ADITUM
Enrich your Research

Journal of Veterinary Medicine And Science

Review Article

A Review on Bovine Brucellosis and Its Public Health Significance in Ethiopia

Mulugeta Abera
Jimma University College of Agriculture and Veterinary Medicine School of Veterinary Medicine.

Article Info

Received: June 23, 2024
Accepted: June 26, 2024
Published: June 28, 2024

***Corresponding author:** Mulugeta Abera, Jimma University College of Agriculture and Veterinary Medicine School of Veterinary Medicine.

Citation: Abera M. (2024) "A Review on Bovine Brucellosis and Its Public Health Significance in Ethiopia." Journal of Veterinary Medicine and Science, 1(1); DOI: 10.61148/JVMS/002

Abstract:
Brucellosis is economically important zoonotic bacterial disease caused by the genus *Brucella*. Brucellosis occurs worldwide, except for few countries that have been successfully eradicated. The Aborted fetus, fetal membrane and uterine discharges are considered as the major source of infection. Bovine brucellosis is mainly transmitted to animals by ingestion of contaminated feed and water, by contact with infected aborted fetus, fetal membrane and genital discharges and by artificial insemination from infected bulls. The bacteria are preferentially localized mainly in the reproductive tract of pregnant animals and consequently cause late abortion, retained fetal membrane and infertility, whereas *orchitis* and *epididimitis* are seen in males. The overall studies of bovine *brucellosis* in Ethiopia range from 1.1% to 22.6% and 0.05% to 15.2% in intensive and extensive management systems respectively. Brucellosis is mainly transmitted to humans through the consumption of unpasteurized dairy products, occupational case direct contact with infected animal and their discharges. The prevalence of *Bovine brucellosis* depends on the standards of environmental hygiene, animal husbandry practices and species of the causative agent. Brucellosis has been widely reported from cattle in Ethiopia. This requires formulating effective control strategies are needed that includes surveillance to identify infected animals, prevention of transmission to non-infected animals and removal of the reservoir to eliminate the source of infection.

Key Words: animal; brucellosis; economic zoonosis

1. Introduction

Ethiopia has the largest livestock population in Africa with an estimate of 65 million cattle, 4O million sheep, and 51 million goats 8 million Camels 49 million Chickens (CSA, 2020) and 11.1 million equines (CSA, 2017). However, the country has not used this resource effectively owing to various limitations. Animal disease, management problems, poor genetics, and nutritional deficiency are among the foremost impediments to cattle production in the country (Ibrahim *et al.*, 2010). Among the infectious diseases, *Brucella* infection is widely prevalent and causes extensive economic losses, and brucellosis is one of the most serious zoonotic diseases in Ethiopia (Jergefa *et al.*, 2009; Asgedom *et al.*, 2016) .

The introduction of higher-yielding cattle breeds is one of the major strategies to increase milk production in the country. However, brucellosis is the main challenge to the development of dairy farming in different parts of Ethiopia, since the disease causes reproductive inefficiency and pregnancy loss in cattle (Asmare *et al.*, 2010; Tulu *et al.*, 2018). *Brucella* infection causes huge financial losses and community health concerns in

countries around the world, including Ethiopia (Asfaw *et al.*, 2016).

Brucellosis is one of the economically important diseases of livestock caused by members of the genus *Brucella* (OIE, 2020a). The disease is characterized by reproductive disorders such as abortion, stillbirth and birth of weak offspring in females and orchitis and epididymitis in male animals causing transient or permanent infertility (Constable *et al.*, 2017). The genus *Brucella* currently comprises six classical species primarily affecting domestic animals and rodents including *B. melitensis* of small ruminants, *B. abortus* of cattle, *B. suis* of pigs and hares, *B. ovis* of small ruminants, *B. canis* of dogs and *B. neotomae* of desert wood rats; and six novel species identified from marine mammals *(B. penippedialis and B. ceti)*, red foxes (*B. vulpes*), baboons (*B. papionis)*, a human breast implant (*B. inopinata*) and rodents (*B. microti)* (Scholz *et al.*, 2018). While *Brucella* species are host-adapted to preferred hosts, they are capable of infecting other species for instance, *B. abortus*, which is host-adapted to cattle, can infect small ruminants and wildlife (Godfroid, 2018). Camels are known to be infected by both *B. melitensis* and *B. abortus* when they are reared in close contact with small ruminants and cattle respectively (Gwida *et al.*, 2012; OIE, 2016).

Brucella abortus, *B. melitensis*, and *B. suis* are the major causes of bovine brucellosis. The disease is known to cause abortion in the last stage of pregnancy, followed by retention of the fetal membrane and infertility in succeeding pregnancies in cattle (Robi, 2020) . Office International des Epizooties (OIE) declares *brucellosis* as multiple species disease, infection and infestation (OIE, 2016). The etiological agent of bovine brucellosis is a Gram-negative *coccobacillus*, *Brucella abortus* and occasionally by *Brucella melitensis* and *Brucella suis* (OIE, 2016; Khurana *et al.*, 2021; CFSPH, 2018a).

Human *brucellosis* is popularly known as undulant fever, Crimean fever, Mediterranean fever, remitting fever, Maltese fever, goat fever, Gibraltar fever and bovine *brucellosis* is called as contagious abortion or Bang's disease (Hayou *et al.*, 2020). *Brucella* species are among those pathogenic bacteria which have propensity to those pathogenic bacteria which have propensity to adapt to new host and they can either be naturally transmitted to their primary hosts by direct or indirect contact or sometimes inadvertently to other susceptible hosts (Moreno, 2014). Mixed farming of cows, buffaloes, sheep and goats has increased the risk of brucellosis where small ruminants act as primary hosts for *B. melitensis* and cattle as spillover host (El-Wahab *et al. 2019*).

The direct economic impact of the disease is associated with loss of replacement stock, reduction in milk production and culling of valuable reproductive age animals further constraining herd expansion. In countries like Ethiopia, where the export of live animals is one of the sources of foreign exchange earnings, brucellosis hinders access to lucrative international markets. Where market accesses are permitted, the requirements by importing countries of testing every individual animal at export quarantines and rejection of those testing positive, further adds up to the economic loss (Franc *et al.*, 2018).

Brucellosis is also one of the significant zoonotic diseases affecting 0.83 million individuals worldwide, annually (WHO, 2015). In Ethiopia, a country with an estimated population of 112,078,730 (The World Bank, 2020) 60.9 million Heads of cattle, 31.3 million sheep, 32.7 million goats (CSA, 2018), 1.2 million camels and 11.1 million equines (CSA, 2017). serological studies conducted so far demonstrated that the disease is endemic across greater areas of the country (Asmare *et al.*, 2013; Regassa *et al.*,2009; Sintayehu *et al.*, 2015; Teshome *et al.*, 2003). However, no official figures are available both for livestock and human brucellosis. Only the presence, absence or suspected statuses of the disease during the various years between 1996 and 2019 in livestock and humans had been reported to the World Organization for Animal Health (OIE) (OIE, 2020b).

No control strategy including vaccination is so far been implemented against brucellosis in any of the livestock species in Ethiopia. Studies conducted to estimate the prevalence of brucellosis in the country were conducted by individual researchers, in research organizations or higher education institutions. But they were fragmented and are limited in space, time and scope; as a result, there is a need for summarizing such data to make them useful in understanding the disease burden and its distribution at a national level to devise appropriate intervention strategies. Then the objective of this review is to illustrate the potential predictors and the disease's spatial distribution pattern along the bovine and describe the public health impact .Finally, it suggests the way forward with a contextual intervention strategy to reduce the economic and public health impact of bovine brucellosis in Ethiopia.

2. LITERATURE REVIEW

2.1. Etiology

Bovine brucellosis is usually caused by *Brucella abortus*, less frequently by *B. melitensis*, and rarely by *B. suis*. However; humans, sheep, goats, and other domestic animals can also be infected by *Brucella abortus*. Cattle are infected with *B. suis* and *B. melitensis* when they graze together with infected pigs, goats, or sheep (Godfroid *et al*., 2011, Robi and Gelalcha, 2020). Each *Brucella* species tends to infect a particular animal species and they have a predilection for both female and male reproductive organs in sexually mature animals. The target organs and tissues of *Brucella* species are placenta, mammary glands and epididymis in animal reservoir host (Quinn *et al*., 2002). *Brucella* organism persist in targeted organ of the reservoir host and Persistent (lifelong) infection is a characteristic of its facultative intracellular organism, with shedding in reproductive and mammary secretions (Radostits *et al*., 2007).

2.2. Characteristics of *Brucella* Organism

They are Gram-negative, aerobic, facultative intracellular rods or coccobacilli, which lack capsules, endospores or native plasmids. The bacterium has a diameter of 0.5–0.7µm and has 0.6-1.5µm length, partial acid fast with oxidase, catalase, nitrate reductase and urease activity. The *Brucellae* are able to survive freezing and

thawing, but are susceptible to most of the common disinfectants. The bacterium remains viable in environment for months especially in cool and wet conditions. Pasteurization can effectively kill Brucella in milk (Dhanashekar *et al.*, 2012).

A total of six classical species (Table 1) and seven novel *Brucella* species have been recognized from a wide spectrum of susceptible hosts. Species affecting terrestrial animals are seven in number including *B. abortus, B. melitensis, B. suis, B. ovis, B. canis, B. neotomae* and *B. microti* (Scholz *et al*., 2008); two other species, *B. ceti* and *B. pinnipedialis* affect marine mammals (Foster *et al*., 2007). *B. papionis* isolated from baboons and *B. vulpis* from red foxes were also added to the list of genus *Brucella* (Scholz *et al*., 2016). Seven biovars have been recognized for *B. abortus*, three for *B. melitensis* and five for *B. suis*. Rest of the species has not been characterized into biovars. The recovery of distinct Brucella strains from marine mammals and human beings recently indicates the significance of zoonotic transmission (El-Sayed and Awad, 2018).

Table 1: A six classical Brucella species

Strain	Principle Host	Other Hosts	Symptoms	Transmission	Human Disease
Brucella abortus	Cattle	Sheep, goats, pigs, horses, dogs, humans, wild ungulates	Abortion after 5 Months	Ingestion, some venereal	undulant fever-control with antibiotics
Brucella melitensis ,	Sheep goats. Buffalo	cattle, pigs, dogs, humans, camels	Later term abortion, weak young, mastitis (goats)	Ingestion	Malta fever: can be fatal in human
Brucella ovis	Sheep		most often effects rams, rare abortions		
Brucella suis Brucella canis	Pig Dogs	cattle, horses dogs, humans reindeer, caribou Humans	Abortion and infertility abortions at 40-60 day	ingestion and venereal Venereal	extremely deadly in human mild disease in humans

Sources: Int. J. Adv. Res. Biol. Sci. (2023). 10(4): 140-158

2.3. Source of infection

The concentration of the bacteria is highest in pregnant uterus. The aborted fetus, placental membranes or fluids, and other uterine discharges were considered as major source of infection (FAO, 2010). Infected animals also shade organisms in milk which serve as source of infection for the new born. Contaminated feed can spread the infection from infected pasture over long distance. Invading *Brucella* usually localize in the lymph nodes, draining the invasion site, resulting in hyperplasia of lymphoid and reticulo endothelial tissue and the infiltration of inflammatory cells. Survival of the first line of defense by the bacteria results in local infection and the escape from the lymph nodes in to the blood. During bacteriamic phase, bones, joints, eyes and brain can be infected, but the bacteria are most frequently isolated from supra-mammary lymph nodes, milk, iliac lymph nodes, spleen and uterus. In bulls, the predilection sites for infection are also the reproductive organs and the associated lymph nodes. During the acute phase of infection, the semen contains large number of *Brucella* but as the infection becomes chronic, the number of Brucella excreted decreases. However, it may also continue to be excreted for years or just become intermittent (Acha and Szyfers, 2001).

2.4. Transmission

Brucellosis is typically transmitted to other cattle by direct or indirect interaction with diseased cattle discharges (OIE, 2009). The spread of brucellosis in cattle occurs through the ingestion of contaminated feed and drinking water contaminated by the bacteria that are present in massive amounts in birth products and discharge (Acha and Szyfres, 2001) . Moreover, cattle typically lick their fetuses and newborn calves, which can have very high levels of bacteria and are the major source of infection.(FAO, 2010). *Brucella* infection can also be transmitted by feeding pooled colostrum to newborn calves. *Brucella* infection is rarely spread through sexual contact in cattle. However, artificial insemination has been shown to spread the infection from infected cattle to healthy cattle (Robinson, 2003).

Humans acquire *Brucella* infection via the ingestion of unpasteurized milk or milk products. Interaction of the mucosal abrasions with the fluid or tissues of aborted fetuses of diseased cattle can also be a source of disease in humans (Quinn *et al.*,2002; Fugier *et al*., 2007) . Work-related contact with cattle or their products is the major risk, for human brucellosis. Abattoir, farm, and laboratory workers as well as veterinarians, known risk groups for *Brucella* infection (Memish and Mah, 2001).

In the herd animals, the infection can be due to introduction of an infected animal that subsequently gives birth or aborts a fetus,

whereupon pasture or water becomes contaminated by these excretions. Transient disease such as abortion can also develop following administration of a live *Brucell*a vaccine, particularly the *B abortus* vaccine strain 19. The organisms have been recovered from fetal and manure samples that remained in a cool environment for longer than 2 months. However, exposure to sunlight kills the bacterium within a few hours, and the organism is susceptible to many common disinfectants (Waring, 2005).

2.5. Epidemiology of Brucellosis

2.5.1. Geographical Distribution of Brucellosis

Brucellosis is endemic in many developing countries and is caused by *Brucella* species that affect man, domestic, some wild animals and marine mammals (Molla, 1989). The majority of human and animal brucellosis is found in sub-Saharan Africa with large pastoral communities has been recorded at herd level, within-herd level and individual animal level .The persistent disease was observed in most countries in the Sahel, with Ethiopia, Chad, Tanzania, Nigeria, Uganda, Kenya, Zimbabwe and Somalia reporting brucellosis in humans attributed to domestic cattle, camels, goats and sheep calculated an estimated seroprevalence of 16.2% with in cattle in sub-Saharan African (Mangen, *et al.*, 2002) . It is more prevalent in developing countries and considered to be a serious health problem due to lack of effective public health measures, domestic animal health programs, and appropriate diagnostic facilities. Furthermore, the situation is also worsened by the resemblance of the disease with other diseases leading to misdiagnosis and under reporting (Aworh *et al.*, 2013). The management systems as well as ecological conditions greatly influence the spread of brucella infection Haileselassie *et al.*, 2010).

2.5.2. Risk Factors for Bovine Brucellosis

The occurrence of Brucella infection is affected by a variety of factors associated with the management system, host, and environment. These include the age, sex, and breed of cattle, herd size and type, and agro ecology (Radostits *et al.*, 2007, Mcdermott *et al*., 2002, Gul, and Khan, 2007). Age has been stated as the intrinsic factor related to *Brucella* infection. A higher seroprevalence of *Brucella* organisms has been determined in adult cattle than in young cattle (Ashagrie *et al.*, 2011, Borba *et al.*, 2013). Sexually mature and pregnant cattle are more prone to being infected with *Brucella* than sexually immature cattle (Matope *et al.*, 2011). This is because the *Brucella* organism confers a response in the reproductive tract owing to the concentration of erythritol sugar, generated within the fetal tissues of cattle, which stimulates the growth of *Brucella* organisms (Radostits *et al.*, 2007). However, the higher prevalence of *Brucella* in adults has also been related to longer interaction with diseased cattle. This could also be vital in the herd, while not culling the positive cattle (Megersa *et al.*, 2011).

The effect of sex on the occurrence of *Brucella* infection in cattle has been stated previously (Munoz *et al.*, 2010) Female cattle are more likely than males to have *Brucella* infection (Talukder *et al.*, 2012). Although this is not easy to elucidate, it may be related to the biology of the *Brucella* organism and tropism to the fetal tissues (Radostits *et al.*, 2007). Because *Brucella* infection in males confers symptoms such as epididymitis and orchitis, the incidence in males may be lower than in females; as a result, they may be culled more quickly (Coelho *et al.*, 2013) . However, the absence of symptoms such as abortion or metritis in non-pregnant diseased females may also mean that there is a higher prevalence in females. Moreover, brucellosis becomes chronic in non-pregnant cattle. This has important epidemiological consequences as, after the initial immune response in cattle that are symptomless carriers, the antibodies disappear from the circulation, and it can be challenging to identify them with standard serological methods (OIE, 2009).

Herd size is another risk factor for Brucella infection, with the risk being highest in large herds (Ibrahim *et al.*, 2010, Muma *et al.*, 2007).The rise spread of brucellosis by interaction among members of the herd, the use of common grazing lands or inadequate cleaning and disinfection techniques on big farms (Reviriego *et al.*, 2000). The low incidence of Brucella infection in small herds may be related to herd and/or management (Coelho *et al.*, 2013). Thus, small herds often graze nearby pastures, allowing interactions with other herds to be controlled, or using communal methods (Center for Food Security & Public Health 2009). A small herd can be simply managed during delivery, and cattle are frequently removed from the herd throughout parturition. This is extremely important in the case of abortion, to prevent contamination of the pasture. In small herds, substitutions are typically made by relocating animals and economic trade is uncommon. Hence, the lower rate of cattle movement reduces the chances of disease transmission. In contrast, cattle movement in large herds is common, both for replacement and for trade, thus increasing the risk of Brucella infection (Claudia *et al.*, 2015) .

Herding several species within a herd has been characterized as a risk factor for brucellosis (Robi *et al* 2020, Megersa *et al.*, 2011) although there is no indication of the higher susceptibility of particular species to *Brucella* infection. As a result, the reason for the increased prevalence of brucellosis when various species mix is unclear, but it may be related to a higher probability of being infected with brucellosis owing to various sources of the disease. Brucella infection is seldom spread from small ruminants to cattle (Jackson *et al.* 2004). Nevertheless, the threat to cattle on farms that also keep small ruminants suggests that some cases of bovine brucellosis may have originated from small ruminants, because *B. melitensis* biovar 3 has been isolated from cattle milk (Smits *et al.*, 2013).

Dairy cattle have a far greater probability of not only acquiring *Brucella* infection but also spreading it more rapidly than beef cattle. Cattle housed in small areas come into close contact with each other during feeding and milking (Sammartino *et al.*, 2006). Dairy cattle are exposed to additional stress on farms, causing conditions that are more conducive to *Brucella* infection (Kataria *et al.*, 2010). Cattle purchase is considered as a risk for brucellosis and will increase the chance of introducing diseased cattle into the herd (Matope *et al*., 2011). Most infectious disease in previously brucellosis-free herds starts with the purchase of diseased cattle from unidentified sources (Islam *et al.*, 2013).

The effect of agro ecology is also recognized as a *Brucella* infection risk factor, with a higher prevalence in dry areas (Silva *et al.,* 2000). Because of a shortage of pasture in dry areas, cattle are put out to pasture over large areas, indicating uncontrolled cattle to cattle interaction with the potential risk of transmission. In addition, transmission through aerosol inhalation of contaminated dust from fetal discharges or abortions is likely (Claudia *et al.,* 2015). Large herd sizes are likely to be related to intensive management systems, which are generally tougher to manage and permit closer interactions between cattle and their surroundings, which can increase the probability of exposure to *Brucella* organisms (Talafhah *et al.,* 2003). In addition, the stressful conditions of an intensive production system may make cattle more prone to infections. However, an extensive production system may also increase the risk of *Brucella* infection. This may be related to the management of abortions, identification of diseased cattle, and interactions among cattle (Coelho *et al.,* 2013).

Since an extensive system implies rearing many cattle over a large area and sharing common pastures, the contamination of pastures with discharges from the reproductive tract may lead to brucellosis in the herds. Risk factors relating to farming and ecological conditions that affect the spread of brucellosis include giving birth, breeding in semi-dark settings, confined areas, and high cattle populations (Talafhah *et al.,* 2003).

The intensive system is another risk factor for brucellosis. This may be related to airborne transmission of disease-causing bacteria indoors (Claudia *et al.,* 2015). Similarly, the seasons have an influence on animal husbandry and nutrition, principally in pastoral areas (Reviriego *et al.,* 2000).

2.5.3. Status of Brucellosis in Ethiopia

Ethiopia, located in Eastern Africa, is predominantly an agrarian country with over 85% of its population engaged in agricultural activity. Since the first report of brucellosis in the 1970s in Ethiopia, the disease has been noted as one of the important livestock diseases in the country (Ibrahim *et al.,* 2010; Kebede *et al.,* 2008). A large number of studies on bovine have been reporting individual brucellosis sero-prevalence ranging from 1.1% to 22.6% in intensive livestock management systems (Kebede *et al.,* 2008) and 0.05% to 15.2% in extensive management systems (Megersa *et al.,* 2011; Degefa *et al*., 2011).

Most brucellosis study report for highland agro-ecology was concentrated at urban and pre urban dairy farms. According to different authors herd level sero-prevalence ranged between 2.9% and 45.9%. The highest sero-prevalence (50%) was documented using ELISA in Dididtuyura Ranch (Alem *et al.,* 2002). 2.91% in indigenous Borena breed cows in Borena zone in Southern Ethiopia (Benti and Zewdie, 2014).

In South Eastern Ethiopian pastoral zones of the Somali and Oromia regional state herds, sero-prevalence per species which were 1.4% were reported (Gumi *et al*., 2013). Another study from Addis Ababa, Ethiopia found a prevalence of 10% (Eshetu *et al.,* 2005). A study conducted on smallholder farmers of central Ethiopia (Wuchale Jida district) reported a prevalence rate of 11% (Kebede *et al.,* 2008).

The overall seroprevalence of bovine brucellosis in pastoral and agro pastoral regions of East Showa Zone, Oromia Regional State, was 11.2% by the Rose Bengal Plate Test (RBPT). This report was within the range 10 to 15% that was estimated for any assumed brucellosis seroprevalence for East Africa (Mangen *et al.,* 2002). According to study of bovine Brucellosis in cattle under traditional production system in North- West Ethiopia Benishangul-gumuz, among the 1,152 cattle screened for *B. abortus* antibodies, 14 (1.2%) tested positive by RBPT of these, 11 animals (79 %;) were confirmed positive by complement fixation test (CFT), giving an apparent seroprevalence of 1.0% in the study area (Adugna and Alga, 2013; Sintayehu *et al.,* 2015).

2.6. Pathogenesis

The *Brucella* spp to cause disease requires a few critical steps during infection. *Brucella* spp can invade epithelial cells of the host, allowing infection through mucosal surfaces: M- cells in the intestine have been identified as a portal of entry for *Brucella* spp. usually through the digestive or respiratory tract, they are capable of surviving intra cellular within phagocytic or non-phagocytic host cells and replicate within the phagocyte ,then release to circulation and colonization of the bacteria in multiple tissues ,like lymphoid tissues, mammary gland and reproductive tract (CarvalhoNeta *et al.,* 2010).

Invading *Brucella* usually localize in the lymph nodes, draining the invasion site, resulting in hyperplasia of lymphoid and reticulo endothelial tissue and the infiltration of inflammatory cells. Survival of the first line of defense by the bacteria results in local infection and the escape from the lymph nodes in to the blood. During bacteriamic phase, bones, joints, eyes and brain can be infected, but the bacteria are most frequently isolated from supra-mammary lymph nodes, milk, iliac lymph nodes, spleen and uterus. In bulls, the predilection sites for infection are also the reproductive organs and the associated lymph nodes. During the acute phase of infection, the semen contains large number of *Brucella* but as the infection becomes chronic, the number of *Brucella* excreted decreases. However, it may also continue to be excreted for years or just become intermittent (Acha and B. Szyfers, 2001).

2.7. Clinical Signs

Primary clinical manifestations of brucellosis among livestock are related to the reproductive tract in highly susceptible non vaccinated pregnant cattle. The principal symptoms of *Brucell*a infection are abortion in the last stage of pregnancy in female cattle and orchitis and bursitis in male (Folitse *et al.,* 2014).

Females usually abort only once, presumably due to acquired immunity. In general, abortion with retention of the placenta and the resultant metritis may cause prolonged calving interval and permanent infertility. In cattle, *B abortus* causes abortions, stillbirths and weak calves. The placenta may be retained and lactation may be decreased. Epididymitis, seminal vesiculitis, orchitis and testicular abscesses are sometimes seen in bulls. Infertility occurs occasionally in both sexes, due to metritis or

orchitis/epididymitis. Hygromas, particularly on the leg joints, are a common symptom in some tropical countries. Arthritis can develop after long-term infections. Systemic signs usually do not occur in uncomplicated infections and deaths are rare except in the fetus or newborn. Infections in non-pregnant females are usually asymptomatic, but pregnant adult females infected with *B abortus* develop placentitis, which normally causes abortion between the fifth and ninth month of pregnancy. Even in the absence of abortion, there is heavy shedding of bacteria through the placenta, fetal fluids and vaginal exudates. The mammary gland and regional lymph nodes can also be infected and bacteria can be excreted in milk (OIE, 2010).

2.8. Diagnosis

2.8.1. Bacteriological diagnosis

Isolation of the organism is considered the gold standard diagnostic method for brucellosis since it is specific and allows biotyping of the isolate, particularly if the direct examination supported by other tests. Occasionally, bacteria can be recovered from the cerebrospinal fluid, urine or tissues. *Brucella* spp can be isolated on a variety of plain media, or selective media such as Farrell's medium. Samples for *Brucella* spp isolation from cattle include fetal membranes, particularly the placental cotyledons where the number of organisms tends to be very high. In addition, fetal organs such as the lungs, bronchial lymph nodes, spleen and liver, as well as fetal gastric contents, milk, vaginal secretions and semen are samples of choice for isolation (Lage *et al.*, 2008; Poester *et al.*, 2006).

Vaginal secretions should be sampled after abortion or parturition, preferably using a swab with transporter medium, allowing isolation of the organism up to six weeks post parturition or abortion (Poester *et al.*, 2010). Milk samples should be a pool from all four mammary glands. Non- pasteurized dairy products can also be sampled for isolation (Poester *et al.*, 2010; Lage *et al.*, 2008). Samples of choice in slaughter houses include mammary, iliac, pharyngeal, parotids cervical lymph nodes, and spleen. Samples must be immediately sent to the laboratory, preferentially frozen at -20°C, and they must be identified as suspect of *Brucella* spp. infection (Poester *et al.*, 2010).

Vaginal swabs, semen and seminal fluid have low numbers of viable organisms, and therefore isolation is more difficult, often resulting in false negative results. Enrichment media containing selected antibiotics can improve the sensitivity in these cases (De Miguel *et al.*, 2011; Her *et al.*, 2010).

2.8.2. Serological Tests

Several commercial serological tests are available for humans and animals (WHO, 2006) The Rose Bengal test (RBT) has been recommended as a suitable screening test at the national or local level for diagnosis of brucellosis in animals (WHO, 2006). Enzyme-linked immunoassays (ELISA) and the fluorescent polarization assay (FPA) have recently been added as prescribed tests. They are simple, but robust, tests which can be conducted with a minimum of equipment and are therefore also suitable for smaller laboratories. Further serological tests (e.g. the Combs' test, the serum or plate agglutination test and the immune-capture test) are available, and have specific advantages and disadvantages (OIE, 2008).

Rose Bengal Plate Test (RBPT): Often used as a rapid screening test; the sensitivity is very high (>99%) but the specificity is disappointingly as low as 68.8%. RBPT is a rapid, slide-type agglutination assay performed on serum. The general principle of this test is the agglutination of serum antibodies with Rose Bengal dye-stained *B. abortus* whole cells buffered at a pH of 3.65 to inhibit nonspecific agglutinins. Due to its simplicity and low cost, it is the most common test used for brucellosis screening purposes, especially in laboratories with limited resources. However, this is of value as a screening test in high risk rural areas where it is not always possible to perform the other tests (WHO, 2006; Mantur *et al.*, 2006).

2.8.3. Milk Ring Test (MRT)

The MRT has been explained as a satisfactory and inexpensive test for the surveillance of dairy herds for brucellosis. The MRT is easy, simple and takes low time to perform (Radostits *et al.*, 2000). When positive test result is obtained, all animals contributing milk should be tested for seroprevalence. It detects lacteal anti *Brucella* IgM and IgA bound to milk fat globules (OIE, 2004). Milk that contains low concentration of lacteal IgM and IgA or that lacks the fat-clustering factors can give false negative results. Because lacteal antibodies rapidly decline after abortion or parturition, MRT, using 1ml milk, to detect *Brucella* antibodies in individual animal or in tank milk is strongly discouraged. In large herds (>100 lactating animals), the sensitivity of the test becomes less reliable (Radostits *et al.*, 2000). False positive reactions may also occur in animals vaccinated 4 months prior to testing and in samples containing abnormal milk (colostrum) or in case of mastitis (OIE, 2004).

2.8.4. Polymerase Chain Reaction

Polymerase chain reaction (PCR) assays can be used to detect *Brucella* DNA in pure cultures and in clinical specimens, i.e. serum, whole-blood and urine samples, various tissues, cerebrospinal, synovial or pleural fluid, and pus (Colmenero *et al.*, 2010 Direct detection of *Brucella* DNA in brucellosis patients is a challenge because of the small number of bacteria present in clinical samples and inhibitory effects arising from matrix components. Basic sample preparation methods should minimize inhibitory effects and concentrate the bacterial DNA template (Queipo-Ortuño *et al.*, 2008).

2.9. Treatment

Due to the intracellular localization of Brucella and its ability to adapt to the environmental conditions encountered in its replicative niche e.g. macrophage (Seleem et al., 2008). Treatment of domestic animals with antibiotics is not usually successful. Even though, treatment failure and relapse rates are also high in humans, treatment depend on the drug combination of doxycycline with streptomycin which is currently the best therapeutic option with less side effects and less relapses, especially in cases of acute and

localized forms of brucellosis (Seleem *et al.*, 2009). A combination of doxycycline treatment (6 weeks duration) with parentally administered gentamicin (5 mg/kg) for 7 days is also considered an acceptable alternate regimen (Glynn and Lynn, 2008).

2.10. Prevention and Control

In Ethiopia the source of human brucellosis is direct or indirect exposure to infected animals or their products, prevention must focus on various strategies that will mitigate infection risks. There have been no national programs proposed for prevention and control of brucellosis in Ethiopia. Rather than teaching or giving awareness to population similarly, at regional levels, no strategy is in place to control brucellosis. This is largely a result of lack of facilities and budget to run such program. Moreover, many responsible bodies may not recognize the significance of brucellosis given the contradictory and sometimes low prevalence data. However, now, it is crucial to define geographical extent of the problem and then allocate resources and funds to initiate prevention and control strategies in Ethiopia and other countries with similar economic situations (Yohannes *et al.*, 2013).

Based on Prevalence In areas where the disease is less prevalent (for example livestock seroprevalence of less than 1%), cull policy with compensation may be recommend. For areas with high and moderate prevalence (>5%) under well-organized farming systems, we may recommend test and segregation policy by which animals with brucellosis will be isolated and products consumed after pasteurization, with animals being disposed of properly at the end of their productive live (Yohannes *et al.* 2013).

2.10.1. Vaccination

Brucellosis is a bacterial zoonosis caused by *Brucella* spp. which can lead to heavy economic losses and severe human diseases. Thus, controlling brucellosis is very important. Vaccine against brucellosis in animals plays a crucial role in the management of the disease in animals as well as in humans. The most common *Brucella* spp., viz., strain 19, RB51 and Rev1 are widely used as vaccine strains to protect against Brucella infection and related abortions in livestock. However, their use in other susceptible animals needs further studies and requires the development of novel effective vaccines in near future (Masjedian Jezi *et al*., 2019).

B. abortus strains 19 and RB51 are very efficient and common vaccines being used against bovine brucellosis. The best vaccine for the prevention of brucellosis in goats and sheep presently is *B. melitensis* strain Rev1 (Benkirane *et al*., 2014). *B. abortus* vaccine should also be able to give cross-protection against *B. melitensis*. Aninfluenza viral vector- *B. abortus* vaccine completely protected against abortions in pregnant heifers. An excellent level of cross-protection (90-100%) in the heifers, their calves or fetuses was observed upon challenge with *B. melitensis* 16 M. Influenza viral vector-B. abortus vaccine provided equivalent protection when compared with *B. abortus* S19 vaccine (Tabynov *et al*., 2015). These two vaccines were found to provide high degree of immunity against *B. melitensis* 16 M infection (Tabynov *et al*., 2015).

The economic analysis showed that a vaccination program covering the vaccination with S19 vaccine in 90% of the replacement heifers of 3–8 months of age provides excellent economic returns in a brucellosis vaccination program in bovines (Alves *et al,*. 2015). *B. abortus* S19 vaccine, an intermediate rough strain, was found to be safe, immunogenic and also has the potential to be used as strategy vaccine for prevention and control of bovine brucellosis (Lalsiamthara *et al,*. 2015).

2.10.2. Appropriate Hygienic Measures

Good hygiene and protective clothing/equipment are very important in preventing occupational exposure. Precautions should be taken to avoid contamination of the skin, as well as inhalation or accidental ingestion of organisms when assisting at a birth, performing a necropsy, or butchering an animal for consumption (Pappas *et al.*, 2006). .

Care should be taken when handling an aborted fetus or its membranes and fluids. Risky agricultural practices such as crushing the umbilical cord of newborn livestock with the teeth or skinning aborted fetuses should be avoided. Application of farm bio-safety measures: Implementation of measures to reduce the risk of infection through personal hygiene, adoption of safe working practices, protection of the environment and food hygiene (Pappas *et al.*, 2006).

Under appropriate conditions, *Brucella* organisms can survive in the environment for prolonged periods. The proper handling and burying of abortion materials to prevent contamination of water sources and pasture is of paramount importance. Furthermore, the common practice of feeding abortion materials to dogs should be avoided as this increases the risk of transmission to other animals. It is imperative to education on risks for infection to these populations to influence behavioral practices that will reduce risks of transmission (Walker, 1999).

2.10.3. Pasteurization

Brucella abortus is inactivated by pasteurization and Pasteurization of dairy products is an important safety measure to prevent Human brucellosis where this disease is endemic. Unpasteurized dairy products and raw or undercooked animal products (including bone marrow) should not be consumed. Main source of transmission of *B. abortus* to human is through consumption of unpasteurized or raw milk or milk products including butter, whey, cheese, yogurt, ice-cream, etc. (Dhanashekar *et al.*, 2012).

2.11. Disease spectrum in humans

Human brucellosis is primarily caused by *B. melitensis* globally. *B. abortus*, *B. suis* and *B. canis* also cause human brucellosis worldwide (Khurana *et al*., 2021). Sheep, goats and their products are major sources of *B. melitensis* infection in human beings (Corbel, 2006). Main source of transmission of *B. abortus* to human is through consumption of unpasteurized or raw milk or milk products including butter, whey, cheese, yogurt, ice-cream, etc. (Dhanashekar et al. 2012).

2.12. Public Health and Economic Significance

Brucellosis, particularly *B. melitensisis* is thought to be one of the most prevalent re-emerging zoonotic diseases globally with an estimated incidence of more than 50,000 human cases per year (Gwida *et al.* 2010). The zoonotic importance of brucellosis as zoonosis is increasing owing to tremendous increase in global trade in animal products, rapid deforestation, unplanned and unsustainable development, urbanization, intensive farming, having migratory/nomadic animal husbandry and increased international tours and travel (Memish and Balkhy, 2004; Bayeleyegn, 2007).

Even the exhaustive and advanced surveillance and control measures have not been able to reduce the prevalence of brucellosis in most of the developing countries due to poor hygiene, lack of sanitation, poverty, lack of proper administration and political will (Pappas *et al.*, 2006). Brucellosis badly affects livestock welfare and economy. The collective economic losses are the cumulative effect of reduction in the production of milk, abortions, losses of newborn calves resulting from abortions and stillbirths, culling of brucellosis affected animals, hindrance in export and trade of animals, loss of human effort in terms of man-days wasted, veterinary and medical expenses, administrative and governmental expenses on research and control programs (Georgios *et al.*, 2005).

3. Conclusions And Recommendations

Bovine Brucellosis is a bacterial zoonotic disease, which has both public health and economic importance. This disease can be transmitted from infected animal to healthy one through fetal discharges, contaminated feed and water, licking of vaginal discharges or secretions or newly born infected calves in animals. In human, brucellosis can be transmitted via consumption of unpasteurized milk and cheese, direct contact with infected animal and handling of specimen that contaminated with *Brucella* species. Brucellosis is a worldwide disease both in developed and developing country even though; this disease is eradicated in developed countries with vaccination program and screening method of livestock, this disease poses serious problem in developing countries mainly African countries including Ethiopia. This disease causes abortion, delayed heat, loss of calve, infertility, reduce milk production and meat production and still birth in cattle due to absence of regular screening method and vaccination programs in most developing country mostly in sub Saharan Africa. Since brucellosis is a leading zoonotic bacterial disease that affects human health and economy due to trade ban attention must be given to control or prevent this disease in developing country through routinely screening method, regular vaccination and tackling the mode of transmission of this disease may reduce risk posed. In addition, at regional, national and international level strict regulation should be devised to control or prevent as well as to eradicate this disease. Therefore, I recommend:-

- ❖ Effective control and prevention strategies should be formulated and applied.
- ❖ Public education on the source of infection and transmission of the disease as well as awareness creation should be applied.
- ❖ Implementation of control and prevention measures of brucellosis in animals, to stop human infections.
- ❖ Good hygiene and protective clothing/equipment are very important in preventing occupational exposure.
- ❖ Avoid eating or drinking unpasteurized milk, and milk product

LIST OF ABBREVIATIONS

B. abortus	Brucella *abortus*
B. canis	Brucella *canis*
B. melitensis	Brucella *melitensis*
B. microti	Brucella *microti*
B. neotome	Brucella *neotome*
B. ovis	Brucella *ovis*
B. suis	Brucella *suis*
B. vulpis	Brucella *vulpis*
Brucella spp	Brucella Species
CFT	complement fixation test
CSA	Central statistical agency
DNA	*Deoxyribo Nucleic Acid*
ELISA	Enzyme-Linked *Immunosorbent* Assay
FAO	Food and Agricultural Organization
FPA	Fluorescent polarization assay
IgA	Immunoglobulin A
IgM	Immunoglobulin M
Kg	Kilogram
Mg	Milligram
MRT	Milk Ring Test
WOAH	World organization for animal health
PCR	Polymerase chain reaction
PH	Potential of Hydrogen
RBPT	Rose Bengal Plate test
RBT	Rose Bengal test
WHO	World Health Organization

4. References

1. Abd El-Wahab, E.W., Hegazy, Y., El-Tras, W.F., Mikeal, A., Kapaby, A.F., Abdelfatah, M., Bruce, M. and Eltholth, M.M., 2019. Knowledge, attitudes and practices (KAPs) and risk factors of brucellosis at the human-animal interface in the Nile Delta, Egypt. *bioRxiv*, p.607655.
2. Acha, P. N., & Szyfres, B.(2001). Zoonoses and Communicable Diseases Common to Man and Animals: Volume 3: Parasitoses (Vol. 580). Pan American HealthOrg.
3. Adel El- Gohary, M. B, Abd-El Haleem, M, El-Gohary, F., & El-Deen, M. M. In vitro evaluation of commonly, used disinfectants and antiseptics in veterinary practice against Brucella abortus.
4. Adugna, K. E., Agga, G. E., & Zewde, G. J. R. S. T. (2013). Seroepidemiological survey of bovine brucellosis in cattle under a traditional production system in western Ethiopia. Rev Sci Tech, 32(3), 765-73.
5. Alem,W., & Solomon, G (2002). Aretrospective sero-epidemiology study of Bovine Brucellosis in different Production Systems in Ethiopia. In Proceeding of 16th Annual

Conference, Addis Ababa, Ethiopia (pp. 53-57).
6. Al-Talafhah, A. H., Lafi, S. Q & Al-Tarazi. Y(2003). Epidemiology of Ovine Brucellosis in Awassi sheep,in Northern Jordan. Preventive Veterinary Medicine, 60(4), 297-306.
7. Alves, A. J. S., Rocha, F., Amaku, M., Ferreira, F., Telles, E. O., Grisi Filho, J. H. D. H. & Dias, R. A. (2015). Economic analysis of vaccination to control bovine brucellosis in the States of Sao Paulo and Mato Grosso, Brazil. Preventive Veterinary Medicine, 118(4), 351-358.
8. Asgedom, H, Damena, D., & Duguma, R (2016) . Seroprevalence of bovine brucellosis and associated risk factors in and around Alage district, Ethiopia. SpringerPlus, 5, 1-8.
9. Ashagrie, T., Deneke, Y., & Tolosa, T. (2011). Seroprevalence of caprine brucellosis and associated risk factors in South Omo Zone of Southern Ethiopia. African Journal of Microbiology Research, 5(13), 1682-1
10. Asmare, K., Asfaw, Y., Gelaye, E., & Ayelet, G(2010). Brucellosis in extensive management system of Zebu cattle in Sidama Zone, Southern Ethiopia. African Journal of Agricultural Research, 5(3), 257-263.476.
11. Asmare, K., Sibhat, B., Molla, W., Ayelet, G., Shiferaw, J., Martin, A. D., & Godfroid, J. (2013). The status of bovine brucellosis in Ethiopia with special emphasis on exotic and cross bred cattle in dairy and breeding farms. Acta tropica, 126(3), 186-192.
12. Aworh, M. K., Okolocha, E., Kwaga, J., Fasina, F., Lazaru s, D., Suleman, I, & Nsubuga, P. (2013). Human brucellosis: seroprevalence and associated exposure factors among abattoir workers in Abuja, Nigeria-2011. The Pan African Medical Journal, 16.
13. Bayeleyegn, M.(2007). Advanced veterinary public health lecture note. FVM, AAU, Debre-Zeit, Ethiopia, 2007, 10-32.
14. Benti, A. D., & Zewdie, W.(2014). Major reproductive health problems of indigenous Borena cows in Ethiopia. Journal of Advanced Veterinary and animal research1 (4),182-188.
15. Borba, M. R., Stevenson, M. A., Goncalves, V. S. P., Neto, J. F., Ferreira, F., Amaku, M.,& Dias, R. A. (2013). Prevalence and risk-mapping of bovine brucellosis in Maranhão State, Brazil. Preventive veterinary medicine, 110(2), 169-176.
16. CarvalhoNeta, A. V., J.P. Mol, M.N. Xavier,T.A. Paixao, A.P. Lage and R.L. Santos,2010. Pathogenesis of bovine brucellosis.Veterinary Journal, 184: 146–155.
17. Center for Food Security & Public Health. Bovine Brucellosis: Brucella Abortus. Ames, Iowa: College of Veterinary Medicine, Iowa State University; 2009
18. CFSPH. 2018a. Brucellosis: Brucella suis. http://www.cfsph.iastate.edu/Factsheets/pdfs/ brucellosis_suis.pdf. Accessed 20th April 2020.
19. Coelho, A. C., Díez, J. G., & Coelho, A. M. (2015). Risk factors for Brucella spp. in domestic and wild animals. In Updates on brucellosis. London, UK: IntechOpen.
20. Colmenero, J. D., Morata, P., Ruiz-Mesa, J. D., Bautista, D., Bermúdez, P., Bravo, M. J., & Queipo-Ortuño, M. I. (2010). Multiplex real-time polymerase chain reaction: a practical approach for rapid diagnosis of tuberculous and brucellar vertebral osteomyelitis. Spine, 35(24), E1392-E1396.
21. Constable, P. D., Hinchcliff, K. W., Done, S. H., & Grünberg, W. (2017). Infectious diseases primarily affecting the reproductive system. Veterinary medicine: A textbook of the diseases of cattle, horses, sheep, pigs, and goats, 1761-1784.
22. Corbel, M. J. (2006). Brucellosis in humans and animals. World Health Organization.
23. CSA(Central Statical Agency) (2020) Agricultuiral Sample Survey. ,.Rep.ort on Livestkock an d Livesokck Characteritics.Volume II Stastiocal Bulletionm587.ddioa Ababa, 90 -11.
24. CSA. Central Statistical Agency. Report on livestock and livestock characteristics. Stat Bulettin. 2018;II:587.
25. De Miguel, M. J., Marín, C. M., Muñoz, P. M., Dieste, L., Grilló, M. J., & Blasco, J. M. (2011). Development of a selective culture medium for primary isolation of the main Brucella species. Journal of clinical microbiology, 49(4), 1458-1463.
26. Degefa, T., Duressa, A., & Duguma, R. (2011). Brucellosis and some reproductive problems of indigenous Arsi cattle in selected Arsi zones of Oromia regional state, Ethiopia. Global Veterinaria, 7(1), 45-53.
27. Dereje, T., Benti, D., Feyisa, B., & Abiy, G. (2018). Review of common causes of abortion in dairy cattle in Ethiopia. Journal of Veterinary Medicine and Animal Health, 10(1), 1-13.
28. Dhanashekar, R., Akkinepalli, S., & Nellutla, A. (2012). Milk-borne infections. An analysis of their potential effect on the milk industry. Germs, 2(3), 101.
29. El-Sayed, A., & Awad, W. (2018). Brucellosis: Evolution and expected comeback. International journal of veterinary science and medicine, 6, S31-S35.
30. Eshetu, Y., Kassahun, J., Abebe, P., Beyene, M., Zewdie, B., & Bekele, A. (2005). Seroprevalence study of brucellosis on dairy cattle in Addis Ababa, Ethiopia. Bulletin of Animal health and Production in Africa, 53(3), 211-214.
31. FAO. (2010). Brucella melitensis in Eurasia and the Middle East. In FAO Technical Meeting in Collaboration with WHO and OIE.
32. Folitse, R. D., Boi-Kikimoto, B. B., Emikpe, B. O., & Atawalna, J. (2014). The prevalence of Bovine tuberculosis and brucellosis in cattle from selected herds in Dormaa and Kintampo Districts, Brong Ahafo region, Ghana. Archives of clinical Microbiology, 5(2).
33. Foster, G., Osterman, B. S., Godfroid, J., Jacques, I., & Cloeckaert, A. (2007). Brucella ceti sp. nov. and Brucella pinnipedialis sp. nov. for Brucella strains with cetaceans and seals as their preferred hosts. International journal of systematic and evolutionary microbiology, 57(11), 2688-2693.
34. Franc, K. A., Krecek, R. C., Häsler, B. N., & Arenas-Gamboa, A. M. (2018). Brucellosis remains a neglected disease in the developing world: a call for interdisciplinary action. BMC public health, 18(1), 1-9.
35. Fugier, E., Pappas, G., & Gorvel, J. P. (2007). Virulence factors in brucellosis: implications for aetiopathogenesis and treatment. Expert Reviews in Molecular Medicine, 9(35), 1-10.
36. Genene, R., Desalew, M., Yamuah, L., Hiwot, T., Teshome, G., Asfawesen, G., ... & Smits, H. L. (2009). Human brucellosis in traditional pastoral communities in

Ethiopia. International Journal of Tropical Medicine, 4(2), 59-64.
37. Glynn, M. K., & Lynn, T. V. (2008). Zoonosis update. AVMA, 233, 900-908.
38. Godfroid, J. (2018). Brucella spp. at the wildlife-livestock interface: an evolutionary trajectory through a livestock-to-wildlife "host jump"?. Veterinary sciences, 5(3), 81.
39. Godfroid, J., Scholz, H. C., Barbier, T., Nicolas, C., Wattiau, P., Fretin, D., ... & Letesson, J. J. (2011). Brucellosis at the animal/ecosystem/human interface at the beginning of the 21st century. Preventive veterinary medicine, 102(2), 118-131.
40. Gul, S. T., & Khan, A. (2007). Epidemiology and epizootology of brucellosis: A review. Pakistan veterinary journal, 27(3), 145.
41. Gumi, B., Firdessa, R., Yamuah, L., Sori, T., Tolosa, T., Aseffa, A., ... & Schelling, E. (2013). Seroprevalence of brucellosis and Q-fever in southeast Ethiopian pastoral livestock. Journal of veterinary science & medical diagnosis, 2(1).
42. Gwida, M., Al Dahouk, S., Melzer, F., Rösler, U., Neubauer, H., & Tomaso, H. (2010). Brucellosis–regionally emerging zoonotic disease?. Croatian medical journal, 51(4), 289-295.
43. Gwida, M., El-Gohary, A., Melzer, F., Khan, I., Rösler, U., & Neubauer, H. (2012). Brucellosis in camels. Research in veterinary science, 92(3), 351-355.
44. Hayoun MA, Smith ME, Shorman M. 2020. Brucellosis. In: StatPearls [Internet]. Treasure Island (FL): Stat Pearls Publishing. Available from: https://www.ncbi.nlm.nih.gov/books/NBK441831/
45. Her, M., Cho, D. H., Kang, S. I., Cho, Y. S., Hwang, I. Y., Bae, Y. C., ... & Yoo, H. (2010). The development of a selective medium for the Brucella abortus strains and its comparison with the currently recommended and used medium. Diagnostic microbiology and infectious disease, 67(1), 15-21.
46. Hirsh, D. C., & Zee, Y. C. (1999). Veterinary Microbiology. USA, Black well science. Inc. Pp, 115-249.
47. Ibrahim, N., Belihu, K., Lobago, F., & Bekana, M. (2010). Sero-prevalence of bovine brucellosis and its risk factors in Jimma zone of Oromia Region, South-western Ethiopia. Tropical Animal Health and Production, 42, 35-40.
48. Islam, M. A., Khatun, M. M., Werre, S. R., Sriranganathan, N., & Boyle, S. M. (2013). A review of Brucella seroprevalence among humans and animals in Bangladesh with special emphasis on epidemiology, risk factors and control opportunities. Veterinary Microbiology, 166(3-4), 317-326.
49. Jackson, R., Pite, L., Ward, D., Kennard, R., Stack, J., Domi, X., & Dedushaj, L. (2004). Survey of the seroprevalence of brucellosis in ruminants in Kosovo. Veterinary record, 154(24), 747-751.
50. Jergefa, T., Kelay, B., Bekana, M., Teshale, S., Gustafson, H., & Kindahl, H. (2009). Epidemiological study of bovine brucellosis in three agro-ecological areas of central Oromiya, Ethiopia. Revue scientifique et technique, 28(3), 933.
51. Kahn, C. M. (2005). The Merck Veterinary Manual 9th ed. White house station, NJ, USA, Merck & CO. INC, pp2201-2206.
52. Kataria, N., Kataria, A. K., Maan, R., & Gahlot, A. K. (2010). Evaluation of oxidative stress in brucella infected cows. Journal of Stress Physiology & Biochemistry, 6(2), 19-25.
53. Kebede, T., Ejeta, G., & Ameni, G. (2008). Seroprevalence of bovine brucellosis in smallholder farms in central Ethiopia (Wuchale-Jida district). Revue de Médecine Vétérinaire, 159(1), 3.
54. Khurana, S. K., Sehrawat, A., Tiwari, R., Prasad, M., Gulati, B., Shabbir, M. Z., & Chaicumpa, W. (2021). Bovine brucellosis–a comprehensive review. Veterinary Quarterly, 41(1), 61-88.
55. Khurana, S. K., Sehrawat, A., Tiwari, R., Prasad, M., Gulati, B., Shabbir, M. Z., ... & Chaicumpa, W. (2021). Bovine brucellosis–a comprehensive review. Veterinary Quarterly, 41(1), 61-88.
56. LAGE, A. P., POESTER, F. P., PAIXÃO, T. A., SILVA, T. M., XAVIER, M. N., MINHARRO, S., ... & SANTOS, R. L. (2008). Brucelose bovina: uma atualização. Revista brasileira de reprodução animal, 32(3), 202-212.
57. Lalsiamthara, J., Gogia, N., Goswami, T. K., Singh, R. K., & Chaudhuri, P. (2015). Intermediate rough Brucella abortus S19Δper mutant is DIVA enable, safe to pregnant guinea pigs and confers protection to mice. Vaccine, 33(22), 2577-2583.
58. Livestock, C. S. A. (2017). Livestock characteristics, agricultural sample survey. Addis Ababa, Ethiopia. Statistical Bulletin, 2(583), 9-13.
59. Mangen, M. J., Otte, J., Pfeiffer, D., & Chilonda, P. (2002). Bovine brucellosis in sub-Saharan Africa: estimation of sero-prevalence and impact on meat and milk offtake potential. FAO Livestock policy discussion paper, 8, 1-58.
60. Mantur, B. G., Biradar, M. S., Bidri, R. C., Mulimani, M. S., K, V., Kariholu, P., ... & Mangalgi, S. S. (2006). Protean clinical manifestations and diagnostic challenges of human brucellosis in adults: 16 years' experience in an endemic area. Journal of medical microbiology, 55(7), 897-903.
61. Masjedian Jezi F, Razavi S, Mirnejad R, Zamani K. 2019. Immunogenic and protective antigens of Brucella as vaccine candidates. Comp Immunol Microbiol Infect Dis. 65:29–36.
62. Matope, G., Bhebhe, E., Muma, J. B., Oloya, J., Madekurozwa, R. L., Lund, A., & Skjerve, E. (2011). Seroprevalence of brucellosis and its associated risk factors in cattle from smallholder dairy farms in Zimbabwe. Tropical animal health and production, 43, 975-982.
63. McDermott, J. J., & Arimi, S. M. (2002). Brucellosis in sub-Saharan Africa: epidemiology, control and impact. Veterinary microbiology, 90(1-4), 111-134.
64. Megersa, B., Biffa, D., Abunna, F., Regassa, A., Godfroid, J., & Skjerve, E. (2011). Seroprevalence of brucellosis and its contribution to abortion in cattle, camel, and goat kept under pastoral management in Borana, Ethiopia. Tropical animal health and production, 43, 651-656.
65. Megersa, B., Biffa, D., Niguse, F., Rufael, T., Asmare, K., & Skjerve, E. (2011). Cattle brucellosis in traditional livestock husbandry practice in Southern and Eastern Ethiopia, and its zoonotic implication. Acta Veterinaria Scandinavica, 53(1), 1-8.
66. Mekonnen, H., Kalayou, S., & Kyule, M. (2010). Serological survey of bovine brucellosis in barka and arado breeds (Bos indicus) of Western Tigray, Ethiopia. Preventive Veterinary

Medicine, 94(1-2), 28-35.
67. Memish, Z. A., & Balkhy, H. H. (2004). Brucellosis and international travel. Journal of travel medicine, 11(1), 49-55.
68. Memish, Z. A., & Mah, M. W. (2001). Brucellosis in laboratory workers at a Saudi Arabian hospital. American journal of infection control, 29(1), 48-52.
69. Microbiology Research, 7(14), 1150-1157.
70. Minda, A. G., Gobena, A., Getachew, T., Angella, A., & Gezahegne, M. K. (2016). Seropositivity and risk factors for Brucella in dairy cows in Asella and Bishoftu towns, Oromia Regional State, Ethiopia. African Journal of Microbiology Research, 10(7), 203-213.
71. Molla, B. (1989). Seroepidemiological survey of bovine Brucellosis in Arsi region. DVM thesis, Faculty of Veterinary Medicine, Addis Ababa University, Debre Zeit, Ethiopia.
72. Moreno, E. (2014). Retrospective and prospective perspectives on zoonotic brucellosis. Frontiers in microbiology, 5, 213.
73. Muma, J. B., Samui, K. L., Oloya, J., Munyeme, M., & Skjerve, E. (2007). Risk factors for brucellosis in indigenous cattle reared in livestock–wildlife interface areas of Zambia. Preventive veterinary medicine, 80(4), 306-317.
74. Muñoz, P. M., Boadella, M., Arnal, M., de Miguel, M. J., Revilla, M., Martínez, D., ... & Gortázar, C. (2010). Spatial distribution and risk factors of Brucellosis in Iberian wild ungulates. BMC infectious diseases, 10(1), 1-14.
75. OIE, A. (2008). Manual of diagnostic tests and vaccines for terrestrial animals. Office international des epizooties, Paris, France, 1092-1106.
76. OIE, A. (2008). Manual of diagnostic tests and vaccines for terrestrial animals. Office international des epizooties, Paris, France, 1092-1106.
77. OIE. (2016). Brucellosis (Brucella abortus, B. melitensis and B. suis)(infection with B. abortus, B. melitensis and B. suis). Manual of diagnostic tests and vaccines for terrestrial animals, 1-44.
78. OIE. 2016. Brucellosis (Brucella abortus, B. melitensis and B. suis) (Infection with Brucella abortus, B. melitensis and B. suis). http://www.oie.int/filead min/Home/fr/Healthstandards/tahm/2.01.04_BRUCELLOSIS .pdf Accessed 20th April 2020
79. OIE. Office international des épizooties. (2004). Manual of Diagnostic Tests and Vaccines for Terrestrial Animals: 2004. OIE. Office international des épizooties.
80. Padilla Poester, F., Nielsen, K., Ernesto Samartino, L., & Ling Yu, W. (2010). Diagnosis of brucellosis. The Open Veterinary Science Journal, 4(1).
81. Pal, M., Gizaw, F., Fekadu, G., Alemayehu, G., & Kandi, V. (2017). Public health and economic importance of bovine Brucellosis: an overview. Am J Epidemiol, 5(2), 27-34.
82. Pappas, G., Christou, L., Akritidis, N., & Tsianos, E. V. (2006). Quinolones for brucellosis: treating old diseases with new drugs. Clinical Microbiology and Infection, 12(9), 823-825.
83. Pappas, G., Solera, J., Akritidis, N., & Tsianos, E. (2005). New approaches to the antibiotic treatment of brucellosis. International journal of antimicrobial agents, 26(2), 101-105.
84. Plowright, W., Thomson, G. R., Neser, J. A., Coetzer, J. A. W., & Tustin, R. C. (1994). Infectious diseases of livestock, with special reference to southern Africa.
85. Poester, F. P., Gonçalves, V. S., Paixao, T. A., Santos, R. L., Olsen, S. C., Schurig, G. G., & Lage, A. P. (2006). Efficacy of strain RB51 vaccine in heifers against experimental brucellosis. Vaccine, 24(25), 5327-5334.
86. Queipo-Ortuño, M. I., De Dios Colmenero, J., Macias, M., Bravo, M. J., & Morata, P. (2008). Preparation of bacterial DNA template by boiling and effect of immunoglobulin G as an inhibitor in real-time PCR for serum samples from patients with brucellosis. Clinical and Vaccine Immunology, 15(2), 293-296.
87. Quinn, P. J., Carter, M., Dnnelly, C. J., & Leonard, C. F. (2002). Veterinary microbiology and microbial diseases. Great Britain.
88. Radostits, O. M., Gay, C. C., Blood, D. C., & Hinchcliff, K. W. (2000). A textbook of the diseases of cattle, sheep, pigs, goats and horses. Veterinary medicine, 9, 603-700.
89. Radostits, O. M., Gay, C. C., Hinchcliff, K. W., & Constable, P. D. (2007). Veterinary Medicine: a textbook of the disease of cattle, sheep, goats, pigs and horses. London: Balliere Tindall, 1223-1227.
90. Reviriego, F. J., Moreno, M. A., & Domınguez, L. (2000). Risk factors for brucellosis seroprevalence of sheep and goat flocks in Spain. Preventive Veterinary Medicine, 44(3-4), 167-173.
91. Robi, D. T. (2020). Epidemiology, economic and public health importance of small ruminant Brucella infection in Ethiopia. World J Vet Sci, 2(1), 1007.
92. Robi, D. T., & Gelalcha, B. D. (2020). Epidemiological investigation of brucellosis in breeding female cattle under the traditional production system of Jimma zone in Ethiopia. Veterinary and animal science, 9, 100117.
93. Robinson, A., & Production, A. (2003). Guidelines for coordinated human and animal brucellosis surveillance. Rome, Italy:: Fao.
94. Sammartino, L. E., Gil, A., & Elzer, P. (2006). Capacity building for surveillance and control of bovine and caprine brucellosis. FAO Animal Production and Health Proceedings-AO/WHO/OIE Expert and Technical Consultation. Rome, 7, 55.
95. Scholz, H. C., Banai, M., Cloeckaert, A., Kämpfer, P., & Whatmore, A. M. (2015). B rucella. Bergey's Manual of Systematics of Archaea and Bacteria, 1-38.
96. Scholz, H. C., Hubalek, Z., Sedlácek, I., Vergnaud, G., Tomaso, H., Al Dahouk, S., ... & Nockler, K. (2008). Brucella microti sp. nov., isolated from the common vole Microtus arvalis. International journal of systematic and evolutionary microbiology, 58(2), 375-382.
97. Scholz, H. C., Revilla-Fernández, S., Dahouk, S. A., Hammerl, J. A., Zygmunt, M. S., Cloeckaert, A., ... & Hofer, E. (2016). Brucella vulpis sp. nov., isolated from mandibular lymph nodes of red foxes (Vulpes vulpes). International journal of systematic and evolutionary microbiology, 66(5), 2090-2098.
98. Seleem, M. N., Boyle, S. M., & Sriranganathan, N. (2008). Brucella: a pathogen without classic virulence genes. Veterinary microbiology, 129(1-2), 1-14.
99. Seleem, M. N., Jain, N., Pothayee, N., Ranjan, A., Riffle, J. S.,

& Sriranganathan, N. (2009). Targeting Brucella melitensis with polymeric nanoparticles containing streptomycin and doxycycline. FEMS microbiology letters, 294(1), 24-31.
100. Silva, I., Dangolla, A., & Kulachelvy, K. (2000). Seroepidemiology of Brucella abortus infection in bovids in Sri Lanka. Preventive Veterinary Medicine, 46(1), 51-59.
101. Sintayehu, G., Melesse, B., Abayneh, D., Sintayehu, A., Melaku, S., Alehegne, W., ... & Abera, K. (2015). Epidemiological survey of brucellosis in sheep and goats in selected pastoral and agro-pastoral lowlands of Ethiopia. Revue scientifique et technique (International Office of Epizootics), 34(3), 881-893.
102. Smits, H. L. (2013). Brucellosis in pastoral and confined livestock: prevention and vaccination. Revue scientifique et technique (International Office of Epizootics), 32(1), 219-228.
103. Tabynov K, Ryskeldinova S, Sansyzbay A. 2015. An influenza viral vector Brucellaabortus vaccine induces good cross-protection against Brucellamelitensis infection in pregnant heifers. Vaccine. 33(31):3619–3623.
104. Talukder, B. C., Samad, M. A., & Rahman, A. K. M. (2011). Comparative evaluation of commercial serodiagnostic tests for the seroprevalence study of brucellosis in stray dogs in Bangladesh. Bangladesh Journal of Veterinary Medicine, 9(1), 79-83.
105. Teshome, H., Molla, B., & Tibbo, M. (2003). A seroprevalence study of camel brucellosis in three camel-rearing regions of Ethiopia. Tropical Animal Health and Production, 35, 381-390.
106. Uddo, T. B. (2021). The Contemporary Situation of Elderly People in Ethiopia and the Ethiopian Evangelical Church Mekane Yesus.
107. World Health Organization. (2015). WHO estimates of the global burden of foodborne diseases: foodborne disease burden epidemiology reference group 2007-2015. World Health Organization.
108. World Health Organization. (2019). Food and Agriculture Organization of the United Nations, World Organisation for Animal Health. Taking a multisectoral, one health approach: a tripartite guide to addressing zoonotic diseases in countries, 166.
109. Yohannes, M., Degefu, H., Tolosa, T., Belihu, K., Cutler, R. R., & Cutler, S. (2013). Brucellosis in Ethiopia. African Journal of Benkirane A, Idrissi AH, Doumbia A, de Balogh K. 2014. Innocuity and immune response to Brucella melitensis Rev.1 vaccine in camels (Camelus dromedarius). Open Vet J. 4(2):96–102.

by Proskura *et al* (2015). The *F-KER* gene is coding for keratin is related to the quality of the feathers, which may influence flight. (Proskura et al 2017). A relation between variability in cryptochrome 1(*CRY1*) genotype and race performance has been suggested (Dybus *et al* 2021). *CRY1* is involved with magneto-reception (Du et al 2014) and therefore variability in genotypes could potentially have impact on special orientation. Other genes related to orientation include *LRP8* and *GSR* in which mutations were identified in racing pigeons (Shao et al 2020). *LRP8* and *GSR* may play a role in race performance. *LRP8* is a gene that encodes for the LDL receptor related protein 8 that is involved the spatial memory and learning ability. *GSR* (encoding glutathione-disulfide reductase) may be involved in magneto-reception and may be part of the biocompass pathway in the homing pigeon. The myostatin (*MSTN*) gene encodes a transforming growth factor that controls the growth and development of muscle tissue. It has been hypothesized that variability in *MSTN* may be related to increases in muscle mass and superior racing performance (Dybus et al 2013, Małgorzata et al 2018).

While a favorable profile for race performance was reported previously (Kolvenbag et al 2022), this was with birds from a closely bred family. It is of interest to demonstrate or confirm such profile in unrelated racing pigeons. In 2023, the top 10 performing birds over 4 races in 8 of the largest one loft races were reported (Table 1); For example the number 1 bird in the USA won 4th place against 750 birds in the race over 150 mile, 17th against 695 birds (186 mile), 9th against 650 birds 238 miles and 5th against 518 birds (350 miles). Genetic profiles were obtained for 7 of the top 10 birds in the USA in 2023. This report presents the genetic profiles for 7 of the Top10 USA OLR birds against the general population background prevalence.

OVERALL RANK	PERCENTAGE	LOFT NAME	BIRD	COLOR	SEX	ONE LOFT RACE
1	1.332%	Da-Dong	AU 23 TENT 3050	BBWF	H	Orlando Golden Classic
2	2.889%	Royal Heir Loft	AU 23 AA 10303	BC	H	Crooked River Challenge
3	2.991%	Ignacio Family Loft	AU 23 HRPC 3512	BC	H	Orlando Golden Classic
4	3.221%	Los 4 Loft	AU 23 ARPU 5802	BC	C	Orlando Golden Classic
5	4.033%	99 Problems	AU 23 EMIL 36	BBWF	H	Hoosier Classic Million Dollar Race
6	4.538%	Nemelka Racing Pigeon Loft	AU 23 NRPL 3035	BB	H	Crooked River Challenge
7	4.626%	Yang Loft	AU 23 YANG 0136	BB	H	USA Pigeon Derby
8	4.840%	Linda Loft	IF 23 LIND 1004	BB	H	USA Pigeon Derby

9	5.180%	Denis Loft	AU 23 AA 6007	BB	H	USA Pigeon Derby
10	5.354%	003 loft	AU 23 ARPU 81205	BCWF	H	USA Pigeon Derby

Abbreviations: One Loft Race (OLR)

Table 1: Top 10 ace champion birds in the USA OLRs in 2023.

Methods:

All 10 owners of the Top 10 Ace Champion birds in the USA OLRs in 2023 were invited to participate, 7 responded with donating feathers. Samples were obtained by the owner and shipped to the author. Feathers were send for analysis at Feanix Biotechnologies, 39 Glendale Ave, Suite 102, Asheville, NC 28803, tel (530) 205-3588), email: info@feanixbio.com

General prevalence allele frequencies were provided by Feanix Biotechnologies from 100 random samples. Comparison of profiles of top 7 birds versus general prevalence was performed by goodness of fit tests for each of the gene categories (Snedecor et al 1989). The general prevalence data was used as the control and assessed, whether or not the distribution of the genotypes from the 7 pigeons are marking the different from the general prevalence data. P value of the Chi–square goodness of fit test is provided.

Results

The largest differences observed comparing the profiles of the 7 top race birds versus the expected profiles are in gender, *LDHA*, *LRP8* and *GSR*, while no or small differences were seen in the other genes (Table 2).

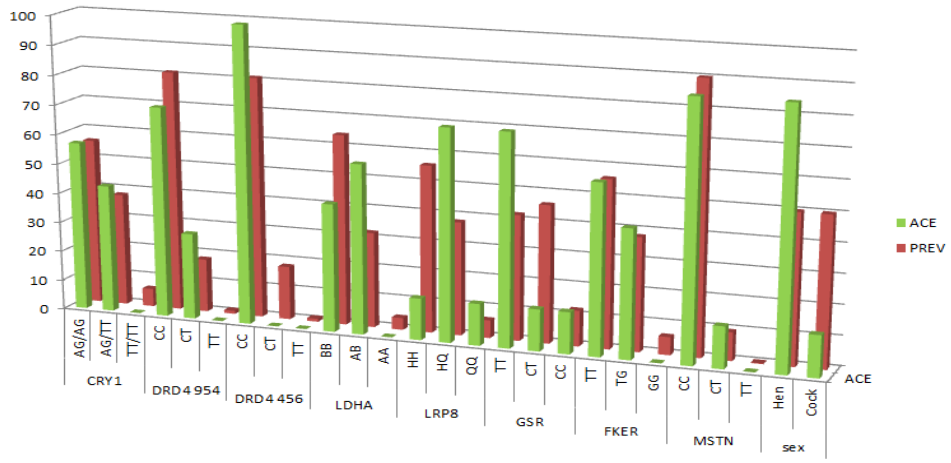
Gene	**Genotype**	**ACE (n=7)**	**% of n**	**PREV (n=100)**	**Expected n**	**(O-E)^2/E**	**Chi-square**	**p-value**
CRY1	*AG/AG*	4	57.1%	56%	3.92	0.002		
	AG/TT	3	42.9%	38%	2.66	0.043	0.465	0.793
	TT/TT	0	0.0%	6%	0.42	0.420		
DRD4 954	*CC*	5	71.4%	81%	5.67	0.079		
	CT	2	28.6%	18%	1.26	0.435	0.584	0.747
	TT/TT	0	0.0%	1%	0.07	0.070		
DRD4 456	*CC*	7	100.0%	81%	5.67	0.312		
	CT	0	0.0%	18%	1.26	1.260	1.642	0.440
	TT	0	0.0%	1%	0.07	0.070		
LDHA	*BB*	3	42.9%	64%	4.48	0.489		
	AB	4	57.1%	32%	2.24	1.383	1.942	0.379
	AA	0	0.0%	1%	0.07	0.070		
LRP8	*HH*	1	14.3%	58%	4.06	2.306		
	HQ	5	71.4%	38%	2.66	2.058	5.166	0.076
	QQ	1	14.3%	6%	0.42	0.801		
GSR	*TT*	5	71.4%	42%	2.94	1.443		
	CT	1	14.3%	46%	3.22	1.531	3.004	0.223
	CC	1	14.3%	12%	0.84	0.030		
F-KER	*TT*	4	57.1%	56%	3.92	0.002		
	TG	3	42.9%	38%	2.66	0.043	0.465	0.793
	GG	0	0.0%	6%	0.42	0.420		

MSTN	*CC*	6	85.7%	90%	6.3	0.014	0.233	0.890
	CT	1	14.3%	10%	0.665	0.169		
	TT	0	0.0%	0%	0.05	0.050		
SEX	Hen	6	85.7%	50%	3.5	1.786	3.571	0.059
	Cock	1	14.3%	50%	3.5	1.786		

Abbreviations: lactate dehydrogenase A (*LDHA*), dopamine receptor (*DRD*), myostatin (*MSTN*), feather keratin (*F-KER*), cryptochrome 1 (*CRY1*), LDL receptor related protein 8 (*LRP8*), encoding glutathione-disulfide reductase (*GSR*), Ace champion bird (ACE), general population prevalence (PREV)

Table 2: Genotype comparison of the 7 OLR ace champions versus expected prevalence.

No statistical significant differences were observed in the comparison of the genotypes of the 7 ace champions compared to the prevalence in the general population. Of interest are the trends towards a difference observed for gender and for *LRP8*. There was no suggestion for a difference in the other genes including *CRY1*, *DRD4*, *LDHA*, *GSR*, *F-KER* and *MSTN*. There were 9 hens in the Top 10 (see Figure 1). Unfortunately from 3 no samples were received, hence the analysis and report is based on 7 birds. In the analysis of gender a 9:1 ratio would have been in favor of hens and statistically significant (p=0.001).



Abbreviations: lactate dehydrogenase A (*LDHA*), dopamine receptor (*DRD*), myostatin (*MSTN*), feather keratin (*F-KER*), cryptochrome 1 (*CRY1*), LDL receptor related protein 8 (*LRP8*), encoding glutathione-disulfide reductase (*GSR*), Ace champion bird (ACE), general population prevalence (PREV)

Figure 1: Bar chart of genotype frequency of Ace Pigeon (ACE) population and general population **(PREV)**

Discussion:

The hypothesis that race performance is determined by one or more genotypes still needs to be proven. Reports to date have created hypotheses (summarized by Kolvenbag et al 2022), but to date, no firm concluding evidence has been delivered. Partly because large prospective studies have not been performed and / or the actual genes causatively involved in race performance have not yet been identified.

Reports on individual genes have made suggestion about a gene to be associated with race performance, most often limited to a certain distance eg *LDHA* and *CRY1* on the short races (Proskura et al 2014, Dybus et al 2021), or for *F-KER* in long distance races (Proskura et al 2017). Our previous study in a closely related family of racing pigeons generated a hypothesis for birds with the genotypes *DRD4 CCCT* and *F-KER TT* would relate to consistent race results (Kolvenbag et al 2022). This was subsequently observed in an individual bird from the same family with consistent race performance two years later (Kolvenbag 2024). However, the current study of the unrelated 7 top USA OLR birds, did not confirm this; with no observed difference in prevalence for variability in the *DRD4* and *F-KER* genotypes compared to the distribution in the general population. Thus this hypothesis is still to be confirmed or rejected in a large prospective study.

We recognize the limitations of our current study having a small sample size of n=7. However, these were 7 birds in the top 10 Ace Champion birds in the 2023 OLRs in the USA. A distinction from the overall population, when existing, could be expected. It is striking that 6 of the 7 birds (or 9 out of 10) had the female gender. This contradicts our previous study in a close family of birds in which there was no difference in gender (Kolvenbag G and Scott

M (2022). This indicates that one has to be careful studying performance in a closely related family of birds as the results may not be applicable to the wider general population. The result of our study rejects the working hypothesis that *LRP8 HH* is related to better race performance than *LRP8 QQ;* it is surprising to see that all 6 hens had at least one *Q* allele for the *LPR8* gene, and the only male bird had *LPR8 HH*. It is unknown if there is a gender relationship with *LRP8* genotypes.

Another unknown is how close the reference allele frequencies used in this comparison are the real life numbers. Reference frequencies were provided by Feanix Biotechnologies from a random 100 birds from the USA sample size based on feathers submitted to their commercial laboratory for analysis. This could include a selection bias as one could expect that only feathers from good performing birds are submitted for analysis. Either way, the frequencies provided by Feanix Biotechnologies were largely consistent with the frequencies previously reported in our study (Kolvenbag et al 2022). Nevertheless, larger sample sizes need to be analyzed and reported to be able to reference the normal distribution of genotypes in racing pigeons.

The results in this report are not completely in line with the previous reported data and hypothesis on individual genes and genotype profiles related to race performance. This could be due to differences in pigeon population (eg related or not), the sample size (most studies have a small sample size), and or results reported to-date are random findings as the true causative genes related to performance may still need to be identified.

In conclusion, this report showed that in the population of racing pigeons studied, there was a suggestion there may be a difference in profiles for USA OLR Ace Champion birds versus the general population; i.e. hens with at least one *Q* allele for the *LPR8* gene was typical of the Ace Champions. Given this observation and reports made to-date, the call should be made for large prospective studies involving birds that are not family related. In parallel, research should continue to identify other genes or gene profiles potentially related to race performance. In the meantime, selection of racing pigeons remains an art.

Author contributions: GK: Conceptualization; Data curation; Investigation; Methodology; Project administration; Resources; Writing-original draft manuscript

Artificial intelligence (AI): No AI-assisted technologies were used in the generation of this manuscript.

Funding: The author declares that no funds, grants, or other support were received neither for this study nor during the preparation of this manuscript.

Ethics Statement: Study was conducted by an independent scientist without institutional relationship. No human subjects were involved in this study. Ethics review was not available. Study was conducted following high standard and generally accepted practice to breed and race racing pigeons.

Consent to participate: N/A: no human subjects were involved in this study.

Data availability: N/A: All data are incorporated into the article.

Acknowledgements: Mark Scott for providing statistical analysis; Feanix Biotechnologies for providing allele prevalence information; Owners of the Champion birds (Thomas Baldwin, Lance Crockett, Yunier Aquila, Eugen Danilet, Leo Yang, Lu Nick and Denis Martinez) for providing feather samples.

Conflict of interest: Author declares no conflict of interest

References:

1. Du, X.L.; Wang, J.; Pan, W.S.; Liu, Q.J.; Wang, X.J.; Wu, W.J. (2014) Observation of magnetic field effects on transient fluorescence spectra of cryptochrome 1 from homing pigeons. Photochem. Photobiol. , *90*, 989–996.
2. Dybus A, Pijanka J, Cheng Y-H, Sheen F, Grzesiak W and Muszynska M (2006). Polymorphism within the *LDH-A* gene in the homing and non-homing pigeons. Journal of Applied Genetics, 47: 63-66.
3. Dybus A., Proskura W., Sadkowski S., Pawlina E. (2013): A single nucleotide polymorphism in exon 3 of the myostatin gene in different breeds of domestic pigeon (Columba livia var. domestica) 32. Veterinarni Medicina, 58: 32-38.
4. Dybus A., Proskura W., Pawlina E., & Nowak B. (2018): Associations between polymorphisms in the myostatin, αA-globin and lactate dehydrogenase B genes and racing performance in homing pigeons. Veterinarni Medicina, 63, 2018 (08): 390–394.
5. Dybus A, Kulig H, Yu YH, Lanckriet R, Proskura W, Cheng YH. (2021) *CRY1* Gene Polymorphism and Racing Performance of Homing Pigeons. Animals (Basel). Sep 7;11(9):2632. PMID: 34573598; PMCID: PMC8466513.
6. Kolvenbag G and Scott M (2022) The Impact of Gender in Young Bird Pigeon Racing. Corpus J Vet Dairy Sci 3: 1034
7. Kolvenbag G, Scott M, de Kloet A, de Kloet E. (2022) Prospective study relating genotype profiles with race performance in racing pigeons. J Appl Genet. Sep;63(3):563-570. Epub 2022 May 4. PMID: 35505002; PMCID: PMC9365715.
8. Kolvenbag, G. (2024) Consistent race performance in racing pigeons: A case report confirming a favorable genetic profile. Corpus J Vet Diary Sci 5 (1) 1062.
9. Małgorzata A Gazda, Pedro Andrade, Sandra Afonso, Jolita Dilytė, John P Archer, Ricardo J Lopes, Rui Faria, Miguel Carneiro, (2018) Signatures of Selection on Standing Genetic Variation Underlie Athletic and Navigational Performance in Racing Pigeons, Molecular Biology and Evolution, Volume 35, Issue 5, Pages 1176–1189.
10. Proskura, W.S., Cichon, D., Grzesiak, W., Zaborski, D., Sell-Kubiak, E., Cheng, Y-H., Dybus, A. (2014). Single nucleotide polymorphism in the *LDHA* gene as a potential marker for the racing performance of pigeons. J. Poult. Sci., 51(4), 364–368.
11. Proskura, W.S., Kustosz, J., Dybus, A., & Lanckriet, R. (2015): Polymorphism in dopamine receptor D4 gene is associated with pigeon racing performance. Animal Genetics, 46, 586-587.

12. Proskura, W.S., Dybus, A., Łukaszewicz, A., Hardziejewicz, E., Pawlina, E. (2015a). The single nucleotide polymorphisms in lactate dehydrogenase-A (*LDHA*) and feather keratin (*F-KER*) genes and racing performance of domestic pigeon. Zesz. Nauk. UP Wroc. Biol. Hod. Zwierz., 76(608), 37–42.
13. Proskura, W., Lukaszewicz, A., Dzierzbae., Cichon, D., Zaborski, D., Grzesiak, W., & Dybus A. (2017): The Cys83Gly amino acid substitution in feather keratin is associated with pigeon performance in long-distance races. Veterinarni Medicina, 62, (04): 221–225.
14. Snedecor, George W. and Cochran, William G. (1989), Statistical Methods, Eighth Edition, Iowa State University Press.
15. Yong Shao, Hang-Yu Tian, Jing-Jing Zhang, Hamed Kharrati-Koopaee, Xing Guo, Xiao-Lin Zhuang, Ming-Li Li, Hojat Asadollahpour Nanaie, Elahe Dehghani Tafti, Bahador Shojaei, Mohammad Reza Namavar, Narges Sotoudeh, Adeola Oluwakemi Ayoola, Jia-Li Li, Bin Liang, Ali Esmailizadeh, Shu Wang, Dong-Dong Wu, (2020) Genomic and Phenotypic Analyses Reveal Mechanisms Underlying Homing Ability in Pigeon, Molecular Biology and Evolution, Volume 37, Issue 1, Pages 134–148,