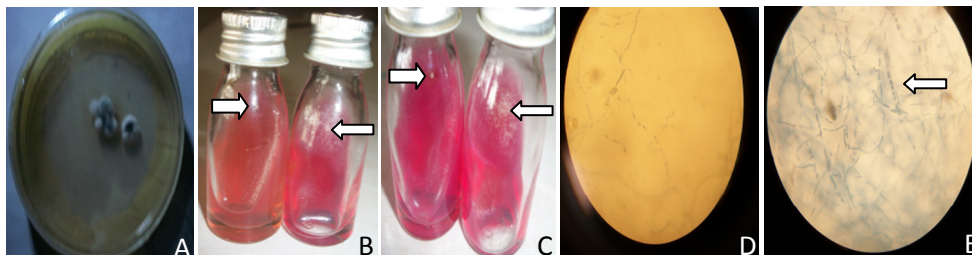
Characters that have been employed in identifying dermatophytes include colony pigmentation, texture, growth rate, and distinctive morphological features such as microconidia, macroconidia and nodular organs (Weitzmann and Summerbell, 1995). The macroscopic features observed were typical for *Trichophyton* spp.



**Figure 1.** (A) Initial lesion of raised hair patches and focal alopecic patch (arrow). (B) Annular (circumscribed) alopecic, greyish, shining characteristic "ringworm" lesion on the hind limb. (C) Coalesced lesions on the same limb after 2 weeks. (D) Crusty, coalesced alopecic lesion on the head.



**Figure 2.** Primary culture, (A) Obverse: Growth of two dermatophyte species (arrows) at spots where hair strands touched the agar after 8 days of incubation at 25°C; (B) Reverse: Wine red and colourless pigmentation of the growths. Pure culture, (C) Obverse: White, powdery, folded, velvety with central folding similar to both isolates after 12 days of incubation at 25°C; (D) Reverse: Colourless (left) and wine red pigmentation (right with arrow) of the isolates; (E) Reverse of the isolates on SDA agar plates.



**Figure 3.** (A) Heaped "button" morphology of *T. verrucosum* cultured at 37°C after 4 days of incubation; (B) Urease positive test of *T. verrucosum* (left) and *T. mentagrophytes* after 7 days of incubation at 25°C, observe that *T. verrucosum* which is glabrous (left) showed slow hydrolysis; (C) Complete hydrolysis after 15 days of incubation (arrows point at fungal growth); (D) *T. verrucosum* chlamydospores in "antler-like" arrangement; (E) *T. mentagrophytes* multinucleated smooth, thin-walled clavate "cigar shaped" macroconidia (x400).

*T. verrucosum* colonies have been reported to be glabrous, folded, heaped, velvety, wrinkled and white, with an unpigmented (colourless/tan) reverse (Quinn and Markey, 2003).

Enhanced growth and formation of "button-shaped" and/or heaped colonies on culturing at 37°C, has been widely utilized in differentiating *T. verrucosum* from other

*Trichophyton* spp. (Khosravi and Mahmoudi, 2003). *T. mentagrophytes* complex, both zoophilic (var. *interdigitale*) and anthropophilic (var. *mentagrophytes*) strains have been described. The former strains produce colonies that are granular and off-white colour, while the latter are white and fluffy with wine red colour pigment on the reverse side (Weeks et al., 2003). Again, growth on salt-

supplemented SDA has been used to differentiate *T. mentagrophytes* from other *Trichophyton* spp. (Weitzmann and Summerbell, 1995). These characters were observed in the present case.

The microscopic “antler-like” arrangement of chlamydospores (chlamydoconidia) observed in the present case has been a feature used for identifying *T. verrucosum* (Quinn and Markey, 2003; Shams-Ghahfarokhi et al., 2009). It is also well documented that this *Trichophyton* spp. rarely produces macroconidia and often times do not produce microconidia (Weitzmann and Summerbell, 1995; Quinn and Markey, 2003). *T. mentagrophytes* produce macroconidia which are clavate, multicelled (multi-septated), cylindrical, singly-borne, and smooth-thin walled (Weitzmann and Summerbell, 1995; Quinn and Markey, 2003). Both species have also been reported to produce urease enzyme (as virulence factor) which hydrolyses urea (Issa and Zangana, 2009; Weeks et al., 2003). Weitzmann and Summerbell (1995) reported that urease test for *T. verrucosum* is slow to develop. These features were very similar to our observations, and thus enabled us to identify the isolates as *T. verrucosum* (colourless pigment) and *T. mentagrophytes* (wine red pigment).

Although, there are slight resemblance in cultural (observe) and microscopic features of *Trichophyton* spp. incriminated in equine dermatophytosis (Weeks et al., 2003), we were able to distinguish our isolates from *T. equinum* because its cultures usually have a deep-yellow reverse with dark red centre (Amor et al., 2001; Ural et al., 2009). It also produces microconidia which usually forms nodular bodies (Weitzmann and Summerbell, 1995).

The horse in the present case most likely contracted the infection from the previously-infected horse, which may have served as source of contamination of the formites, trees and the ground where these animals lie and scratch themselves. Trichophytosis is a highly contagious disease which affects horses of all ages and transmission is by direct (especially when animals are grouped together) or indirect contact with a source of infection (Shimozawa et al., 1997; Quinn and Markey, 2003). It is also possible that the spores of the dermatophytes contaminated and persisted in the farm environment (Arslan et al., 2007).

The ticks and their bite wounds observed may have also facilitated infection, by serving as vector and providing portal of entry for the fungal agents. *Trichophyton* infection relies upon the presence of active (live) spores and skin damage (Pascoe, 1979; Hainer, 2003; Weeks et al., 2003). It has been noted that biting insects could be potential vectors for dermatophytes (Weitzmann and Summerbell, 1995). As observed in the present case, initial lesions in equine *Trichophyton* infections appear as erect hairs in circular areas often with a degree of localized inflammation, resulting in thickening of the skin within the infected area (Radostis et

al., 1997). The annular and coalesced lesions observed later in this case have been reported to be the characteristic of *Trichophyton* infection in horses, the infected areas expand centrifugally and may lose the circular appearance, becoming diffuse and ill-defined (Radostis et al., 1997).

For each animal species, the dermatophytes involved depend on the host studied and on the geographical, environmental conditions and husbandry (Bernado et al., 2005; Shams-Ghahfarokhi et al., 2009). The two isolates obtained in the present case have been reported to occur worldwide (Quinn and Markey, 2003; Weeks et al., 2003). However, although *Trichophyton* infection is common in horses, this report describes an unusual mixed infection involving two different species.

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## Case Report

# An incidence of cystic endometrial hyperplasia - pyometra complex in a Nigerian local breed bitch treated with medroxyprogesterone acetate (MPA) as a contraceptive

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Accepted 28 March, 2013

A four year-old Nigerian local breed bitch was presented to the Small Animal Unit of the Ahmadu Bello University Veterinary Teaching Hospital (ABUVTH) Zaria, with distended abdomen despite decreased appetite and persistent vaginal discharge, pale mucus membrane, fever and vomiting, after medroxyprogesterone acetate (MPA) treatment. Hematological examination revealed anemia, neutrophilia, hypoproteinemia and increased serum progesterone concentration on assay. There were abundant neutrophils and few intermediate cells on exfoliative vaginal cytology. *Streptococcus* spp was isolated from the vaginal discharge on bacterial culture. Ovariohysterectomy was performed and the gross findings were enlarged uterus with thickened wall, gray to tan sticky material in the lumen of the uterus. Histopathologically, there was hyperplasia of the surface of the endometrium, infiltration of the endometrial stroma with inflammatory cells and fibrosis of the myometrium.

**Key words:** Cystic endometrial hyperplasia (CEH), pyometra, bitch, medroxyprogesterone acetate, contraceptive.

## INTRODUCTION

Cystic endometrial hyperplasia (CEH) and pyometra in the bitch are luteal phase syndromes, supposed to be caused by hormonal disturbances and changes in endometrial steroid hormone (Kim and Kim, 2005). They are also associated with progestins administration (Concannon and Verstegen, 2005). During the luteal phase of the estrous cycle, serum progesterone concentration rises in pregnant and non pregnant bitches (Goodman, 2001). Progesterone stimulates the endometrial glands secretion, formation of cystic structures which are very important for feeding the embryos, and if the female is not pregnant they normally regress towards the end of the luteal phase leaving the endometrium to

to regenerate and be ready for the next chance for a pregnancy (Romagnoli, 2003).

However, repeated progestational stimulation (as it occurs with age in the bitch and queen) may lead to areas of the endometrium with these cystic structures not re-gaining normal status during the last part of diestrus and anestrus (Romagnoli and Concannon, 2003). These accumulated effects after several cycles explain the higher incidence of CEH in females of middle and advance age. CEH is thought to predispose to pyometra, as both can occur independently of the other. Although CEH usually precedes the development of pyometra, the CEH does not inevitably progress to pyometra in all

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bitches, the same way, while all bitches develop CEH with age, only some of them develop pyometra (Angulo, 2009). CEH is an incidental finding, and its natural incidence is not known. Exposure of the endometrium to progestin causes proliferation of the superficial layers of the endometrium with increased secretory activity of the endometrial glands, which can lead to CEH (Romagnoli and Concannon, 2003). Subclinical infection or a uterine endometrial irritation by foreign bodies during the end of estrus or early diestrus may stimulate an exaggerated hypertrophy-hyperplasia of the endometrium, similar to what occurs during the implantation (Angulo, 2009). Progesterone also reduces endometrial resistance which encourages bacterial proliferation (Noakes and Allen, 1986; Concannon and Romagnoli, 2003). CEH often predisposes the dog to pyometra (Feldman, 2000; Angulo, 2009) due to movement of normal vaginal bacterial flora into the uterus when the cervix is still open at the beginning of diestrus (Kustritz, 2005). The bacteria most frequently isolated in cases of pyometra is *Escherichia coli*, but *Staphylococcus* spp., *Streptococcus* spp., *Pseudomonas* spp., *Proteus* spp. contamination can also be found. Pyometra could either be closed cervix or open cervix (Romagnoli, 2003). Clinical signs of pyometra in bitches will depend on the patency of the cervix. Treatment of choice for pyometra is ovariohysterectomy (Kustritz, 2005; Angulo, 2009). The aim of this article is to report an incidence of cystic endometrial hyperplasia-pyometra complex in a bitch treated with medroxyprogesterone acetate.

## CASE REPORT

A four year-old Nigerian local breed bitch was presented to the Small Animal Unit of the Ahmadu Bello University Veterinary Teaching Hospital (ABUVTH) Zaria, with the chief complaint of vomiting, distended abdomen despite decreased appetite and persistent yellowish vaginal discharge. She had been given an injection of MPA at a dose rate of 1.5 mg/kg every 3 months to prevent estrous cycle. A month after the second dose of medroxyprogesterone acetate (MPA), the bitch developed those signs mentioned above. The reproductive history of the bitch was that she cycles twice a year. She usually gives birth to 4 to 6 puppies, hence MPA was administered. The contraceptive was administered about 2 months after her last whelping. The dosage of the contraceptive administered was 150 mg/ml (Depo-Provera<sup>®</sup> Pharmacia and Upjohn Company Kalamazoo, MI 49001, USA).

On physical examination, rectal temperature was 40.1°C and the mucus membrane was pale. Blood sample (5 mls) was collected from the cephalic vein- 3 mls for complete blood count and 2 mls for serum progesterone assay using ELISA 90 test kit (Fortress Diagnostic Ltd BT41 IQS USA). A swab sample of the

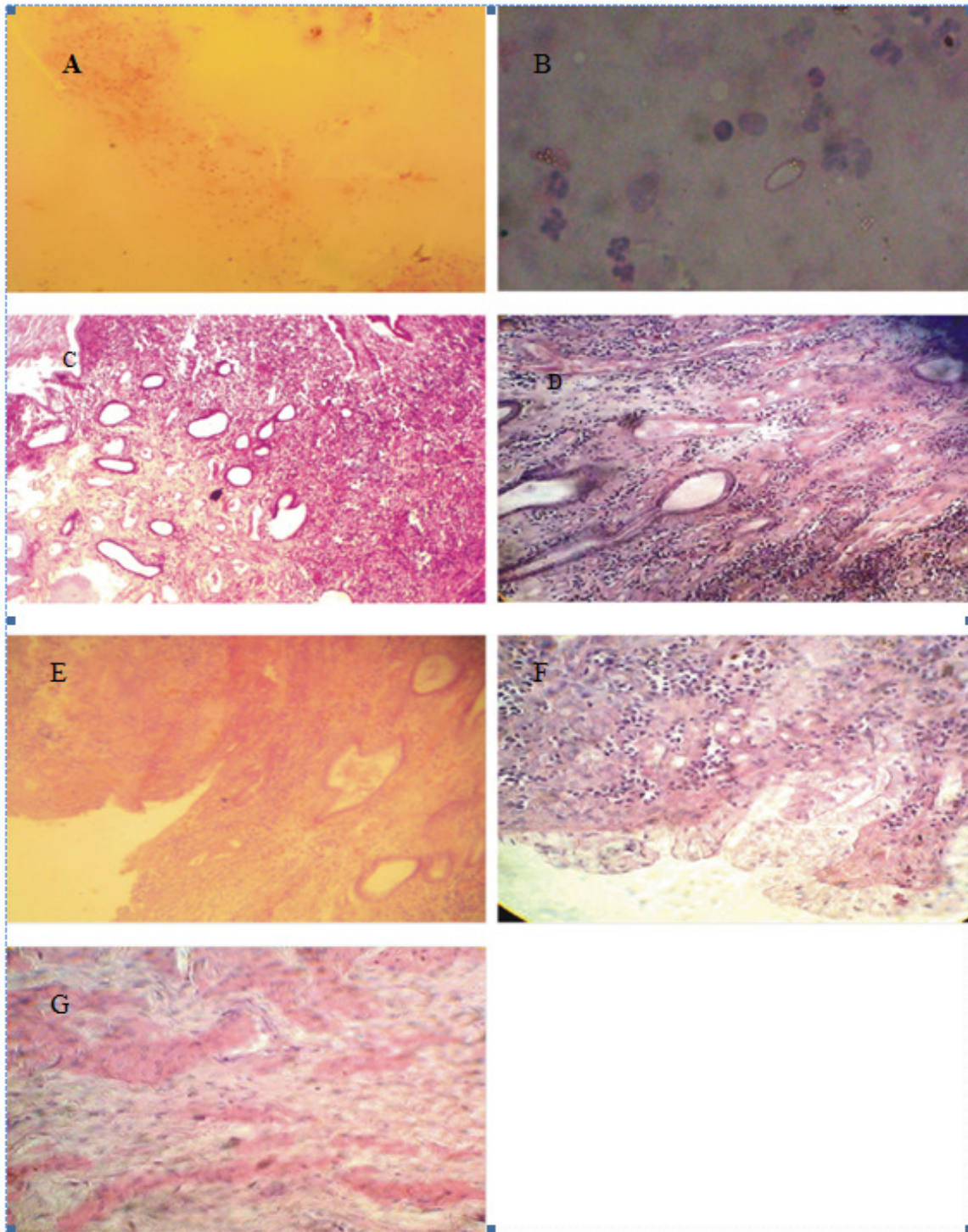
vaginal discharge was taken for bacterial culture. Vaginal swab was also taken for exfoliative vaginal cytology. The smear made from the vaginal swab was fixed in 95% ethanol, and then stained with Papanicolaou stain.

Ovariohysterectomy was performed, which revealed an enlarged uterus with thickened wall. The lumen of the uterus was filled with gray to tan sticky material trimmed sections of the surgically removed uterus were fixed in 10% formalin and stained with H&E stain for histopathological examination. Blood sample taken revealed anaemia (PCV 11%), leucocyte count was ( $6.0 \times 10^9/L$ ), neutrophils (75%), lymphocytes (19%) total protein (7.0 g/dl), eosinophils (4%) serum progesterone concentration (8 ng/ml). *Streptococcus* spp was isolated on bacterial culture. The predominant cells on exfoliative vaginal cytology were abundant neutrophils and few intermediate cells. Histopathologically, there was hyperplasia of endometrium, proliferation of inflammatory cells in the endometrial stroma and fibrosis of the myometrium. The bitch made complete recovery following ovariohysterectomy.

## DISCUSSION

Progestins are widely used in small animal medicine, with indications ranging from dermatological to behavioral problems, but the main use involves the control of the reproductive cycle. Exogenous progestin commonly used in dogs and cats are medroxyprogesterone acetate (MPA), proligestone (PR), chlomidione acetate (CMA), megestrol acetate (MA), delmadione acetate (DMA), norethisterone acetate (NTA) and melengestrol acetate (MGA) (Romagnoli, 2006). The use of these compounds are associated with side effects such as mammary tumor, decreased adrenocorticosteroid, increased prolactin and growth hormone, insulin resistance, increased appetite, weight gain, polydipsia, slight depression, decreased libido in males.

Progestin can cause masculinization of female fetuses when administered in the last trimester of pregnancy (Romagnoli and Concannon, 2003; Concannon, 2004; Romagnoli 2006). Clinical considerations for safe use of progestins are that a treatment period of 6 to 12 months is safe in healthy individuals. Treatment periods of over 12 months may worsen some subclinical conditions such as diabetes, microscopic mammary lesions or cystic endometrial hyperplasia. Progestin treatment should be restricted to bitches in anoestrus or early proestrus (Romagnoli and Concannon, 2003, Concannon, 2004). Bitches in late prooestrus, oestrus, or dioestrus if treated may result in abnormal stimulation of the reproductive tract and uterine pathology due to the presence of increased progesterone (Romagnoli and Concannon 2003). MPA is an exogenous progestin commonly used in temporal estrous prevention in dogs and cats in which future breeding are desired. However, proliferation of



**Figure 1.** (A and B) Vaginal cytology showing neutrophils ( $\times 10$  and  $\times 100$  respectively). (C and D) showing endometrial glands ( $\times 10$  and  $\times 400$ , respectively). (E and F) showing showing proliferation of the superficial layer of the endometrium and infiltration of the stroma with polymorphonuclear cells ( $\times 10$  and  $\times 400$  respectively). (G) showing fibrosis of the myometrium ( $\times 400$ ).

endometrium and mammary hyperplasia are the side effects of the exogenous progestin (Chatdarong et al., 2008). CEH is difficult to diagnose because it is not

usually associated with clinical signs unless accompanied with pyometra (Feldman, 2000). Pyometra is the most common sequel to CEH (Feldman, 2000; Angulo, 2009).

Incidence of pyometra is thought to be greater in the bitch than the queen because dogs are more exposed to natural progesterone frequently than the cat (Davidson, 2000). Bitches older than 7 years of age are prone to CEH than pyometra due to repeated exposure to progesterone, thus it is unlikely that CEH is the cause of pyometra in bitches younger than 6 years of age (Feldman, 2000). Some breeds are more predisposed to the condition like the Rottweiler, Saint Bernard, Chow Chow, Golden Retriever, Miniature Schnauzer, Terriers, Collie, The Alsatian, Dachshund, and other hounds however are at lower risk (Angulo, 2009). Open cervix pyometra is easily recognized because it is associated with vaginal discharge which is usually purulent, creamy, reddish-brown to green in colour and foul smelling (Kustritz, 2005). Closed cervix pyometra is not easily recognized. It is usually associated with abdominal distension and when it is accompanied with septicemia and toxemia, can result in progressive dehydration, shock and death (Feldman, 2000). Some bitches may suffer uterine rupture (Davidson, 2000). Other common signs of pyometra include pyrexia, lethargy, depression, inappetance or anorexia, polyuria, polydipsia, vomiting and diarrhoea (Feldman, 2000; Kustritz, 2005).

This case report recorded slight distended abdomen due to uterine enlargement. Grossly the surgically operated uterus was enlarged with thickened walls. On complete blood count, the bitch was anaemic with PCV at 11% and leucocytic neutrophilia. Predominant cells on vaginal cytology were neutrophils (Figure 1A, B). There was enlargement of endometrial glands (Figure 1C, D), proliferation of the superficial layer of the endometrium and abundant polymorphonuclear cells in the stroma of the endometrium (Figure 1E, F) fibrosis of the myometrium (Figure 1G).

## Conclusion

CEH–Pyometra complex can occur in normal and bitches undergoing contraceptive treatment. Despite administering the drug at a low dose and for a short period of time, the bitch came down with a uterine pathology. There is the likelihood of a pre-existing CEH.

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Full Length Research Paper

## Identification and characterization of *Salmonella* species in whole egg purchased from local markets in Addis Ababa, Ethiopia

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Accepted 16 April, 2013

Currently, salmonellosis is one of the major food borne pathogen both in developing and developed countries. Humans encountered this problem by consuming raw or undercooked food especially of poultry and egg products. The objective of the study was to identify and characterize *Salmonella* species in trans-ovarian contaminated eggs purchased from local markets in Addis Ababa. The study was conducted by using a standard laboratory diagnostic procedure. Isolation of *Salmonella* species from eggs was done both in solid and liquid media, and among three hundred eighty four (384) clean and non-cracked eggs examined, twenty eggs (5.21%) were positive for *Salmonella enteritidis* using selenite broth and Rappaport vassilidies broth as liquid media and xylose lysine desoxycholate (XLD) agar, MacConkey, *Salmonella Shigella* agar as solid media. *S. enteritidis* positive eggs (n = 20) when subjected to biochemical test using lysine iron agar (LIA) identified eighteen (4.69%) positive and two (2) negative samples. In this research, some commercial eggs yielded a number of *S. enteritidis*. This can be attributed to different causes but the most important one is transovarian transmission which implicate the possibility of poor animal health in layer farms. Storage time/temperature play the most significant role for its multiplication.

**Key words:** Egg, *Salmonella enteritidis*, Addis Ababa.

### INTRODUCTION

*Salmonella* is a rod-shaped, motile, aerobic and facultative anaerobe, non-spore forming and gram-negative organism. It can grow from 5°C up to 47°C, with an optimum temperature of 37°C. *Salmonella* is heat sensitive and can be readily destroyed at pasteurization temperature. *Salmonella* is a general name used for a group of more than 2,000 closely related bacteria that cause illness by reproducing in the digestive tract. Each *Salmonella* serotype shares common antigens and has

its own name; *Salmonella enteritidis* was the commonest serotype isolated from human clinical specimens (D'Aoust, 2000).

Food borne salmonellosis constitutes a major health problem in many countries (Persson and Jendteg, 1992). Globally, food borne infection and intoxications have been estimated that one billion cases of acute diarrhea occur annually in children under the age of 5 years in Africa, Asia (except China) and Latin America; approximately

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5 million of these cases were proved fatal (Vernam and Evans, 1991). Poultry has been widely acknowledged to be a reservoir for *Salmonella*. Egg contents may be contaminated with salmonellae by two routes: trans-ovarian (vertical transmission) or trans-shell (horizontal transmission) (Food and Agriculture Organization (FAO), 2002).

In trans-ovarian transmission, *Salmonella* are introduced from infected reproductive tissues to eggs prior to shell formation. *Salmonella* serotypes associated with poultry reproductive tissues includes *S. Enteritidis*, *S. Typhimurium* and *S. Heidelberg*. Among these, *S. enteritidis* may have better invasive properties and therefore, found more frequently in reproductive tissues (Advisory Committee on the Microbiological Safety of Food (ACMCF), 2001).

Investigations in a number of countries have revealed that when fresh, positive eggs contain about < 50 *S. enteritidis* per egg, growth can occur due to storage related changes and become rapid once *Salmonella* gain access to the egg yolk (ACMCF, 2001). *S. enteritidis* infection in egg laying hens and broiler chickens has important implication on public health worldwide (Humphrey, 1991). Gast and Beard (1990) showed that *S. enteritidis* can be recovered from cloaca, ceca, liver, spleen, ovaries and oviducts in hens exposed by either inoculation or horizontal contagion. *S. enteritidis* (anti-serum group D) and *Salmonella* serotype Typhimurium (anti-serum group B) are the most commonly reported serotypes involving in human salmonellosis. According to the data provided by the Department of Health (DH), *S. enteritidis* was the most common serotype isolated from human clinical specimens followed by *S. Typhimurium* and *Salmonella derby* during the years of 1997 to 2001 (HKSAR, 2004). From 1974 to 1981, Gebreyes conducted a study to identify the prevalent serovars and their susceptibility pattern to antibiotics in Addis Ababa. This study serves as a base-line data for all subsequent surveillance studies in Ethiopia. *Salmonella* strains were isolated from adult patients referred to the Central Laboratory and Research Institute, Addis Ababa, between January, 1974 and October, 1981. Of 216 *Salmonella* isolates studied, 54.6% were from stool and 45.4% from invasive sites: blood 34.7%, pus 5.6%, and urine 5.1%. There were 26 different serovars, of which *S. Typhimurium* (48.6%) was the most common, followed by *Salmonella concord* (12.5%), *S. typhimurium* (11.1%) and *S. Paratyphi B* (5.6%) (Gebreyes and Altier, 2002). The high isolation rate of *Salmonella concord* in Ethiopia is unusual and is in contrast to the other regions in Africa where *S. Typhimurium* or *S. Enteritidis* are more common (Nisbet and Ziprin, 2001).

The aim of this research is therefore to isolate and characterize *Salmonella* species in eggs contaminated due to trans-ovarian transmission.

## MATERIALS AND METHODS

### Study area

The study was conducted in Addis Ababa which is located 2,408 m.a.s.l and receives an annual mean rainfall of 1,200 mm, with average minimum and maximum annual temperature of 9.4 and 23.2°C, respectively (National Metrological Service Agency, 2002). Based on the preliminary 2007 census results, Addis Ababa has a total population of 2,738,248, consisting of 1,304,518 men and 1,433,730 women. The city is fully urban, with no rural dwellers within the city's administrative boundaries. Addis Ababa contains 22.9% of all urban dwellers in Ethiopia. With an estimated area of 530.14 square kilometers, this chartered city has an estimated density of 5,165.1 inhabitants per square kilometer (Central Statistical Agency of Ethiopia, 2008).

### Study materials

Whole eggs produced from both local and exotic breeds were purchased at local markets in Addis Ababa.

### Study design

Experimental study was conducted to identify and characterize *S. Enteritidis* in egg yolk. Isolation of *S. Enteritidis* from egg yolks indicates that this organism infects birds' reproductive organs and thereby transmitted through the egg.

### Sample size

Sample size was defined using the formula (Thrusfield, 2007) with expected prevalence taken as 50% (because there was no research conducted previously) at 95% confidence interval and significance level of 5%. A total of three hundred and eighty four (384) clean non-cracked eggs were collected from markets, supermarkets, and smaller grocery stores located in different zones of Addis Ababa regardless of the data of lay or storage type.

$$N = \frac{Z^2 P (1 - P)}{d^2}$$

Where Z = statistics for a level of confidence, P = expected prevalence or proportion, d = precision.

$$N = \frac{(1.96)^2 (0.5) (1-0.5)}{(0.05)^2} = \underline{\underline{384}}$$

### Study methodology

Whole eggs were washed and rinsed in alcohol for 20 min for decontamination followed by egg yolk separation and mixing with 225 ml of peptone water (under hood) and incubated for 24 to 48 h at 32°C. When the mixture becomes turbid, 1 ml of turbid solution was transferred into test tubes containing 10 ml of selenite broth and Rappaport vassilidies broth, respectively. Test tubes were incubated for 24 h until it becomes turbid and then transferred to

Salmonella Shigella agar, MacConkey agar and XLD agar using streaking loop and were incubated for 24 to 48 hours at 32°C. Colonies which are H<sub>2</sub>S producing and non lactose fermenters on the Salmonella Shigella agar, non lactose fermenters on MacConkey agar and those red colonies with H<sub>2</sub>S production on XLD agar were isolated and transferred to trypticase soy yeast (TSY) broth. When the solution becomes turbid, it was transferred to lysine iron agar using streaking needle (stab the butt, and streak on the slant). If purple alkaline production on the slant and blacking or acidic (H<sub>2</sub>S) production in the butt is observed, it was confirmed as *S. Enteritidis* (SOP Bacteriological Inter laboratory Comparison Study IX, 2005).

### Data analysis

The data were filled in a sheet of paper then descriptive statistics and prevalence was used to analyze the data manually. The prevalence was calculated by dividing the number of positive samples on biochemical test by the total number of egg sampled.

## RESULTS

The numbers of positive samples before and after biochemical tests are shown in Table 1. Among three hundred eighty four eggs examined, 20 became positive for *S. Enteritidis* using selenite broth and Rappaport vassiliadis broth as liquid media and XLD, MacConkey, Salmonella Shigella agar as solid media. Out of the twenty eggs which were identified as *S. Enteritidis* and biochemically tested using LIA, only eighteen *S. Enteritidis* positive eggs with two eggs became negative. As Table 1 shows, there are eighteen eggs which are positive on biochemical test. These eighteen eggs were isolated as *S. Enteritidis* using XLD, MacConkey, Salmonella Shigella Agar. From this solid media, XLD isolates 16 of the sample as salmonella positive whereas Salmonella Shigella agar isolated 9 of the samples as *Salmonella* positive and MacConkey agar isolated 4 of the sample as *S. enteritidis*.

From twenty nine positive samples grown in one or the other solid media (XLD, MacConkey and or *S. shigella* agar) which are biochemically tested positive samples, twenty five (25) of them were found using selenite broth enrichment and the rest four (4) were found using Rappaport Vassiliadis enrichment liquid media.

## DISCUSSION

Of the total 384 eggs tested, eighteen eggs (4.69%) were found positive as *S. enteritidis* that were confirmed by biochemical test on LIA agar. *S. enteritidis* isolates obtained in this research confirm the presence of this particular bacterium in commercial eggs for human consumption in Addis Ababa. These results agree with the US Food Safety Inspection Service, and Food and Drug Administration (FSIS, FDA) information, regarding the

presence of *S. Enteritidis* in table eggs, a highly popular food (Martinez et al., 2005). Before conducting biochemical test, there were twenty (20) egg samples which means 5.21% of the total sample size were found as *S. Enteritidis* positive samples on solid media (XLD, MacConkey and Salmonella Shigella Agar); then after biochemical test, two of the samples became negative on LIA. LIA aids in the differentiation of enteric bacilli on the basis of their ability to decarboxylate lysine, to deaminate lysine and to produce hydrogen sulfide, thus producing blackening of the butt.

The attempt to isolate *S. Enteritidis* from eggs using different culturing media (XLD, SS and MacConkey Agar) and nutrient broth (selenite broth and Rappaport-Vassiliadis broth) showed that this particular bacterium (*S. Enteritidis*) has its own behavior on this different culturing solid and liquid media as the result shows in Tables 2 and 3. Xylose lysin desoxycholate agar was used to identify 55.17%, *S. shigella* agar was used to identify 31.03% and MacConkey agar was used to identify 13.79% of the biochemically positive samples. Considering the liquid media, selenite broth was used to enrich 86.21% and Rappaport-Vassiliadis was used to enrich 13.79% out of a total of biochemically tested positive samples.

Most food-borne infections caused by *Salmonella* in humans were associated with foods such as mayonnaise, ice cream and frozen desserts which are consumed without being cooked after raw egg is added. Of course few *Salmonella* organisms are present in egg contents which multiply in a few minutes during storage at room temperature (Dugid and North, 1991). These results demonstrated that improvements are needed in controlling transovarian transmission of *S. Enteritidis* control program at farm level because the current farming system have not been able to prevent the introduction of *S. Enteritidis* on poultry farms, as well as egg contamination.

The abilities of this organism to asymptotically infect hen ovaries and to transmit to the internal contents of eggs, and to persist in farm environments, allowed for its unchecked spread in an era of increasingly large farms that house tens of thousands of birds. Contaminations of individual eggs with *S. Enteritidis* is infrequent, and out breaks are typically associated with food service situations in which eggs are pooled (Braden, 2006). This bacterium is deleterious for egg quality, and they are hazardous for consumers' health. This fact suggest the importance of establishing good animal health practice in poultry farms and a refrigeration chain throughout egg transportation, storage and commercialization, as practiced in other countries in an attempt to prevent the production of *S. enteritidis* contaminated egg.

The bacteria (*S. enteritidis*) are present in chicks for a long period of time when they are exposed to *Salmonella* at the end of hatchery period or during the first hours of

**Table 1.** Total number of eggs which are positive before and after biochemical test on lysine iron agar (LIA).

Parameter	No. of positive samples (eggs)
Salmonella positive samples before biochemical test on LIA	20
Salmonella positive samples after biochemical test on LIA	18
Total number of eggs sampled	384

**Table 2.** Positive samples in solid media using XLD, MacConkey and Salmonella Shigella agar.

Type of media	Number of positive samples
XLD agar	16
MacConkey agar	4
Salmonella Shigella agar	9

**Table 3.** Positive samples in biochemical test using selenite broth and Rappaport Vassilidias enrichment.

Parameter	Using selenite broth enrichment	Using Rappaport Vassilidias enrichment
Samples which are positive in biochemical test (LIA)	25	4
Total positive samples on biochemical test (LIA)		29

life, and may be disseminated to other susceptible chicks in the same flock or other flock. Therefore, the first step to prevent *Salmonella* introduction in farms is to obtain *Salmonella* free chicks, avoiding lateral transmission (Gast and Holt, 1998).

In this research, some commercial eggs yielded higher number of *S. enteritidis*. This can be attributed to different causes; but the most important one is transovarian transmission which implicate the possibility of poor animal health in layer farm, and time/temperature storage play the most significant role.

## CONCLUSION AND RECOMMENDATIONS

Egg and egg products are safest when stored in the refrigerator individually and thoroughly cooked, and promptly consumed. The larger the number of *Salmonella* present in the egg, the more likely to cause food borne illness. To minimize the potential risk of salmonellosis due to the consumption of egg and egg products, good manufacturing and handling practices should always be observed. Reference can be made to a World Health Organization (WHO) education brochure which outlines the safe procedure for consumers as well as food handlers to follow when handling and preparing eggs and food containing eggs. To further prevent the possibility of infection, some recommendations are thus made.

## Advice to business operators and producer

1. Establishment of good hygiene of housing and health of laying hens and to decrease factors which facilitate the production contaminated eggs with *S. enteritidis*.
2. Application of best prevention and control methods of transovarian transmission of *S. Enteritidis* in the farm level.
3. Considering the major role of eggs and poultry as a vehicle of transmission in human salmonellosis. An assessment of different factors affecting the prevalence, growth and transmission of salmonella in eggs and broiler chicken on the risk of human illness would be useful to risk managers in identifying the intervention strategies that would have the greatest impact on reducing human infections.
4. Adopting a first-in-first-out principle to store raw materials and keep them at appropriate temperatures.
5. Purchasing raw materials from reputable and reliable suppliers.
6. Storing and transporting eggs intended to be served cold at 4°C or below.

## Advice to the consumer

1. Buy food from reputable and reliable suppliers.
2. The elderly, children, pregnant women and persons with

lowered immunity should be careful when choosing food especially high risk food, such as uncooked eggs and egg products.

3. Keep eggs adequately refrigerated to prevent any *Salmonella* present in the eggs from growing to higher numbers, so eggs should be held refrigerated.

4. Discard cracked or dirty eggs.

5. Eat eggs promptly after cooking.

6. Avoid restaurant dishes made with raw or under cooked unpasteurized eggs.

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Full Length Research Paper

## Prevalence and seasonal incidence of bovine trypanosomosis in Birbir valley, Baro Akobo River system, Western Ethiopia

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Accepted 4 March, 2013

The study was conducted in Birbir valley of Oromia Regional State, Western Ethiopia from November 2009 to July 2010 to determine the prevalence and seasonal incidence of bovine trypanosomosis. Blood samples from 2219 randomly selected cattle of both sex and different age groups were collected and examined with conventional hematological and parasitological techniques. Out of the total examined animals, 195 (8.78%) cattle were infected with trypanosomes. Most of the infections were due to *Trypanosoma congolense* (65.6%) followed by *Trypanosoma vivax* (33.8%) and the rest were mixed infections of *T. congolense* and *T. vivax* (0.51%). There was no statistically significant difference ( $P>0.05$ ) in infection between male and female, young and adult animals and altitude levels. However, higher proportion of the infection was detected in adult male animals, during wet season and in lowland areas; 9.03, 10.33, 10.11% respectively. Mean packed cell volume (PCV) value of parasitaemic and a parasitaemic animals was not significantly ( $P>0.05$ ) different. The average seasonal incidence of trypanosome was 21.66, 10, 13.79 and 17.24% during the late rainy, dry, early and wet seasons, respectively. The relative higher incidence rate was observed during the wet seasons of the year. The study revealed that trypanosomosis is the main constraint to livestock production and agricultural activity in Birbir valley, Western Ethiopia. Hence, implementation of integrated tsetse and trypanosome control measures will save greater economic loss of the region in particular and the country in general.

**Key words:** Cattle, epidemiology, *T. congolense*, *T. vivax*, trypanosomosis, Western Ethiopia.

### INTRODUCTION

Trypanosomes multiply in the tsetse fly and are injected into the host when the fly feeds on an animal through tsetse saliva. Tsetse-transmitted trypanosomosis (Nagana) is one of the most ubiquitous and important constraints to agricultural development in the sub-humid and humid zones of Africa including Western and South Western parts of Ethiopia (NTICC, 2004; Enwezor et al., 2006).

African animal trypanosomosis (AAT) is, thus, one of the main constraints to livestock production on the African continent preventing full use of the land to feed the rapidly increasing human population (Murray et al., 1988). AAT is a disease complex caused by *Trypanosoma congolense*, *Trypanosoma vivax* or *Trypanosoma brucei* or simultaneous infection with one or more of these

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organisms. Infection with trypanosomes results in sub-acute, acute or chronic disease characterized by intermittent fever, anaemia, occasional diarrhoea and rapid loss of body condition and often terminates in death (Urquart et al., 1995). Cattle, particularly work oxen, are an integral part of farming in the Birbir valley Western Ethiopia. However, their productivity is severely hampered due to animal trypanosomosis. This study was undertaken in valley of Birbir, Baro Akobo river system from November 2009 to July 2010 to determine the prevalence and seasonal incidence of bovine trypanosomosis; assess the factors regulating the epidemiology of bovine trypanosomosis in Birbir valley and establish an appropriate strategy for its control in the study area.

## MATERIALS AND METHODS

### Study area

The study was conducted in two districts: Dalesadi and Dalewabera of Kellem Wollega Zone in Oromia Regional State in Birbir Valley Baro Akobo River system, Western Ethiopia (Figure 1). The agro-climatic condition of the areas alternates with long summer rainfall (June to September) and winter dry season (December to March) with an annual rainfall ranging from 1300 to 1600 mm. The annual mean minimum and maximum temperature ranges are 11.0 to 15.5 and 26.1 to 33.4°C respectively. The altitude ranges from 1300 to 1800 m.a.s.l. The natural vegetations have been degraded due to intensive cultivation. However, much of the cultivated lands have scattered tree covers and in some fields different vegetation types such as savanna woodland, forest, riverine and bush lands have been grown on soil bunds to provide soil protection. Both vegetation and wild life play very important roles in the transmission of trypanosomosis, the wild life serves as reservoir of the infection and the vegetation as a habitat for the tsetse fly and wild life (NTICC, 1996). The dominant livestock population in Birbir valley is cattle followed by goats and sheep and all of them are raised under traditional management system. Other animals such as equines, poultry and bees are also bred. Mixed livestock and crop farming is the dominant form of production in the livelihood of the people; hence cattle are used for draught power and milk production while sheep, goats, bees and poultry are raised both for household consumption and income generation.

### Study animals

This study was carried out on 2219 local zebu selected by random sampling methods in the two districts of Birbir valley. Information on the sex, species, age of the cattle, and the altitude of the sites, packed cell volume (PCV) from collected blood samples were recorded.

### Parasitological and haematological examinations

Paired blood samples were collected from the peripheral ear vein of each animal using heparinized microhaematocrit capillary tubes that filled 3/4 of the height and sealed with cristaseal. The sealed microhaematocrit capillary tubes containing 70 µl of blood were centrifuged immediately in microhaematocrit centrifuge for 5 min at 12000 rpm. After centrifugation, the pack cell volume (PCV) was read for estimation of anemia using haematocrit reader and the buffy-coat examination done as described in (Murray et al., 1983).

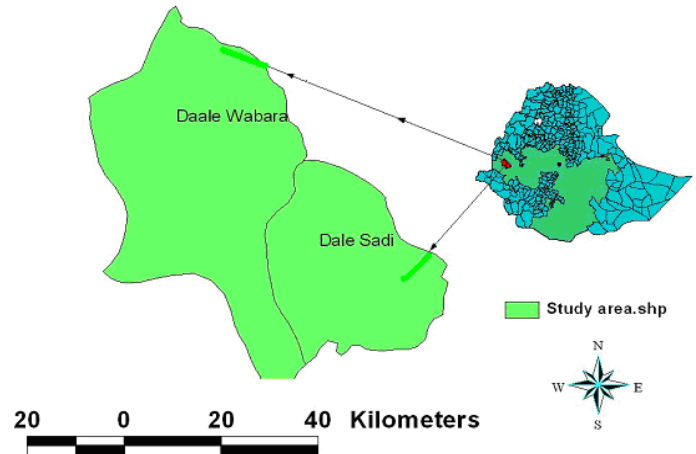


Figure 1. Location map of the study area.

The capillary tube was cut 1 mm below the buffy coat to include the top layer of red cells.

The content of the capillary tube was expressed onto a clean slide, mixed and covered with a 22 x 22 mm cover slip. Then the slide was examined for trypanosomes based on their type of motility in the microscopic field. Confirmations of trypanosome species by morphological characteristics were done after a thin blood smear was prepared from the buffy-coat examination and stained with Giemsa stain and examined under a microscope using oil immersion 100x objective (Murray et al., 1983).

### Incidence rate

Before the treatment, baseline sampling was done to determine point prevalence of the disease (9.3%). The study was conducted by treating all selected cattle with diminazene aceturate at a dose rate of 3.5 mg/kg body weight. Starting after one month (day 0) all selected animals were examined for trypanosome parasite using buffy-coat techniques every 30 days interval until 8 months (December-June) while November is day minus 30 which was when block treatment was given.

### Statistical analyses

The prevalence of trypanosome infection was calculated as the number of parasitological positive animals as examined by the buffy-coat method (Murray et al., 1983) divided by the total number of animals investigated at that particular time. Confidence intervals (95%) for the PCV of trypanosome-infected and non-infected animals were calculated. The prevalence of trypanosomosis under different variables (altitude levels, season, sex and age) was compared by chi-square test. A multivariate computation was conducted using logistic regression analysis in order to establish the effects of different risk factors (age, sex, altitude and season).

## RESULTS

### Parasitological findings

Out of a total of 2219 cattle examined, an overall prevalence rate of 195 (8.78%) was recorded. Relatively higher prevalence was observed during the late rainy and

**Table 1.** Seasonal prevalence of trypanosomosis and relative frequency of trypanosome species in different seasons.

Parameter	Late rainy	Dry	Early rainy	Wet	Total
Examined	701	549	485	484	2219
Infected	72	37	36	50	195
<i>T. congolense</i>	65	30	8	26	129(5.81)
<i>T. vivax</i>	6	7	28	24	65(2.92)
<i>T. congolense</i> and <i>T. vivax</i>	1	0	0	0	1(0.04)
Overall prevalence (%)	10.27	6.73	7.42	10.33	8.78

$\chi^2 = 2.77$ ,  $P > 0.05$ .

**Table 2.** The overall prevalence of trypanosomosis in different sex, age and altitude levels during the study period in Birbir valley, Baro Akobo River system, Western Ethiopia.

Variable	N	Positive	Trypanosome species detected			Prevalence (%)	d.f	$\chi^2$	P-value
			<i>T.c</i>	<i>T.v</i>	Mix				
<b>Sex</b>									
Male	1502	135	83	52	1	8.98	1	2.777	0.337
Female	717	60	46	13		8.36			
<b>Age</b>									
<1 year	133	8	8	0		6.01	2	1.567	0.252
1 to 3 years	492	43	28	13	-	8.7			
>3 years	1594	144	93	52		9.03			
<b>Altitude (m)</b>									
<1500	1266	128	101	26		10.11	1	4.150	0.791
≥1500	953	67	28	39	-	7.03			
<b>Total</b>	<b>2219</b>	<b>195</b>	<b>129</b>	<b>65</b>		<b>8.78</b>			

*T.c* = *T. congolense*, *T.v* = *T. vivax*, Mixed = *T. congolense* and *T. vivax*.

wet seasons at a rate of 10.27 and 10.33%, respectively, than the dry and early rainy seasons 6.73 and 7.42% (Table 1). However, statistically there was no significant ( $P > 0.05$ ) difference between the two. Trypanosome species encountered in the animals (cattle) examined in all seasons belong to the two species of *T. congolense* and *T. vivax*. *T. congolense* was the predominant trypanosome species detected during the study period (5.81%) compared to *T. vivax* (2.92%) ( $P < 0.05$ ).

No tested variables (sex age and altitude) had a significant effect on trypanosome infection rates. However higher proportion of infection was detected in older male animals in lowland areas. The prevalence rate in the lowland areas were 10.11% and in the midland areas 7.03%. The predominant trypanosome species in the lowland areas was *T. congolense* representing 78.9% of all infections (Table 2). *T. congolense* was the predominant tsetse transmitted trypanosome species that causes infection in both lowland and midland areas. The prevalence of trypanosome infection was more or less equal between the lowland and midland areas during the wet season of the study period. *T. vivax* was significantly

dominant in midland areas while *T. congolense* was dominant in lowland areas.

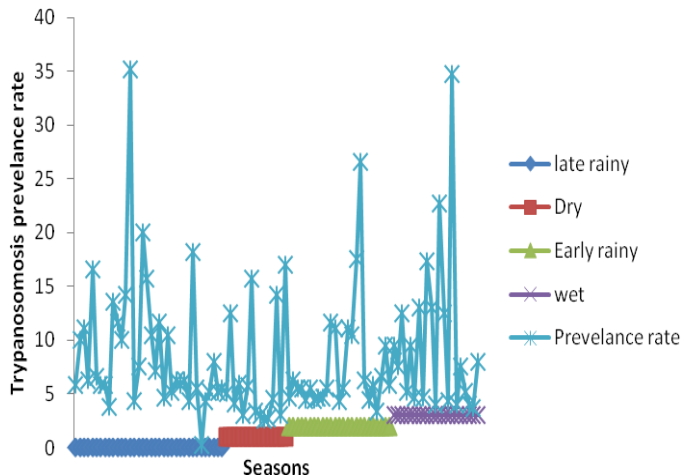
The maximum herd prevalence of trypanosome was 35.8% during late rainy and 34.7% in wet season, respectively (Figure 2). The overall prevalence of sampled animals was 8.78%. All the sampled herds were positive for trypanosome infections. The relationship between herd prevalence of trypanosome infections and herd average PCV were examined by regression analysis using herd average PCV as the dependent variable and the prevalence of trypanosome infections in a herd as independent variable.

During the parasitological survey, animals with  $PCV \leq 24\%$  were considered to be anemic. Mean trypanosome prevalence and PCV were negatively correlated but without statistical significance ( $r = -0.187$ ;  $P > 0.05$ ).

### Haematological findings

The mean PCV (%) values in parasitaemic and aparasitaemic animals during the late rain, dry, early rain





**Figure 2.** Comparison of average PCV value and herd trypanosome prevalence.

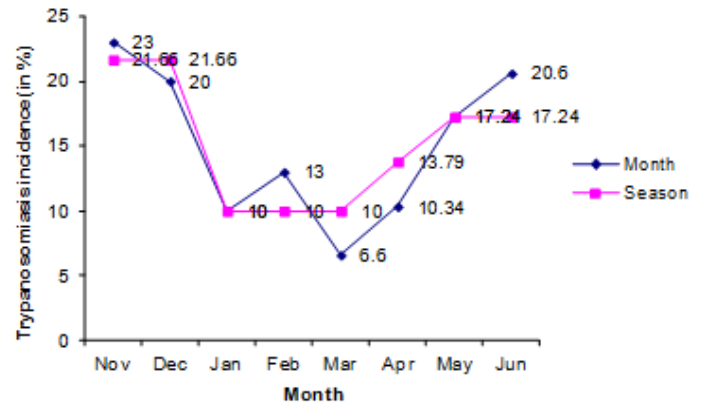
and wet seasons were  $23.04 \pm 2.59$  in parasitaemic and  $26.01 \pm 4.16$  in aparasitaemic,  $22.10 \pm 4.51$  in parasitaemic and  $25.12 \pm 3.53$  in aparasitaemic,  $20.72 \pm 3.59$  in parasitaemic and  $25.87 \pm 3.86$  in aparasitaemic,  $19.84 \pm 2.65$  in parasitaemic and  $25.42 \pm 3.35$  in aparasitaemic, respectively. The overall mean PCV values were also found to have lower difference between parasitaemic and aparasitaemic animals 21.61 and 25.63, respectively.

The mean PCV (%) of animals in the lowland and midland area was  $25.26 \pm 3.84$  standard deviation (SD) (95% confidence interval (CI) = 24.91 - 25.62) and  $26.55 \pm 4.53$  SD (95% CI = 25.97 - 27.12),  $25.17 \pm 3.82$  SD (95% CI = 24.76 - 25.58) and  $24.52 \pm 3.36$  SD (95% CI = 24.0 - 24.97),  $25.32 \pm 3.59$  SD (95% CI = 24.85 - 25.87) and  $25.61 \pm 4.42$  SD (95% CI = 25.06 - 26.16), and  $24.67 \pm 3.47$  SD (95% CI = 24.23 - 25.10) and  $25.03 \pm 3.92$  SD (95% CI = 24.52 - 25.53) during the late rainy, dry, early rainy and wet season respectively.

The range of PCV values in parasitaemic and aparasitaemic animals were from 17 to 29% and 20 to 36%; 13 to 34% and 19 to 38%; 14 to 28% and 20 to 38%; and 16 to 33% and 20 to 38%, during late rainy, dry, early rainy and wet seasons respectively. Generally the PCV of parasitaemic and aparasitaemic animals were within the range of 13 to 34 and 19 to 38, respectively.

### Incidence rate

The result of monthly and seasonal trypanosome incidence rate are presented in (Figure 3). Thus the study of incidence rate revealed that the average seasonal incidence of trypanosome was 21.66, 10, 13.79 and 17.24% during the late rainy, dry, early and wet seasons, respectively. The relative higher incidence rate was observed during the wet seasons of the year.



**Figure 3.** Monthly and seasonal incidence rate of trypanosomiasis.

### DISCUSSION

The prevalence of positive samples by microscopical examination of the buffy-coat for the trypanosomes is within the range of other previous reports of studies conducted in Western Ethiopia by Rowland et al. (1993). *T. congolense* was the most prevalent trypanosome species in the study area that accounts for the overall percentage of about 66.15% (129/195). Similar studies indicate that the most prevalent trypanosome species in tsetse-infested areas of Ethiopia are *T. congolense* and *T. vivax*; Rowland et al. (1993) reported a prevalence rate of 37% for *T. congolense* in Southwest Ethiopia. Abebe and Jobre (1996) reported an infection rate of 58.5% for *T. congolense*, 31.2% for *T. vivax* and 3.5% for *T. brucei* in Southwest Ethiopia. The predominance of *T. congolense* infection in cattle may be due to the high number seroderms of *T. congolense* as compared to *T. vivax* and the development of better immune response to *T. vivax* infected animals (Leak et al., 1999; MacLennan, 1970). *T. congolense* has been incriminated to be the most prevalent species in tsetse area (Langridge, 1976). It was found that in most cases the prevalence of *T. congolense* in cattle was higher than *T. vivax* when specific tsetse areas were considered separately because, sometimes investigations were made after the cattle were treated with trypanocidal drugs such as Diminazene aceturate. After such treatments *T. congolense* predominates over *T. vivax* in prevalence (Wilson et al., 1975). Host's reaction to *T. vivax* may be more adverse than to *T. congolense* because *T. congolense* is more virulent to cattle than *T. vivax*. Higher infection rates were observed in male animals in the present study but the difference was not significant. Similar results have been reported by different works (Afewerk, 1998; Muturi, 1999; Tewelde, 2001). The possible explanation from the present findings would be that male animals are more exposed to traction power and also cross in different vegetations for grazing and watering where tsetse challenge is very high than

females. In this research work, age was not found to be a risk factor but higher infection rates were observed in adults and younger animals in both altitude levels and seasons. This is logically associated to the fact that suckling calves did not go out with their dams but graze at homesteaded until weaned off (Rowlands et al., 1993). And younger animals are naturally protected to some extent by maternal antibodies (Fimmen et al., 1982). On the other hand adult animals travel and cross-different vegetation types for grazing, watering as well as for draught and harvesting crops to tsetse high challenge areas. *T. congolense* infection is a chronic condition that increases infection rates with age. The difference in prevalence of trypanosomosis in lowland and midland areas might be attributed to the difference in tsetse apparent density in the two altitude levels. Trypanosomosis prevalence is influenced by tsetse apparent density and infection rates in tsetse flies (Riordan, 1977) demonstrated that a high tsetse apparent density and infection rates of 50% in tsetse results in 42% trypanosome prevalence in cattle exposed to tsetse flies.

The increase in tsetse apparent density during the wet season has been reported in Ethiopia (Msangi, 1999) in Somalia (Mohammed and Dairri, 1987) and Cote d'ivoire, Togo, Gabon and Zaire (Leak et al., 1988). The increase in apparent tsetse density led to an increase in trypanosome challenge to cattle in the study area, resulting in the observed difference in trypanosome prevalence during the four sampling seasons (in the late rainy season in November 2009, during the dry period in March 2010, in the early rainy season late May 2010 and during wet season late June and early July 2010). Even though relative higher infection was observed in the lowland areas, the mean PCV values between altitude levels did not show any significant difference. The moderate changes in the mean PCV values between altitude levels may be attributed to the higher infection rates observed in the lowland areas and the nutritional imbalance in the mid land areas during the study period. Rowlands et al. (2001) in Ghibe observed an increase in PCV value, as the proportions of positivity decreases and hence mean PCV was a good indicator for the health status of animals in an endemic area. There was lower mean PCV value in parasitaemic animals than is reported by several authors (Leak, 1987; Afewerk, 1998; Muturi, 1999; Tewelde, 2001). The development of anaemia is one of the most typical signs of trypanosomosis caused by *T. congolense* in the susceptible cattle breeds (Murray and Dexter, 1988). The level of anaemia or the PCV usually gives a reliable indication of the disease status and productive performance of an infected animal (Trail et al., 1991). Bovine trypanosomosis control scheme aims at reducing the prevalence of infection with a concomitant increase in the herd average PCV. Therefore, the knowledge of relationship between prevalence of trypanosome infection and herd average PCV could be a useful tool for the assessment of impact

of control intervention. However, the herd average PCV is affected by different factors other than trypanosomosis (Conner, 1994). These different factors are not always identifiable but they are likely to affect both trypanosomosis positive and negative animals. Conner (1994) indicated that anaemia associated with trypanosomosis causes weakness, lethargy and lack of stamina which ultimately reduce efficiency of working animals. Swallow (2000) indicated that animals in tsetse infested area have lower calving rate, milk yield, higher calf mortality and require more treatment with trypanocidal drugs and that trypanosusceptible animal can be devastated by sudden exposure to high levels of trypanosome risk.

The result of parasitological survey revealed that *T. congolense* and *T. vivax* were the most prevalent trypanosome species in the Birbir valley, Baro Akobo River system, Western Ethiopia. Trypanosomosis caused by *T. congolense* and *T. vivax* have chronic and acute causes, respectively. Short-lived disease may have high incidence but low prevalence while lifelong disease has low incidence but high prevalence. In the parasitological survey of this finding, *T. vivax* was less prevalent than *T. congolense*. The chronic form of *T. congolense* was resulted in higher prevalence than *T. vivax* infections.

## Conclusion

Trypanosomosis in domestic livestock is a very common disease in Birbir valley, Baro Akobo River system Western Ethiopia. It is one of the most important diseases which kill more working domestic ruminants. In all the study area whether they are lowland and midland the livestock were all infected with trypanosomosis; however, the infection rates depended on local ecological conditions, the infection rate in tsetse flies, the abundance and activity of the insect vector and cattle management of the individual cattle owners. Community based integrated tsetse and trypanosomosis control should be implemented using impregnated target with baited technology.

## ACKNOWLEDGEMENTS

Ethiopian Ministry of Agriculture National Tsetse and Trypanosomosis Investigation and Control Center (NTTICC) are highly appreciated for the financial support; the Dale wabera and Dalesadi districts, Agricultural office members of the veterinary staff and all NTTICC Technical and administrative staffs are also acknowledged for their materials and technical supports during this research work.

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## Full Length Research Paper

# Do probiotics affect the behavior of turkey poults?

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Accepted 3 May, 2013

With the concept that measuring behavior is often the first step to take when studying how the brain operates, this study was conducted to investigate the effect of probiotic on turkey poult's behavior which will confirm the new concept that gut microbes can influence the brain. Ecobiol® probiotic, spores of *Bacillus amyloliquefaciens* and a carrier as serum of milk with a minimum guaranteed  $1 \times 10^{10}$  CFU/g was given with a dose of 0.01 g/day for each bird in the drinking water to group (P; n=350) and the other group (C; n=350) were kept as controls. Behavioral observations were carried out by direct personal observation without bird disturbance from outside the pen with a good view over the whole pen. Maintenance, comfort behavior, kinesis and agonistic behaviors were recorded. The obtained results indicated that probiotics increased the feeding frequency and duration and decreased distress call and aggressive behaviors in turkey poults.

**Key words:** Ecobiol®, turkey, probiotics, behavior, observation.

## INTRODUCTION

Probiotics are live microbial feed supplements which beneficially affect the host animal by improving its intestinal balance (Gibson and Fuller, 2000) and are nowadays widely used as growth promoters in poultry production. Probiotic foods have been consumed for centuries. A food can be said to be functional if it contains a component (which may or may not be a nutrient) that affects one or a limited number of functions in the body in a targeted way so as to have positive effects on health or if it has a physiological or psychological effect beyond the traditional nutritional effect (Ahmad, 2006).

The stability of the gut micro biota is the key to control intestinal health. The use of probiotics as gut flora stabilizer is a recognized tool to achieve that objective, as the probiotics interact with other bacteria and control them. If these probiotics, besides, interact with the host by producing lactic acid, or enzymes, then the animal not only has a better intestinal health, but can digest better the feed, and hence, improve their feed conversion ratio.

Probiotic composed of spores of *Bacillus amyloliquefaciens*

and it is easy to handle and they thought to act in the intestinal tract of poultry reducing the effect of pathogenic bacteria such as *Clostridium perfringens*, *Escherichia coli*, and *Yersinia*. In addition to enzyme production, such as amylases and proteases that make the feed more digestible, this favors the proliferation of lactic acid bacteria and produces lactic acid as by product of starch fermentation. In broiler chicken, trials of significant improvements in feed conversion rate have been observed for growing period when using supplemented diets with Ecobiol®. Numerical differences were also observed for feed intake; broilers fed with Ecobiol® took less feed, because they perform better with the diet. Mortality of broiler decreases with the inclusion of the probiotic (Diaz, 2007).

Several researchers studied the effect of probiotic administration on the feed conversion ratio (Silva et al., 2000; Opalinski et al., 2007), body weight, feed efficiency (Safalaoh, 2006; Timmerman et al., 2006; Mountzouris et al., 2007; Jouybari et al., 2009; Alkhalf et al., 2010), histology of the intestine (Mongkol and Kohen, 2002; Zhang

et al., 2005) and also its effect on immunity (Ahmad, 2006). However, little is known about the effect of probiotics on the behavior of animals. Recently, Bravo et al. (2011) reported that 'ingestion of *Lactobacillus* strain regulates emotional behavior. Moreover, Sudo et al. (2004), Sudo (2006) and Messaoudi et al. (2011) found that the exposure to probiotic bacteria can reduce stress and depression related behaviors.

In addition, the fact that the brain regulates gut activity is well established in our minds; however, recent attention focused on the reverse pathway and the manner in which gut microbes can influence the brain (Grenham et al., 2011). There are open lines of communication between brains and bowels, these channels allow an individual's gut bacteria to steer their behavior (Yong, 2011). Indeed, measuring behavior is often the first step to take when studying how the brain operates (Tchernichovski and Saar, 2008). Therefore, this study was performed to answer the question; do probiotics affect the behavior of birds? And to confirm the concept that gut microbes can influence the brain.

## MATERIALS AND METHODS

### Accommodation and management of animals

This study was conducted on a private farm in Beni-Suef Governorate. A total number of 700 turkey poults aged 10 days old and each has average weight of 125 g, reared in a deep litter system conventional management conditions. Birds were randomly divided into two groups (n=350) and allocated to identical well ventilated pens, fed a mixed ration from 50 kg maize, 35 kg soya beans and 15 kg concentrates (soya, corn germ, vitamins and minerals) according to the farm system; water were provided *ad libitum*. Poults were vaccinated against influenza, Newcastle at 1, 5 and 19 days old, respectively.

### Probiotic treatment and behavioral observation

Ecobiol® probiotic, spores of *B. amyloliquefaciens* and a carrier as serum of milk with a minimum guaranteed  $1 \times 10^{10}$  CFU/g was given with a dose of 0.01 g/day for each bird in the drinking water to group (P; n=350) and the other group (C; n=350) were kept as controls.

Behavioral observations were carried out by direct, blind personal observation without bird disturbance from outside the pen with a good view over the whole pen three times weekly, three times daily (once in the morning, another at afternoon and the last time in the evening); each observation lasted for 90 min (5 min observation and 5 min rest) using focal observation according to Martin and Bateson (1995). All the actions of one bird were recorded for a specified time period (5 min). This continues until the end of the specified time period (90 min). The behavioral patterns observed were as follows.

### Behavior of maintenance

They are behaviors related to self-maintenance of the animal including eating, standing, drinking, resting and defecation behaviors.

### Comfort behavior

Are heterogeneous groups of behavior related to body care including, scratching, preening and dust bathing behavior.

### Kinesis

Locomotion in the domestic birds was classified into walking and running. Much kinetic activity was invested in pecking for food, but exercise activities were also common.

### Agonistic behavior

Agonistic behavior refer to the complex of aggression, threat and avoidance behaviors that often occur during encounters between members of same species such as biting.

### Statistical analysis

Results were statistically analyzed by the use of non-parametric independent test using Statistical Package for Social Sciences (SPSS) 20 together with least square analysis procedure.

## RESULTS

The obtained results revealed that probiotic administration to turkey poults had variable effects on the different behavioral patterns.

It was observed from Table 1 and Figure 1 that probiotic Ecobiol administration had no prominent effect on the poult ingestive behavior except for feeding duration that was 15.8 min in the P group, while it was 10.7 min in C group during the observation period showing significant ( $p < 0.01$ ) difference between the two groups. However, other maintenance behavioral patterns (lying down, sleeping and standing or elimination) were not significantly affected by probiotics treatment. Table 2 revealed that probiotic administration to turkey poults had no significant effect on locomotion or comfort behavior; only slight increase in walking, preening and dust bathing frequency in P group in comparison with C group.

Concerning the effect of probiotics on the social and agonistic behaviors, Table 3 showed significant ( $p < 0.05$ ) effect of probiotic administration on distress call frequency since it was (0.2) in P group in comparison with 1.7 in C group, while it has no significant effect on the other studied social behavior pattern. Table 3 and Figure 2 demonstrated a significant ( $p < 0.001$ ) effect on biting frequency in poults as it reduced (0.2) in P group, although it was 2.2 in C group and also at  $\alpha = 0.05$  level of significance; there is enough evidence to conclude that there was a difference in the fighting frequency of the two groups.

## DISCUSSION

The aim of the present study was to investigate the effect

**Table 1.** Effect of probiotics on maintenance behavior of turkey poult.

Bird group	Ingestive behavior				Rest and sleep				Elimination	
	Eating		Drinking		Lying down		Sleeping			Standing idle
	Frequency	Duration (min)	Frequency	Duration (min)	Frequency	Duration (min)	Frequency	Duration (min)		Frequency
Control	4.8±0.6	10.7±1.3	2.9±0.4	1.1±0.4	4.3±0.5	2.9±0.5	3.1±0.4	6.7±1.2	1.7±0.3	0.5±0.2
Probiotic treated	5.9±0.4	15.8±1.3*	4.1±0.6	0.8±0.4	3.1±0.5	1.8±0.5	2.6±0.4	3.9±0.8	1.6±0.4	0.3±0.2

Results are expressed as means ± standard error (SE). \*Superscripts within columns indicate significant difference at  $p < 0.01$  at  $df = 34$ . Behavioral patterns were measured as a frequency and duration based on focal observation for each poult.

**Table 2.** Effect of probiotics on Kinesis and comfort behavior of turkey poult.

Bird group	Kinesis		Comfort behavior		
	Walking	Running	Preening	Dust bathing	Scratching
Control	20.3±2.3	2.1±0.7	5.1±0.8	0.5±0.3	1.4±0.5
Probiotic treated	22.9±2.6	1.3±0.5	5.7±0.8	2.0±0.7	1.3±0.4

Results are expressed as means ± standard error (SE) at  $df = 34$ .

**Table 3.** Effect of probiotics on social and agonistic behavior of turkey poult.

Bird group	Social behavior			Agonistic behavior	
	Picking each other	Vocalization		Fighting	Biting
		Distress call	Alarm signal		
Control	8.4±1.4	1.7±0.5*	2.3±0.7	1.4±0.4*	2.2±0.5**
Probiotic treated	10.3±2.0	0.2±0.2	1.8±0.6	0.2±0.1	0.2±0.1

Results are expressed as means ± standard error (SE). \*Superscripts within columns indicate significant difference at  $p < 0.05$  at  $df = 34$ . \*\*Superscripts within columns indicate significant difference at  $p < 0.01$  at  $df = 34$ . Behavioral patterns were measured as a frequency and duration based on focal observation for each poult.

of probiotic Ecobiol administration on the turkey poult's behavior.

The results showed a significant effect of probiotic administration on feeding behavior especially with regard to its duration. Such finding coincides with that obtained by Verdu et al. (2008) who recorded similar effect of probiotic adminis-

tration on the mice feeding behavior. These results may be explained in the light of published reports (Sudo et al., 2004; Sudo, 2006) observing that manipulations of bacteria found in the stomach and intestine can modify neural function and affect mood and behavior as there is an important link and interaction between gut

microbes and the brain (Lee and Chua, 2011). However, it is not agreeable with Diaz (2007) who demonstrated that broilers fed with Ecobiol® take less feed, because they perform better the diet. With regard to effect of probiotic on social behavior, there was a significant reduction in the distress call incidence in P group in comparison

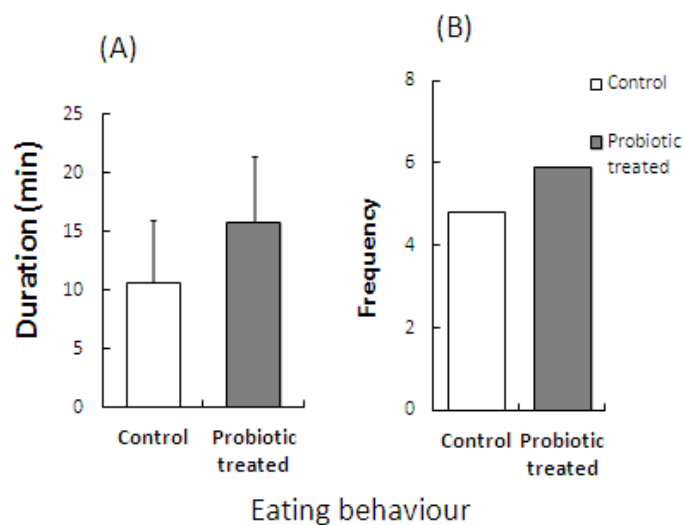


Figure 1. Effect of probiotics on eating behavior of turkey poults.

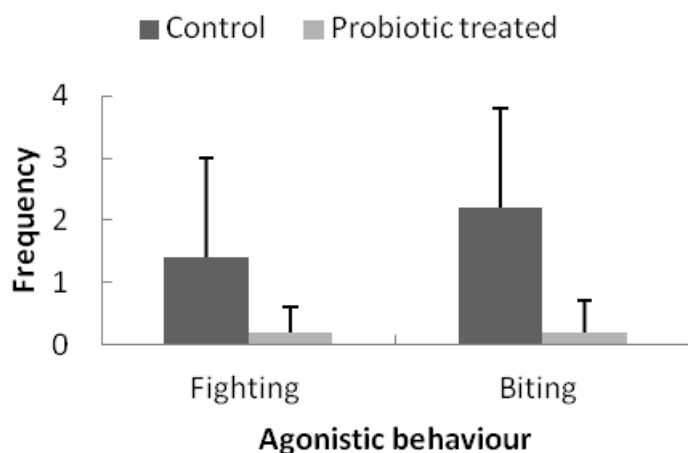


Figure 2. Effect of probiotics on agonistic behavior of turkey poults.

with C group of poults. The assumption of Bravo et al. (2011) that probiotics could have a direct effect on neurotransmitter receptors in the central nervous system (CNS) in normal, healthy animals. Gamma-aminobutyric acid (GABA) is the main CNS inhibitory neurotransmitter and is significantly involved in regulating many physiological and psychological processes.

Alterations in central GABA receptor expression are implicated in the pathogenesis of anxiety and depression and could explain the present data as the change in brain neurotransmitter GABA reduced the anxiety like behaviors such as distress calls.

Regarding the effect of probiotics on agonistic behavior, there was a significant decrease in biting and fighting frequency in P group. These results to some extent agree with that published by Emily (2012) who said

that probiotics may modulate the activity of brain structures involved in the processing of emotions related to anxiety, mood and aggression, and added that probiotics cause a reduction of substance P in the stomach which is a neurotransmitter associated with pain and inflammation, and linked to anxious, depressive and aggressive behaviors, which may clarify the aforementioned results.

In view of the current results, it can be concluded that probiotics increased the feeding frequency and duration and decreased the aggressive behaviors in turkey poults that may explain the probiotic effect on growth performance and immunity.

Further studies should be made to know the exact mechanism by which the microbes influence the brain and which area of the brain is involved. In addition, further studies in probiotics and animal behavior of other animal species and other forms of probiotics should be done.

## ACKNOWLEDGEMENT

The author would like to express her gratitude to all those who helped complete this research, thank Dr. A. M. Abdel Azeem who gave her permission to make the necessary research work on his private farm and Dr. Fatma H. Sayed who helped to stimulate suggestions in writing this research

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